Università degli Studi della Tuscia di Viterbo
Dipartimento di Ecologia e Biologia

PhD Program in

Ecology and Sustainable Management of Environmental Resources
XXXII cycle

Metagenetic profiling of the bacterial communities of extreme or sub-extreme marine environments

(BIO19)

Doctoral dissertation of:
Susanna Gorrasi

Coordinator:
Prof. Roberta Cimmaruta

Tutor:
Prof. Fenice Massimiliano

A.A. 2018/19

Index

It is the time you have wasted for your rose
that makes your rose so important.

(Antoine de Saint-Exupéry, *The Little Prince*)

Chapter 1

INTRODUCTION

1. The marine environment

1.1 General features of the marine environment

Oceans and Seas represent the broadest expanses of water on the planet, covering 3.61 x 10^8 km^2 and containing ca. 97% (1.4 x 10^{18} m^3) of all the water on Earth (Austin, 1988, Munn 2011). The average depth is 3200 m and the maximum depth (ca. 10,920 m) is reached at the bottom of the Challenger Deep, in the Marianas Trench (Nakanishi and Hashimoto, 2011; Speight and Henderson, 2013). The 90% of the volume of the oceans constitutes deep sea, including many habitats, the majority of which is practically unexplored. Five major ocean basins are generally recognised, even if they actually constitute a single interconnected water system (Austin, 1988, Munn 2011; Kennish, 2019).

A large fraction of all planet life is present in the oceans. Marine ecosystem is enormous and provides many different habitats for a wide variety of organisms, which are adapted to different special conditions (Sumich and Morrissey, 2004).

A number of major habitat divisions are recognised in the marine ecosystem, starting from the land towards ocean depths (Fig. 1). The intertidal (or littoral) zone is the part of the coastal zone where land and sea meet, between the limits of the high and low tide. Then, the sublittoral extends from the extreme low water level down to ca. 40 m. From the edge of the continental shelf (the submerged sloping border of the land) the depth increases down the continental slope (marking the edge of the continents), along the continental rise (the gently sloping between the continental slope and the abyssal plain), to reach the abyssal zone (Van den Hove and Moreau 2007, Speight and Henderson, 2013). In general, the marine biome can be divided in three main regions: the intertidal zone; the neritic zone (near the coastline), which extends from the low-tide level to a depth of 200 m; the oceanic zone, which occurs far away from lands (Speight and Henderson, 2013).

Moreover, in the oceans it is also possible to identify (on the basis of major habitats) the benthic and pelagic provinces, involving organisms living on the seafloor and in the water column, respectively (Ross, 1995, Kennish, 2019). The pelagic and benthic environments are generally subdivided into zones based on depth. The benthic province presents five

discrete zones: littoral, sublittoral, bathyal, abyssal, and hadal. Among them, the abyssal zone accounts for the greatest benthic habitat area of the oceans (ca. 75%), while the bathyal and sublittoral zones represents the 16 and 8%, respectively (Kennish, 2019).

Based on depth, the pelagic province is divided into four zones (Speight and Henderson, 2013; Kennish, 2019): epipelagic (0–200 m), mesopelagic (200–1000 m), bathypelagic (1000–4000 m), and abyssopelagic (4000-6000 m) and hadalpelagic (above 6000 m). The hadal zone is the deepest part of the oceans, which includes the ocean trenches.

Moreover, the bathyal and abyssal zones may also present some topographic and geological features such as seamounts (volcanic edifices), hydrothermal vents and cold-seeps (Watling et al., 2013; Rogers, 2015; Kennish, 2019).

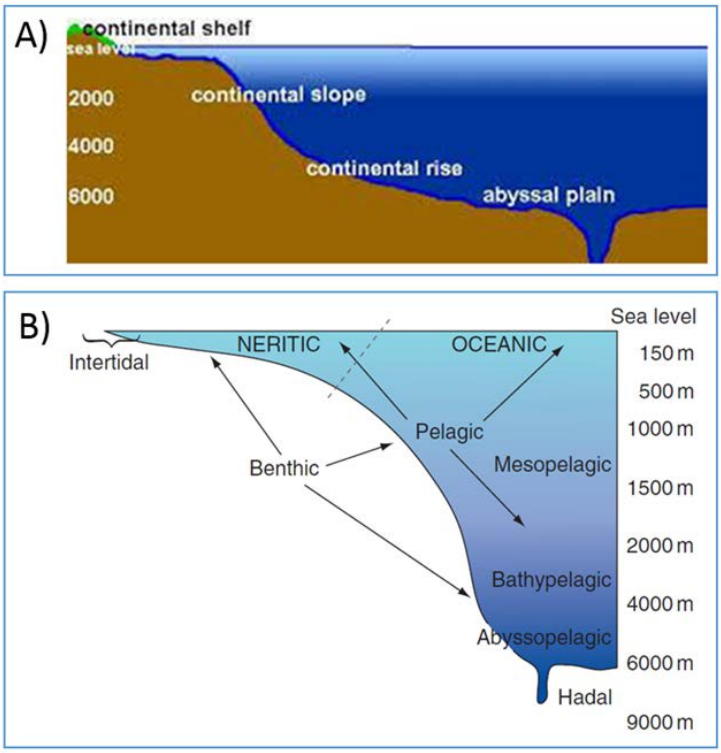

Fig. 1.1 Schematic representation of the major oceanic domains and ecological zones of the marine ecosystem. (A) from Tsili and Zacharopoulos, 2017; (B) from Speight and Henderson, 2013).

Although the shelf area represents only 7% of the total ocean surface, the majority of marine life has been found in these coastal habitats. However, it is recently recognised that life is abundant in the deep seas also (Jamieson et al., 2010; Ramirez-Llodra et al.,

2010; Thurber et al., 2014; Xu et al., 2014; Rogers, 2015; Durden et al., 2015; Malyutina et al., 2018).

The physical and chemical structure of the oceans and the variations of related parameters (i.e. salinity, temperature, light, pressure and nutrient concentration) create distinct conditions to which organisms must adapt.

Salinity – The seawater is a complex mixture of inorganic and organic compounds, including nutrients, and dissolved gases (O_2, CO_2 etc.), the concentration of which changes locally and regionally in relation to geographical and physical factors and affects organisms distribution (Austin, 1988; Speight and Henderson, 2013). Chemical elements are accountable for salinity that ranges in-between 33-37‰, with an average of 35‰ (Austin, 1988; Kennish, 2019); in deep waters (4000 m or deeper), the salinity is relatively uniform (34.6–34.9‰) throughout the world ocean (Mitra and Zaman, 2016). Deviations from average salinity can be recorded at ocean boundaries (due to dilution from river runoff) and at deep-sea hydrothermal vents (due to the emission of mineral-laden water from the oceanic crust). On a regional scale, evaporation and precipitation lead to deviations from average salinity: evaporation causes salinity increase, whereas high rates of precipitation and river runoff reduce it (Kennish, 2019). Salinity increases from coastal to open sea and with depth, and organism distribution reflects this gradient depending on specific tolerance (Austin, 1988; Speight and Henderson, 2013). Estuaries and estuarine systems show more salinity variation (from ca. 0.5 to more than 35‰) compared to the ocean areas. In these coastal zones, salinity changes both temporally (over annual, seasonal, daily, and tidal cycles) and spatially (along longitudinal, vertical, and transverse planes). Moreover, in estuaries and estuarine systems, the mixing between freshwater and seawater is responsible for the occurring salinity variations (Kennish, 2019).

Temperature - Temperature has an important role for the organism distribution in the sea, since many biological processes depends on this factor. In general, the surface temperature of the ocean decreases poleward, since a declining insolation with increasing latitude occurs. On seawater surface there is a latitudinal gradient, with a temperature variation from less than 0°C close to the poles to ca. 25-30°C in the tropics (Speight and Henderson, 2013; Kennish, 2019). The solar radiation produces a thermal stratification, and at 100-150 m depth there is a marked thermocline with strong temperature reductions (to 10°C or less) (Munn, 2011). Temperature is fairly constant in deep ocean waters, ranging from ~0°C to ~4°C in the deep benthic environment (Munn, 2011; Speight and

Henderson, 2013). Temperature recorded for the Antarctic shelf water in connection with the ice shelves base (often at many hundreds meters' depth) reaches −2.0°C or less; but it increases slightly (-1 °C) along the slope to be around 0°C at the bottom of the Antarctic Ocean (Steele et al., 2010, Fig. 1.2).

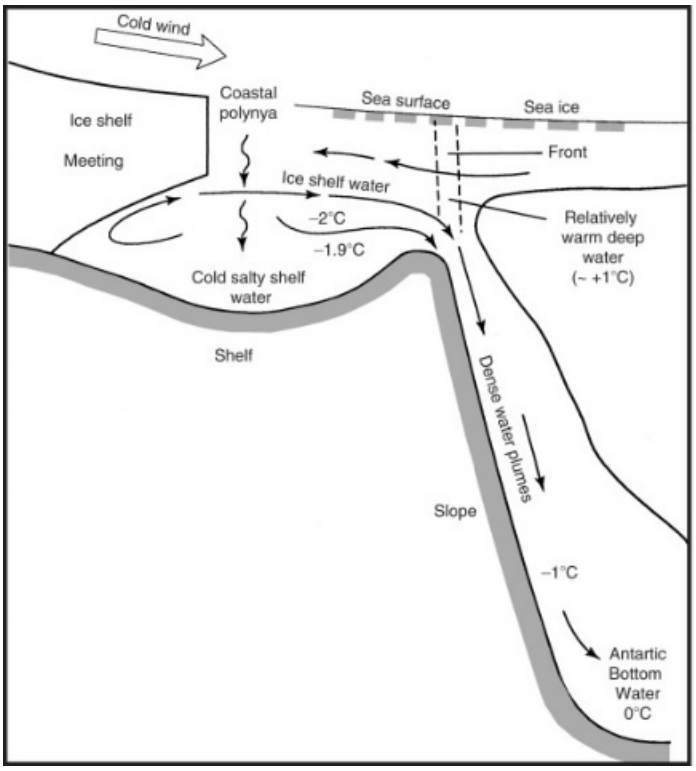

Fig. 1.2 Cold water flows in the Antarctic shelf and bottom (from Steele et al., 2010).

The deep-sea hydrothermal vents represent a notable exception where water can reach local temperatures to over 100°C, and it is about 350°C at the point of emission (it may exceed 400 °C). Instead, unlike the hydrothermal vents, in the cold seeps the temperature recorded (ca. 2 or 3°C) is the same of the surrounding waters (Reysenbach and Cady, 2001; Speight and Henderson, 2013).

Light – Light is another central factor limiting marine organism presence. This is particularly true for phototrophic organisms that are mainly responsible for primary productivity. Based on penetration of light, the oceanic compartment may be principally divided into two distinct zones: the photic zone (up to 150-200 m depth), where light is available; the aphotic zone (usually below 200 m), which is the deeper lightless zone of the ocean (Mitra and Zaman, 2016). Actually, photosynthesis is mainly restricted to the

photic zone, and photosynthetic organisms need various adaptation mechanisms to different light conditions (Sumich and Morrissey, 2004). In general, in coastal waters, scarce light penetrates more than a few meters (limiting the photic zone), since these waters are typically more turbid (due to pigments from land and suspended sediments from rivers) compared to that of the oceanic areas (Speight and Henderson, 2013).

Pressure – The pressure at sea surface is ca. 1 atm and it increases by roughly 1 atm every 10 m of depth. Thus, only organisms that evolved special adaptation (known as piezotolerants or piezophiles) can inhabits deepest waters (Speight and Henderson, 2013). Most of the organisms living in these zones are Bacteria and Archaea, with presence of fungi, fishes and invertebrates (Gage and Tyler, 1991; Herring, 2002; Nagahama, 2006; Van den Hove and Moreau, 2007; Jamieson, 2015; Pernice et al., 2016; Danovaro et al., 2017).

Dissolved oxygen (DO) - DO plays an important role in organism survival. In the open ocean, its concentration is generally high only in the top 10-20 m of water, due to exchange with the atmosphere and oxygenic photosynthesis, while it decreases with depth, reaching a minimum between 200 and 1000 m for its consumption by decomposing aerobic bacteria. At further depths, DO increases again because oxygen consumption is lower; in addition, enhanced oxygen solubilisation is supported by temperature decrease and hydrostatic pressure rise. Moreover, there is a consistent supply of cold and oxygen-rich deep waters from Polar Regions (Munn, 2011). In inshore areas, oxygen concentration in shallow waters can vary both spatially and temporally. In some parts of estuaries, bottom waters are almost anoxic, due to the oxygen consumption by microorganisms. It is worth noting that, the oxygen concentration is one of the physical key-variables determining the diversity and abundance of life in estuarine and shallow coastal waters (Speight and Henderson, 2013).

Nutrient concentration - Seawater environments are generally oligotrophic presenting very low nutrients concentration. Ocean primary production is limited by nutrient availability that, consequently, affects the whole food chain. The principal limiting factors are nitrogen and phosphorus, even if recent studies demonstrate that primary production could be limited by iron also (Moore *et al*, 2001; Speight and Henderson, 2013).

Contribution of marine organisms, in particular microorganisms, to main biogeochemical cycles (i.e. oxygen and carbon) is fundamental. The contribution of marine

phytoplanktonic organisms to global primary net production is about 50%, among them *Cyanobacteria* are responsible of about 25% of carbon fixation and O_2 production. It has been calculated that the picocyanobacteria *Prochlorococcus* spp. and *Synechoccus* spp., living in tropical and temperate oceans, contribute to ca. 40-50% of the photosynthetic primary production by *Cyanobacteria* in oceans (McIntyre, 2010; Munn 2011; Flombaum et al., 2013).

Tides **-** Tides are defined as the periodic rise and fall of the sea level, resulting from the action of the gravitational pull exerted by the sun and the moon (Mitra and Zaman, 2016). They are responsible for the periodic horizontal displacement of coastline and for the movement of tidal currents (driving force for the turbulence and water mixing in most stratified and well-mixed estuarine systems), thus they are one of the most important physical features affecting life in coastal marine environments (Speight and Henderson, 2013; Kennish, 2019).

1.2 Microorganisms and relations with marine habitats

As already stated by the early work of Sieburth (1979), microorganisms are well represented in all marine habitats (Fig. 1.3).

Even if Plankton is traditionally divided in zooplankton (animals) and phytoplankton (plants), prokaryotes represent a significant portion of planktonic biomass. Given their important role in biogeochemical processes, microorganisms play a fundamental role in planktonic marine food webs having a large impact on the whole ecosystem (Giovannoni *et al*., 1990; Gasol *et al*., 1999).

A large number of microorganisms is also found on Neuston, the water-air interface rich in organic matter and microbial life. However, there is no evidence of a stable and unique Neuston community, although predominance of some much specialised bacteria, adapted to intense solar radiation, is possible (Maki, 2002, Agogué *et al*., 2005).

Also the surface of swimming animals (Nekton) and their digestive apparatus are widely colonised by microorganisms constituting peculiar ecosystems. Biofilms, a complex organic matrix containing principally organised microbial communities, could be found on the surfaces of all kind of animals and plants. Many organisms seem to selectively promote surface colonisation of certain microorganisms and inhibit growth and/or attachment of others (Jensen and Fenical 1994). Microbial associations with

marine animals and plants could be neutral or produce mutual benefits (symbiosis) even if some microorganisms are pathogens (Dubilier et al., 2001, Goffredi *et al*., 2008, Munn 2011).

Benthos (the sea-sediment interface, including plants and animals) is widely colonised by microorganisms. Microbial activity in the sediment is intense (sediments are generally less oligotrophic than water column), due to the presence of particulate organic debris and dissolved organic compounds, being of paramount importance for the global carbon and sulfur cycles (Iversen and Joergensen, 1985; Aller and Rude, 1988; Kirschner and Velimirov, 1999; Valentine, 2002). Biofilms and symbioses are present in Benthos also.

The particulate matter constituting the Seston presents lot of microorganisms too. The continue flow of matter, dropping through the water column, is known as marine snow. It consists of aggregates of inorganic particles, living or dead plankton and fecal material pelleted by a polymeric matrix produced by bacteria and phytoplankton (Alldredge and Silver, 1988). Marine snow, mainly produced in the upper layers of the water column (100-200 m), if not degraded by extracellular enzymes and microorganisms, can reach the ocean bottom in matter of days. This is the main mechanism conveying part of primary production to deep waters and seafloor (Karner and Herndl, 1992; Munn, 2011). Organic matter degradation and solubilisation, due to the microbial activity, make nutrients available for other organisms. Thus, most of carbon is re-mineralised during its descent and the remaining material reaches the ocean floor, where it is consumed by benthic organisms or contributes to sediment formation (Munn, 2011).

In all aquatic environments, biofilms cover plants, animals, rocks and anthropic structures/manufactures generating various epibiotic habitats. The biofilm communities adhere to solid surfaces by secretion of extracellular polymers entrapping organic and inorganic components. Biofilms are particularly important in shallow and intertidal waters and are interesting for their complex ecological interactions (Donlan, 2002; Munn, 2011).

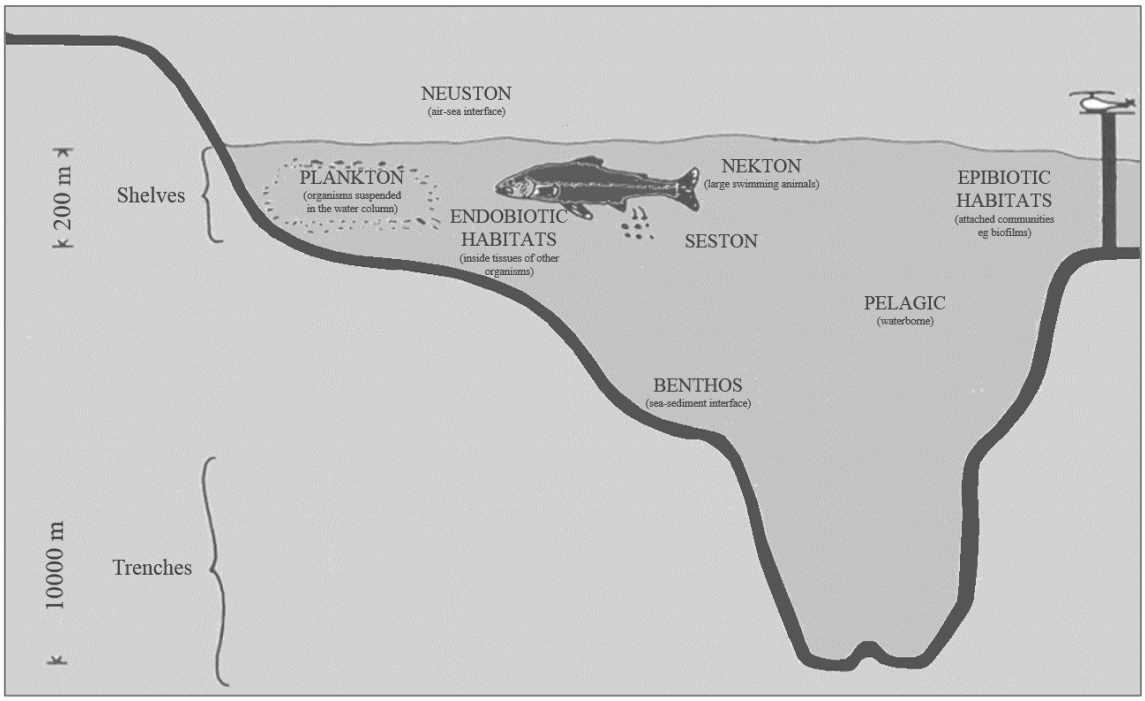

Fig. 1.3 Schematic representation of the habitats for marine microorganisms; based on Sieburth (1979)

Due to the peculiar adaptation strategies, marine microorganisms are important also for their ability to live in extreme habitats were life for other organisms is difficult or even impossible (i.e. cold deep waters and hydrothermal vents).

Microorganisms have also an important role for food chain in sea ice ecosystems of Polar Regions, where both the ecosystem physical phenomena and biological activity contribute to shape the overall habitat. Ecology and biology of sea ice are essentially driven by temperature and salinity, showing high temporal and spatial variability. Sea ice microbiota has a major influence on various trophic levels of the oceanic food web. Bacteria are tightly coupled to primary productivity. Through photosynthesis, microalgae contribute significantly to carbon fixation, while mineralisation of dissolved organic matter is carried out primarily by heterotrophic bacteria (Grossmann and Dieckmann, 1994; Arrigo and Thomas, 2004).

Even if lot of information is available on various marine peculiar habitats, to date, many marine environments are still unknown under the microbiological point of view.

1.3 Extreme habitats in marine environment

Moving from coastal to oceanic areas, as well as along the above-mentioned oceanic zones, various marine extreme or sub-extreme environments/habitats occur. Extreme marine environments could be characterised by very high or rather low temperatures, high-hydrostatic pressure, low or high pH, high salt concentrations. In addition, very often, a combination of two or more extreme parameters lead to poly-extremophilic environments (Bowers et al., 2009; Poli et al., 2017), which force the living organisms to adopt specific adaptation strategies. As demonstrated for other environments, also for the extreme marine habitats microorganisms represent the last boundary of life (Bowers et al., 2009; Zucconi et al., 2016).

- In the coastal areas, various transition zones could be considered as extreme environments both for presence of extreme values of environmental parameters and/or for their broad variations.

-The intertidal zones (littorals) are the coastal zones subject to tides. They are particularly interesting since in these areas all biogeochemical processes are more evident, in particular if tides are very pronounced. Organisms in these zones require adaptation to variable environmental conditions in which factors, such as temperature, water availability, and salinity change frequently. Stress by temperature variations has been considered to be among the fundamental factors of organism distribution (particularly in the rocky littorals), which present both seasonal peaks in correspondence of very high temperatures and peaks in mean daily maxima (defined by Helmuth and Hofmann, 2001 as “acute” and “chronic” high temperatures); highest temperatures are recorded during low tide. Increasing of salinity is often coupled with water evaporation caused by high temperatures and contribute to multiple environmental stress in these areas, particularly in temperate and tropical zones (Helmuth and Hofmann, 2001; Savvichev et al., 2004, Pesciaroli 2012).

-Among marine transition areas, solar salterns represent very interesting extreme marine coastal systems, where physicochemical parameters show intense daily and/or seasonal variations; however, a broad salinity gradient is generally present. As demonstrated for a

few other unstable extreme environments (Reboleiro Rivas et al., 2013; Andrade et al., 2014), rapid and recurrent fluctuations of temperature and water availability submit organisms to great environmental stress, making these sites good models to study some global-change phenomena (Barghini et al. 2018a). In addition, they could represent sources of new microorganisms for possible applications in biotechnology.

Commonly, marine solar salterns are constituted by various shallow pond sequentially connected and showing an increasing gradient of NaCl concentration. The gradient develops from the seawater salinity (i.e. about 35 %), to over 300‰ in the crystallisation ponds, where salt is produced (Barghini et al., 2018a). These features cause sturdy environmental heterogeneity leading to the establishment of composite biological communities distributed along the gradient and including both marine and specialised species well adapted to the hypersaline environment (Halse et al., 2002; Cimmaruta et al., 2010; Barghini et al., 2014). The presence of microorganisms in the various ponds is due to the active growth of taxa adapted to the pond environmental conditions or to external inputs. However, at the highest salinities, communities are dominated by prokaryotes, representing good models for the study of the adaptation strategies adopted to survive under the severe life conditions found in some extreme environments (Salgaonkar et al. 2013; Barghini et al. 2018a).

- From the coastal areas through the shelf and toward the abyssal plain and trenches, it is possible to find some other interesting marine extreme environments, showing very different characteristics. It is worth noting that, within the marine environment, extremophilic conditions increase with depth. The deep seas are characterised by the absence of sunlight and, mostly, by low temperatures and high hydrostatic pressure. However, they are primarily cold environments since most of the water is at about 5 °C or below. Consequently, deep-sea biome harbours principally psychrophilic organisms, which generally show a limited range of temperature for growth. Environmental conditions become even more stressing in very peculiar habitats, such as the deep-sea hydrothermal vents (DHVs), the deep hypersaline anoxic basins (DHABs) and the abyssal trenches with their extremely high pressures. Deep-sea extremophiles can cope with the deep-sea poly-extreme environments that are lethal to other organisms. Most of these organisms are prokaryotes belonging to both Archaea and Bacteria (Jin et al., 2019).

-DHABs are among the most extreme and captivating habitats on the Planet. They are poly-extreme brine areas, discovered at the end of the 70', found on the floor of several oceanic areas, such as the Mediterranean Sea, the Gulf of Mexico, the Black Sea and the Red Sea (Mapelli et al., 2017; Merlino et al., 2018; Guan et al., 2019). These water bodies generally occur within fractures found at the sea floor; they are filled with dense high salinity water (brine) that, due to density difference, does not mix with the overlying water. The high DHABs salinity has been originated from the re-dissolution of evaporitic deposits submerged by sediment layers and exposed to seawater caused by tectonic activities (Cita 2006; Merlino et al., 2018). Thus, upper oxygenated seawater and brine are separated by a dense interface (chemocline/pycnocline), trapping cells and particulate. It has been stated that, due to the lack of mixing, the separation from the upper water layers has been historically extended, and the unique microbial assemblage (mainly prokaryotes with some micro-eukaryotes) harboured by these ecosystems are differentially distributed according to the gradient of oxygen, salinity and nutrients arising in the transition zone (represented by the seawater/brine interface). Microbial life in DHABs have to cope with poly-extreme conditions including high salinity, anoxia, high pressure, and absence of light. This observations demonstrate that life can thrive also under environmental conditions formerly considered incompatible, and supply new perspectives regarding life occurring elsewhere in the Solar System (van der Wielen et al. 2005, Merlino et la., 2018; Mapelli et al., 2017).

-Various fluids are released in sites showing geochemical activity at different depths, from the surface or sub-surface layers up to about 6000 meters. These geochemical activities produce emission of gas and water at different temperatures and with diverse composition (hydrothermal vents). DHV have collected great scientific interest, merging microbiologist and geologist interests, concerning one of the science's key question: "what is the origin of life"? (Martin et al., 2008). DHV host unique ecosystems of endemic animals that do not depend on photosynthesis, but from chemolithoautotrophic bacteria, which for example, due to the oxidation of reduced sulphur compounds, represent a principal driver for carbon fixation (Mitarai et al., 2016; Meier et al., 2017). Based on their temperature, hydrothermal vents could be divided in Black smokers (BSs, high temperatures ~350°C); Lost City systems (LCS, intermediate temperatures 50-90 °C); and Cold Seeps, sometimes called white smokers (CSs, low temperatures 6-23 °C) (Fig. 1.4).

Black smokers, which are placed directly above magma chambers that are located at some kilometers below the sea bottom. Seawater flows through the fissured sea bottom encountering the magma chamber; it becomes very hot before re-emerging at the vents. Hydrothermal vents fields with black smokers have been found up to about 6000 m depth practically in all oceans in correspondence with tectonic boundaries. Thus, Black smokers emanate overheated (up more than 400 °C) seawater, with a strongly modified chemical composition. BSs fluids are usually acidic (pH 2–3) and rich in Fe(II) and Mn(II), CO_2, H_2S and H_2. Variable amounts of methane is also present both due to biogenic and abiogenic processes. Water temperature around BSs is oxygenated and cold (2 °C), thus in a very short space span gradients of temperature and oxygen concentration are established. In these environments, the gases and metals dissolved in the BSs fluid can be used as the base for a short food chain. At the highest temperatures, only Archaea can survive, and some of them seems to be able to replicate up to ca. 121 °C (that is thought as the upper temperature limit for life). With temperature diminution other life forms can flourish (both prokaryotic and eukaryotic), and symbiosis with bacteria are generally the winning strategy to survive. Bacteria oxidise sulphur compounds (i.e. H_2S) to get sufficient energy for the CO_2 organication. Presence of animal life (invertebrates such as molluscs, crustaceans and tube worms and cephalopods, but also fishes contribute to the food chain) is always somehow linked to the primary production carried out by these bacteria (Martin et al., 2008; Mitarai et al. 2016; Meier et al., 2017; Van Audenhaege et al., 2019).

The so-called Lost City systems (or off-axis vents) show very different features compared to the black smokers, even if both produce structures when the emitted fluid are exposed to seawater. First of all, LCS are placed far from the subduction zones, were BS are located; then, the emission is different, very hot acidic for the BS and cooler alkaline for the LCS. In addition, the geothermical/geochemical origin is different, and it is the reason of the differences in the emission temperature and composition. As said, LCS temperature does not exceed about 90°C and their emission has high concentration of H_2 and CH_4, some low-molecular mass hydrocarbons, but almost no carbon dioxide. However, the discussion regarding the geological details about these vents is beyond the aims of this work. It is worth noting that, LCS type vents have been related to the origin of life in our Planet, but composition of their microbial communities could be quite different from those of BS: for example, alkaliphilic instead of acidophilic prokaryotes.

The sites are dominated by methanogens, using several organic compounds, some of which originated from CH_4 oxidation. Methanotrophic, sulphur oxidising and sulphate reducing bacteria are present as well (Martin, 2008; Cardoso and Cartwright, 2017).

Submarine cold seeps involve the seepage and venting of fluids (gas and water) and sediments. Although the term 'cold seep' often refers only to the seeping/venting structures on the seabed, CSs systems are more composite and include three structural elements: the fluid source, the plumbing systems (bringing the fluid to the sea bottom), and the seeping features (venting structures), located at or close to the sea floor (Fig. 1.4) (Talukder, 2012). Sites with presence of CSs have been found to be in association with methane-rich fluid emissions and with a number of submarine structures, such as mud volcanoes, hydrate and carbonate deposits mounds, slabs and/or chimneys. CSs are associated with chemoautotrophic biological communities, supplying spots of primary production and allowing the generation of habitat apt to establish colonisation of a resident macrofauna (mainly constituted by invertebrates such as crabs, clams, mussels and tube-worms) (Bergquist et al., 2003). Very often, invertebrates establish symbiotic relationships with methanotrophic, thiotrophic and/or methylotrophic bacteria (Duperron et al., 2007).

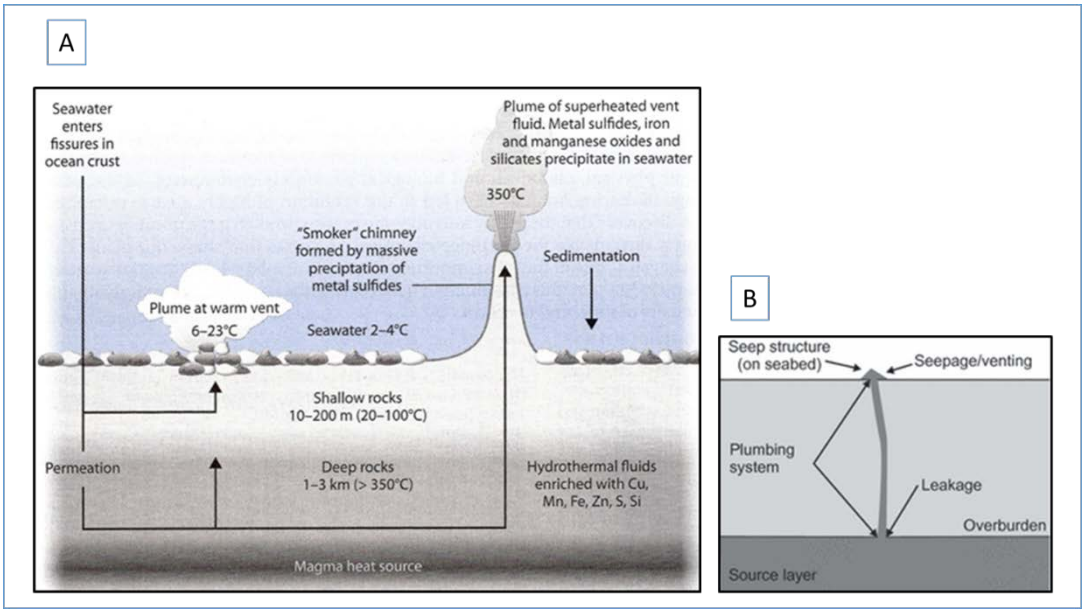

Fig 1.4 (A) simplified geochemical structure of hydrothermal vents (from Munn 2011); (B) details of a cold seep vent (from Talukder, 2012).

-The deepest oceanic environment, in particular the hadal waters and sediments of the oceanic trenches (>6000 m), are all characterised by same extremophilic characteristics: low temperatures and high pressures. Temperature at the bottom of the hadal trenches is considered extraordinarily stable compared to the surface waters (Jamieson, 2015). Its variations with depth is trench-specific and they can be find mainly in the Pacific Ocean Fig. 1.5). The Cayman Trench (4.46-4.49ºC; 6200–6900 m) is the warmest trench, while to the South Sandwich Trench, (0.27-0.09ºC; 6047–7390 m) is the coldest. In any case, the most dramatic environmental feature of these areas is the enormously high hydrostatic pressure, which severely affects the composition and distribution of benthonic life (Tyler, 2003; Mitra and Zaman, 2016).

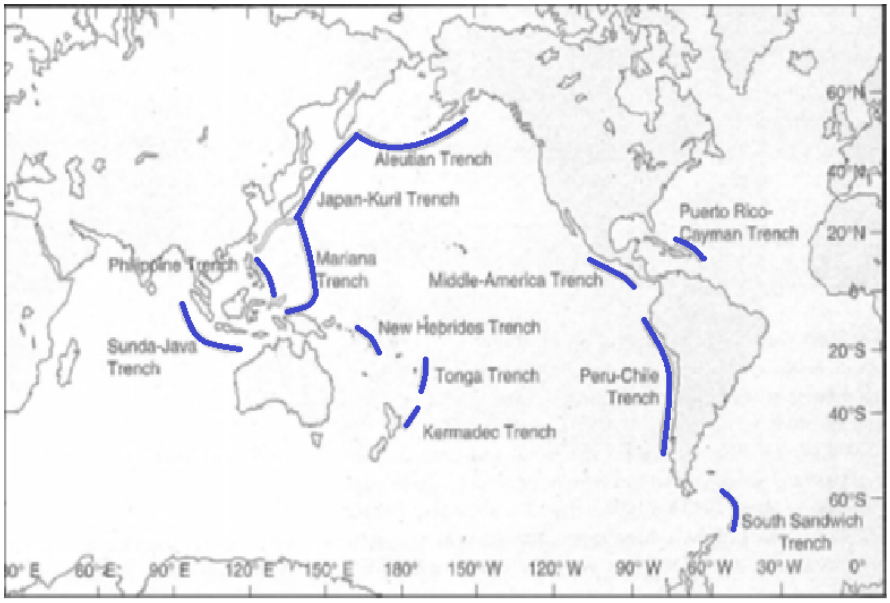

Fig. 1.5 Major trenches on the Planet (modified from Mitra and Zaman 2016)

These environments are quite scarcely explored under the microbiological point of view, due to their remote location and rather broad vastness (they run along the oceans bottom for hundreds km rather than being concentrated in spots), and since their exploration require complex, dedicated and expensive equipment. However, some information are available. In general, microbial diversity, both related to Archaea and

Bacteria, is higher than expected and a rather high number of phyla had been recorded for these prokaryotes. For example, it is known that microbial life in hadal sediment is richer than that of the nearby abyssal plain sediments, due to the increased sedimentation of organic matter (driven by hadal currents) helped by trench topography. Also the presence of fungi has been reported in various sediments of the sea bottom, including those from the Mariana trench (Takami et al., 1997; Xu et a., 2014; Hiraoka et al., 2019).

Chapter 2

STUDY AREAS

2.1 Kandalaksha Bay

Kandalaksha Bay (KB) is one of the three southern bays of the White Sea (WS), which is a rather small semi-enclosed Arctic sea (NW of Russia) and communicates with the Arctic Ocean through the Barents Sea. The water exchanges between the Barents Sea and the WS are limited, but establish a transport system for particulate material, microorganisms and biogeochemical species (Howland et al., 1999; Berger and Naumov, 2000; Pantyulin, 2003). KB is a complex insular estuarine system having a special hydrological regime with rather large sea level differences during tides (shallow, asymmetrical and semi-diurnal), causing great cyclic variations in depths and horizontal displacements of the coastline, and intense mixing of waters within 30 m from the surface (Melnikov et al., 2003; Savvichev et al., 2003). In some areas (i.e. the "Velikaja Salma" strait), both stratified and mixed regions are present. Actually, the topographic features of this area lead to an increase in tide height (up to 2.5 m), but especially in the tidal currents speed that can reach 80–120 cm/s (Pantyulin, 2003).

The pattern of currents is extremely complex and main tidal streams are often split in secondary flows by the various island; moreover, in some areas, peculiar mixing situations are generated also (Tzetlin et al., 1997; Berger and Naumov, 2000; Savvichev et al., 2003). The unique hydrodynamics of the bay is also affected by seasonal intense runoffs of freshwater, due to various rivers, streams and strong precipitations (Howland et al., 1999; Dolotov et al., 2005), which contribute to enrich the sea water with nutrients and microorganisms from the surrounding soils, forests and peatlands. On the other hand, these inputs, carrying particulate and humic substances, cause intense water darkening with consequent strong reduction of the photic zone, which is confined in the first 10 m on average, and could reach 15–20 m in days with the highest solar radiation (Bobrov et al., 1995; Kravchishina et al., 2013).

KB is subject to wide fluctuations of environmental parameters (Savvichev et al., 2003). Annual temperature variability is very high; although global change begins to mitigate it, the winter season is long and quite severe (the sea surface is sheltered by ice for 5–6 months) and the meteorological conditions tend to be very unpredictable. Air temperature may fall to −40 °C, but occasionally rises to few degrees above 0 °C, due to warm Atlantic air streams; water temperature is about −1 °C / −2 °C. In the rather short summer, air temperature can rise up to 30 °C (15–20 °C, on average), while seawater

temperature can reach 15 °C on surface layers, but is subject to a fast drop and remains steadily around 0 °C below a few dozen meters of depth (Berger and Gorbushin, 2001; Pantyulin, 2003; Shaporenko et al., 2005; Vershinin et al., 2006)

In this region, organisms must adapt to variable environmental conditions in which factors (such as temperature and salinity) change frequently (Savvichev et al., 2004; Kravchishina et al., 2008), and communities must be functionally organised to cope with the effects of sudden stressing conditions (Pesciaroli et al., 2015a).

Microbial communities in the White Sea and, particularly in the KB area, have been scarcely investigated and very little is known about their structure, organisation and functionality. In addition, the majority of the studies characterised only the cultivable fraction of the communities (Savvichev et al., 2003, Savvichev et al., 2004; Kravchishina et al., 2008; Pesciaroli et al., 2015b). The sole attempt to depict KB total bacterial community composition and organisation was the preliminary work of Pesciaroli and co-workers (Pesciaroli et al., 2015a), carried out by the PCR-TGGE fingerprinting technique. Although somehow useful for a basic screening, this low-throughput method could only allow for partial characterisation of the community diversity, resulting in incorrect information in relation to its structure. To the best of our knowledge, no study by new generation sequencing techniques has been carried out yet.

2.2 “Saline di Tarquinia” marine salterns

The “Saline di Tarquinia” marine salterns (ST) cover an area of about 135 ha and are located at ca. 80 km NW of Rome, along a low sandy coast of the North Tyrrhenian Sea, from which are separated by an array of low dunes. The salinity gradient is developed along a series of ca. 100 shallow interconnected ponds (Barghini et al., 2018b). The salterns, productive from 1805, dismissed salt production in 1977 to be transformed into a National Natural Reserve (1980). This conversion was accompanied by a dramatic reduction of the anthropic activities and an alteration of the pond management concerning water balance (i.e. irregular control of seawater inputs) and maintenance. Over time, the entire site structure was altered: in particular, the separation between the various ponds became less evident with significant mixing of waters with diverse salinities. In any case, ST can still be considered an extreme environment with broad and sudden variations of

the environmental parameters (in particular salinity and temperature) in the majority of the ponds (Barghini et al., 2018b).

Within the ST plant, aside the various mentioned shallow ponds, there is a rather big pool (ca. 1 ha, 2-3 m depth) that was used as brine storage basin (BSB, named "Vascone"). It was filled at the end of the salt harvesting season with the purpose to have prompt availability of hypersaline waters for the productive crystallisation ponds at the very beginning of the next season.

During the salt production period, this basin interchanged waters with the crystallisation ponds through active pumping systems, but remained completely isolated when salt production was dismissed. It represents now a separated ecosystem showing permanent hypersaline features (Barghini et al., 2018a).

ST biodiversity has been studied since 1976, but the majority of the studies considered the ecology and diversity of plants and/or animals (Alfinito et al., 1990; Frondoni and Iberite 2002; Angeletti et al., 2010; 2017; Bellisario et al. 2010; 2012; 2013; Cimmaruta et al., 2010; Cerfolli et al., 2013).

In 2007, a research laboratory had been established in ST to coordinate and optimise the research activities.

Despite the intense research activity, the site was definitely underrated for its microbial communities. A few studies regarded presence and characterisation of some phytoplanktonic organisms (Alfinito et al., 1990; Pasqualetti et al., 2010; Tempesta et al., 2010; Barghini et al., 2018c). Moreover, a preliminary investigation, carried out both by culture-dependent and culture-independent methods (PCR-DGGE), supplied a first characterisation of the bacterial community found in some ponds, including BSB (Barghini et al., 2014; 2018a). Subsequently, a more detailed investigation of the cultivable fraction was performed, analysing the adaptation ability of various strains to temperature and salinity variations (Barghini et al., 2018b).

2.3 Kuril Kamchatka Trench

The Kuril–Kamchatka Trench (KKT) or Kuril Trench is located in the northwest Pacific Ocean, starting from off the southeast coast of the Kamchatka peninsula, running parallel to the Kuril Islands chain till it meets the Japan Trench east of Hokkaido. It joins with the Aleutian Trench from which it departs making a 90° deviation toward south-west. The area is characterized by high surface productivity, particularly in summer. The deep waters are well oxygenated and present uniform hydrological properties (Zenkevich, 1963; Bogorov, 1972). In this region, two main currents (the Oyashio or Kurile Current and the Kuroshio or Kurile Countercurrent) affect the upper waters (Mitsuzawa and Holloway, 1998; Qiu, 2001). The first is a cold Subarctic current flowing southwards from the Arctic Ocean into the Pacific Ocean, while the second starts off the east coast of Taiwan bringing warm tropical waters northwards (Mann and Lazier, 2009). The region is also partially affected by the currents flowing through the Bussol and the Krusenstern Straits coming from the Sea of Okhotsk through the Kuril Islands into the Oyashio (Belkin and Cornillon, 2003; Zenkevich, 1963). In addition, two bottom currents affect the deep waters; on the landward side of the trench there is the deep boundary current (flowing southwestwards along the slope and entering the Japan Trench); on the oceanward side, the trench counter current is present (flowing along the slope northeastwards) (Mitsuzawa and Holloway, 1998). The KKT region has been investigated through oceanographic expeditions since the late ‘40 abord the RV Vityaz (1949, 1953 and 1966). The studies involved chemical and physical parameters of the area, its productivity and plankton distribution (Bogorov, 1972; Zenkevich, 1963). The benthic fauna has also been described with some details (see Fisher and Brandt, 2015 for more specific literature citation). By contrast, under the microbiological point of view, literature is extremely scarce and it will be discussed later on.

Chapter 3

AIM OF THE WORK

On a global scale, marine environment is extremely huge and covers most of our Planet, harbouring a multitude of living organisms, and containing the highest biodiversity of the Globe.

Actually seas and oceans includes a very broad array of coastal and marine ecosystems, which range from some very stable environments, such as the cold ocean bottoms, to other extremely dynamic ones, such as the estuarine and intertidal zones (transitional areas). Among the extreme marine habitats, it is possible to comprise also the deep hypersaline anoxic basins and various hydrothermal ecosystems generated by geochemical events.

Marine environments represent a subject of study of paramount importance both for basic and applied research; this is particularly true for the most extreme ones. Nevertheless, if compared with the terrestrial extreme environments, they are somehow underrated, mainly due to their vastness and remoteness, causing objective approach difficulties. Moreover, the cost of the research in extreme marine habitats is definitely very high and could be supported just by some very well organised and financed research groups and institutions.

Among the numerous and different marine ecosystems, some extreme or sub-extreme areas have been even more underrated then others. In particular, some extreme marine environments have been investigated only under the oceanological and/or zoological point of view, but very scarce or practically no information is available on the structure and composition of their microbial communities.

This is the case of the sites investigated in the present work:

- Kandalaksha Bay (Russia), an intertidal/estuarine Arctic zone (Gorrasi et al., 2019)
- The “Saline di Tarquinia” marine solar salterns (Italy), a hypersaline environment
- The Kuril Kamchatka Trench (NW Pacific Ocean), an oceanic trench.

The study, performed with metagenetic methods on the DNA extracted from water samples, was carried out in order to increase the knowledge on the bacterial life in these aquatic ecosystems and to establish a solid base for further investigations.

Chapter 4

MATERIALS AND METHODS

4.1 Sample collection and characterisation

Kandalaksha Bay samples

Seawater samples (S1–S6) were collected in September 2008, at various depths and distances from the shore, in the "Velikaja Salma" strait (between Veliky Island and Cape Kindo peninsula), on the south-western coast of KB (Fig. 4.1). Samples were processed at the nearby "Nikolai Pertsov" White Sea Biological Station (WSBS, Moscow State University, "Lomonosov").

Sampling sites were selected according to known flows of tidal currents that represent a branch of the main KB current, which enters the inlet and is further split in secondary flows by several small islands located between the Veliky Island and the shore. An area where tidal stream presents a circular pattern is located in front of the WSBS at ca. 100 m from the Station pier (Tzetlin et al., 1997). S1 and S2 were manually collected, using sterile containers, at the lowest intertidal zone (intertidal pool) and at the nearby coastal line on water surface, ca. 30 and 35 m from the shore, respectively. S3 and S4 were collected using sterile containers by scuba divers in proximity of the mentioned circular pattern of the flow, on water surface and −15 m (ca. 1 m from the sea bottom to possibly avoid cross-contamination with sediments), respectively. S5 and S6 were collected using sanitised Niskin bottles in the inlet tidal current before it is split by the small islands, ca. 600 m from the shore on water surface and −70 m (close to the sea bottom, but distant enough to avoid cross-contamination with sediments), respectively. Table 4.1 reports the GPS coordinates for each sampling site and the environmental parameters recorded during sampling

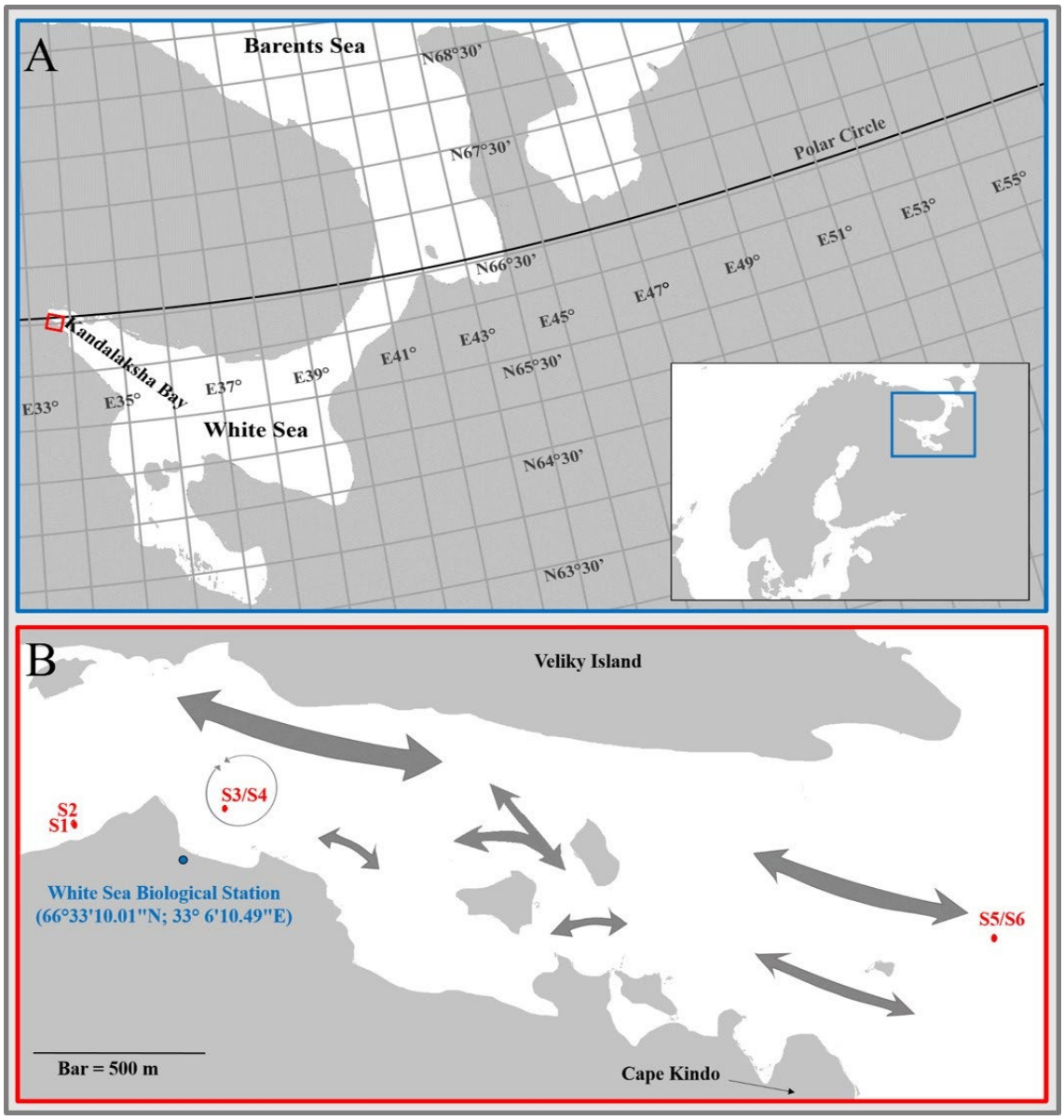

Fig. 4.1 Location of the sampling sites in the Velikaja Salma strait on the south-western shore of Kandalaksha Bay. (A) Overview of the White Sea region where the sampling area is located (red square). (B) Details of the sampling area with indication of the sampling sites (S1-S6) and tidal currents (arrows). Arrow thickness approximately indicates current intensity and the round-shaped thin arrow represents the circular pattern of the current in front of the WSBS. Position and direction of tidal current flows have been superimposed on the map according to Tzetlin et al. (1997). Maps were generated using Google Earth Pro version 7.3.1. and graphically edited.

Table 4.1 GPS coordinates of the sampling sites in the Velikaja Salma strait and environmental parameters recorded during sampling

Sample	Sampling site coordinates (Latitude - Longitude)	Water Temperature (°C)	Salinity (‰)	Depth (m
S1	66°33'13.82"N - 33° 5'42.92"E	8.7	24.9	0.5
S2	66°33'13.82"N - 33° 5'42.92"E	8.4	24.4	0.5
S3	66°33'15.40"N - 33° 6'25.05"E	8.5	23.5	2.5
S4	66°33'15.40"N - 33° 6'25.05"E	6.6	24.2	15
S5	66°33'16.28"N - 33° 8'31.41"E	8.0	24.0	0.5
S6	66°33'16.28"N - 33° 8'31.41"E	0	25.4	70

.

"Saline di Tarquinia" marine saltern samples

Water samples were collected monthly over two years (May 2012-April 2014) from twelve ponds, the neighbouring sea, and from the brine storage basin, within the "Saline di Tarquinia" marine salterns (North Tyrrhenian Sea, Italy; 42°12'07.8"N 11°43'17.8"E) (Aquilanti, 2015). All samples (2 L), obtained pooling 3 different sub-samples (Reboleiro Rivas *et al.,* 2013), were stored in sterile bottles and kept refrigerated (4 °C) during the transport to the ST laboratory to be processed. Then, 1 L of water was vacuum-filtered on sterile membranes (0.22 µm, Millipore, USA), which were washed twice with sterile saline solution to remove possible nutrients from the filters. Membranes were maintained frozen until DNA extraction. The remaining sample aliquots were used for BOD_5 and chlorophyll pigments concentration analyses, which were measured by standard methods.

Environmental parameters (salinity, pH, conductivity and water temperature) were recorded during sampling by common handheld probes. Daily rainfalls and average temperatures of the site were obtained from a local government institution ("Ufficio Idrografico e Mareografico di Roma"; http://www.idrografico.roma.it/annali/home.asp).

Table 4.2 reported the summary of environmental and biological parameters recorded for ST samples.

In this work, environmental DNAs from three ponds (P5, P24 and P37; selected according to their annual range of salinity in order to cover the whole gradient within ST), from the neighbouring sea, and from the brine storage basin were used for metagenetic characterisation of the bacterial communities (Fig. 4.2).

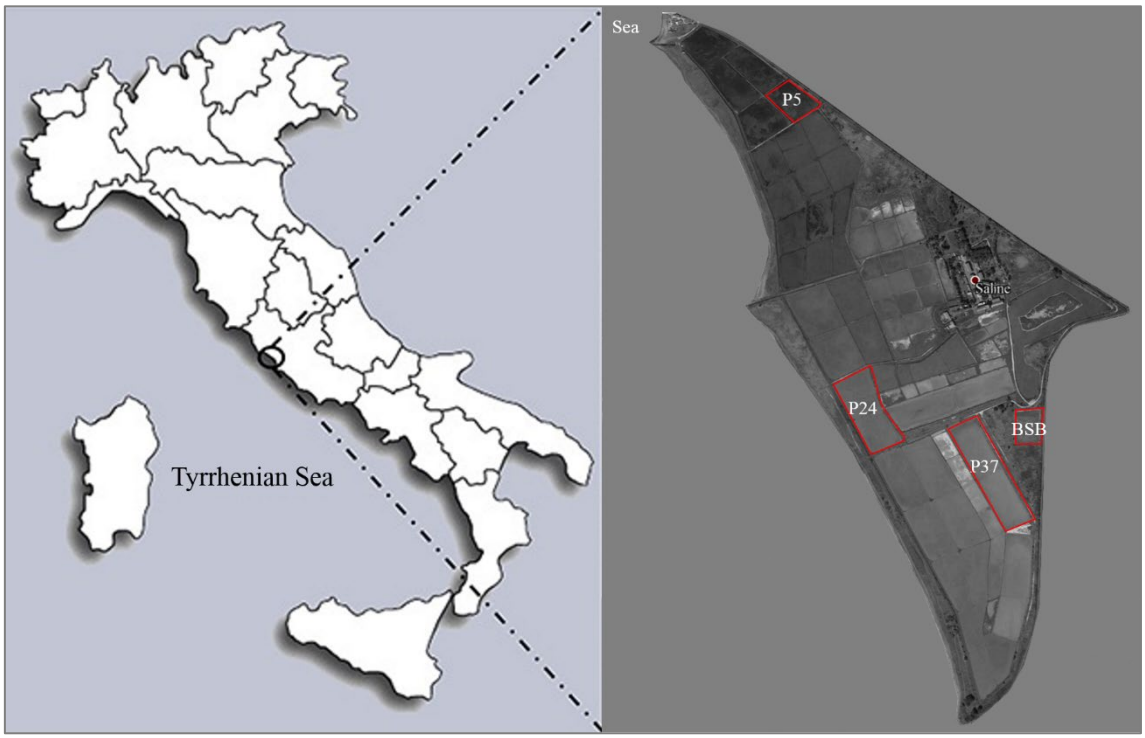

Fig. 4.2 Detailed map of the "Saline di Tarquinia" marine salterns with indication of the sampling sites (Sea, P5, P24, P37 and BSB). Map was generated using Google Earth Pro version 7.3.1. and graphically edited

Table 4.2 Summary of environmental and biological parameters recorded for ST samples

Sample	Salinity (‰)	Water Temperature (°C)	pH	Conductivity (ms/cm)	Chlorophylls (µg/l)	BOD5 (mg/l)
May12-S	38	20.7	8.16	56.10	0.49	2.70
May12-P5	42	18.6	8.16	59.30	0.68	4.30
May12-P24	102	18.5	7.84	129.90	18.36	3.20
May12-P37	180	22.0	7.69	193.60	35.70	4.30
May12-BSB	230	22.0	7.63	236.00	56.80	8.70
Jun12-S	38	25.8	8.28	57.77	0.95	3.80
Jun12-P5	42	27.2	8.31	61.73	0.57	2.70
Jun12-P24	122	32.6	7.50	140.93	46.99	7.60
Jun12-P37	304	36.1	7.20	299.00	30.57	6.50
Jun12-BSB	340	31.7	7.22	305.00	3.19	4.90
Jul12-S	38	23.3	8.12	56.60	0.27	3.20
Jul12-P5	42	24.1	8.65	58.90	1.37	2.70
Jul12-P24	98	27.7	8.05	123.20	24.18	5.40
Jul12-P37	210	31.3	7.89	200.00	170.64	6.50
Jul12-BSB	360	32.7	8.00	178.00	3.65	2.70
Aug12-S	38	27.3	8.10	57.77	1.18	2.10
Aug12-P5	46	25.5	8.50	65.69	0.30	2.70
Aug12-P24	102	25.5	8.15	121.13	54.75	5.40
Aug12-P37	316	29.3	7.60	332.99	10.95	3.80
Aug12-BSB	360	32.0	7.80	416.15	0.00	0.50
Sep12-S	38	22.3	8.15	57.77	2.13	0.50
Sep12-P5	40	22.5	8.37	59.75	3.35	1.00
Sep12-P24	140	23.0	7.86	158.75	16.48	7.60

Table 4.2 (Continued)

Sample	Salinity (‰)	Water Temperature (°C)	pH	Conductivity (ms/cm)	Chlorophylls (µg/l)	BOD5 (mg/l)
Sep12-37	272	27.8	7.72	289.43	15.51	3.80
Sep12-BSB	296	27.4	7.77	313.19	16.43	8.70
Oct12-S	38	20.9	7.90	57.77	0.76	0.50
Oct12-P5	40	20.6	8.30	59.75	0.84	0.50
Oct12-P24	144	24.1	7.90	162.71	21.67	2.70
Oct12-P37	180	25.8	7.90	198.35	28.06	2.70
Oct12-BSB	276	24.4	7.70	293.39	2.28	0.50
Nov12-S	40	16.0	8.10	59.75	1.06	3.20
Nov12-P5	42	13.3	8.30	61.73	1.52	3.20
Nov12-P24	80	15.7	8.15	99.35	39.35	6.50
Nov12-P37	84	17.7	8.44	103.31	28.17	4.90
Nov12-BSB	186	20.0	7.85	176.57	0.11	1.00
Dec12-S	38	17.0	8.15	57.77	0.38	1.60
Dec12-P5	30	17.1	8.17	49.85	3.80	1.60
Dec12-P24	72	17.5	8.08	91.43	30.23	5.40
Dec12-P37	58	17.8	8.20	77.57	15.17	3.80
Dec12-BSB	158	17.1	7.81	204.29	0.00	1.60
Jan13-S	38	10.1	8.20	57.77	1.25	10.40
Jan13-P5	30	9.3	8.10	49.85	1.90	1.00
Jan13-P24	58	10.5	8.10	77.57	14.14	2.70
Jan13-P37	42	9.7	8.20	61.73	7.32	2.70
Jan13-BSB	150	12.8	7.80	168.65	0.15	1.00
Feb13-S	38	11.8	8.15	57.77	0.11	0.50

Table 4.2 (Continued)

Sample	Salinity (‰)	Water Temperature (°C)	pH	Conductivity (ms/cm)	Chlorophylls (µg/l)	BOD5 (mg/l)
Feb13-P5	24	9.4	8.26	43.91	0.15	1.00
Feb13-P24	48	12.2	8.00	67.67	0.23	1.60
Feb13-P37	35	11.5	8.14	54.80	0.38	2.70
Feb13-BSB	140	11.8	7.84	158.75	0.57	0.50
Mar13-S	38	14.2	8.10	57.77	3.19	1.00
Mar13-P5	20	14.5	8.50	39.95	6.54	3.80
Mar13-P24	38	17.7	8.30	57.77	56.08	3.20
Mar13-P37	38	17.6	8.50	57.77	16.20	2.70
Mar13-BSB	120	19.4	7.70	138.95	0.46	0.50
Apr13-S	38	15.0	8.22	57.77	2.62	7.10
Apr13-P5	24	14.7	8.05	43.91	1.37	2.70
Apr13-P24	42	16.5	8.36	61.73	5.93	6.50
Apr13-P37	44	17.3	8.17	63.71	12.32	2.20
Apr13-BSB	148	17.8	7.80	166.67	0.23	2.70
May13-S	38	18.1	8.20	57.77	2.81	0.50
May13-P5	26	20.5	8.50	45.89	2.28	3.20
May13-P24	50	22.2	7.50	69.65	71.86	12.00
May13-P37	76	22.6	7.40	95.39	88.21	18.60
May13-BSB	184	22.3	7.60	202.31	0.57	1.00
Jun13-S	38	27.3	8.10	57.77	1.56	0.50
Jun13-P5	38	27.5	8.10	57.77	2.36	2.10
Jun13-P24	70	28.4	7.50	89.45	20.76	7.60
Jun13-P37	146	30.1	7.50	164.69	18.48	6.50

Table 4.2 (Continued)

Sample	Salinity (‰)	Water Temperature (°C)	pH	Conductivity (ms/cm)	Chlorophylls (µg/l)	BOD5 (mg/l)
Jun13-BSB	208	29.3	7.70	226.07	0.76	2.10
Jul13-S	38	29.0	8.20	57.77	1.43	1.60
Jul13-P5	42	28.1	7.30	61.73	7.19	43.20
Jul13-P24	78	33.2	7.70	97.37	67.87	13.70
Jul13-P37	248	37.3	7.40	265.67	3.65	2.70
Jul13-BSB	272	35.9	7.50	289.43	2.85	3.80
Aug13-S	38	27.4	8.16	57.77	0.59	6.00
Aug13-P5	44	27.9	8.81	63.71	16.96	2.70
Aug13-P24	78	31.1	7.99	97.37	45.34	25.70
Aug13-P37	170	34.7	7.90	188.45	39.35	26.30
Aug13-BSB	320	31.9	7.44	301.31	0.91	0.50
Sep13-S	38	21.7	8.20	57.77	0.27	1.00
Sep13-P5	48	23.9	8.80	67.67	4.56	3.80
Sep13-P24	106	24.8	7.90	125.09	28.33	3.80
Sep13-P37	336	25.4	7.20	352.79	4.56	2.10
Sep13-BSB	360	25.4	7.30	382.49	3.42	1.60
Oct13-S	38	23.5	8.20	57.77	0.91	2.10
Oct13-P5	42	24.0	8.50	61.73	8.14	3.20
Oct13-P24	92	24.3	8.00	111.23	22.58	7.10
Oct13-P37	168	25.9	8.10	186.47	27.88	6.00
Oct13-BSB	274	24.9	7.60	255.77	34.55	2.10
Nov13-S	40	8.0	8.20	59.75	2.66	1.60
Nov13-P5	44	7.7	8.20	63.71	1.37	1.00

Table 4.2 (Continued)

Sample	Salinity (‰)	Water Temperature (°C)	pH	Conductivity (ms/cm)	Chlorophylls (µg/l)	BOD5 (mg/l)
Nov13-P24	86	6.7	8.00	105.29	12.78	4.90
Nov13-P37	110	7.1	8.00	129.05	17.34	5.40
Nov13-BSB	240	19.7	7.60	257.75	5.93	1.00
Dec13-S	40	16.9	8.30	59.75	0.87	1.60
Dec13-P5	42	13.9	7.90	61.73	4.11	2.70
Dec13-P24	84	17.0	8.00	103.31	7.98	4.20
Dec13-P37	100	15.1	8.10	119.15	19.39	2.10
Dec13-BSB	225	16.5	7.50	291.41	1.37	4.90
Jan14-S	38	11.5	8.10	57.77	1.21	1.60
Jan14-P5	42	7.5	7.90	61.73	2.28	3.20
Jan14-P24	78	6.8	8.00	97.37	12.03	3.80
Jan14-P37	68	8.7	8.20	87.47	26.09	4.90
Jan14-BSB	132	10.2	7.70	150.83	0.68	1.00
Feb14-S	38	21.8	8.10	57.77	0.46	1.00
Feb14-P5	40	20.5	8.10	59.75	0.68	2.00
Feb14-P24	54	22.8	8.20	73.61	15.72	7.10
Feb14-P37	40	22.8	8.20	59.75	6.92	4.90
Feb14-BSB	104	14.4	7.60	123.11	0.23	1.00
Mar14-S	40	15.3	8.00	59.75	1.03	1.00
Mar14-P5	32	17.0	8.20	51.83	0.99	2.10
Mar14-P24	54	16.3	8.30	73.61	2.81	3.00
Mar14-P37	46	18.6	8.40	65.69	12.66	4.00
Mar14-BSB	114	16.4	7.70	133.01	0.91	1.00

Table 4.2 (Continued)

Sample	Salinity (‰)	Water Temperature (°C)	pH	Conductivity (ms/cm)	Chlorophylls (µg/l)	BOD5 (mg/l)
Apr14-S	38	17.2	8.10	57.77	1.37	4.30
Apr14-P5	34	18.6	8.50	53.81	1.06	6.50
Apr14-P24	58	19.6	7.90	77.57	7.19	3.00
Apr14-P37	66	21.5	7.90	85.49	2.51	3.00
Apr14-BSB	154	20.8	7.60	172.61	0.57	1.00

Kuril Kamtchatka Trench samples

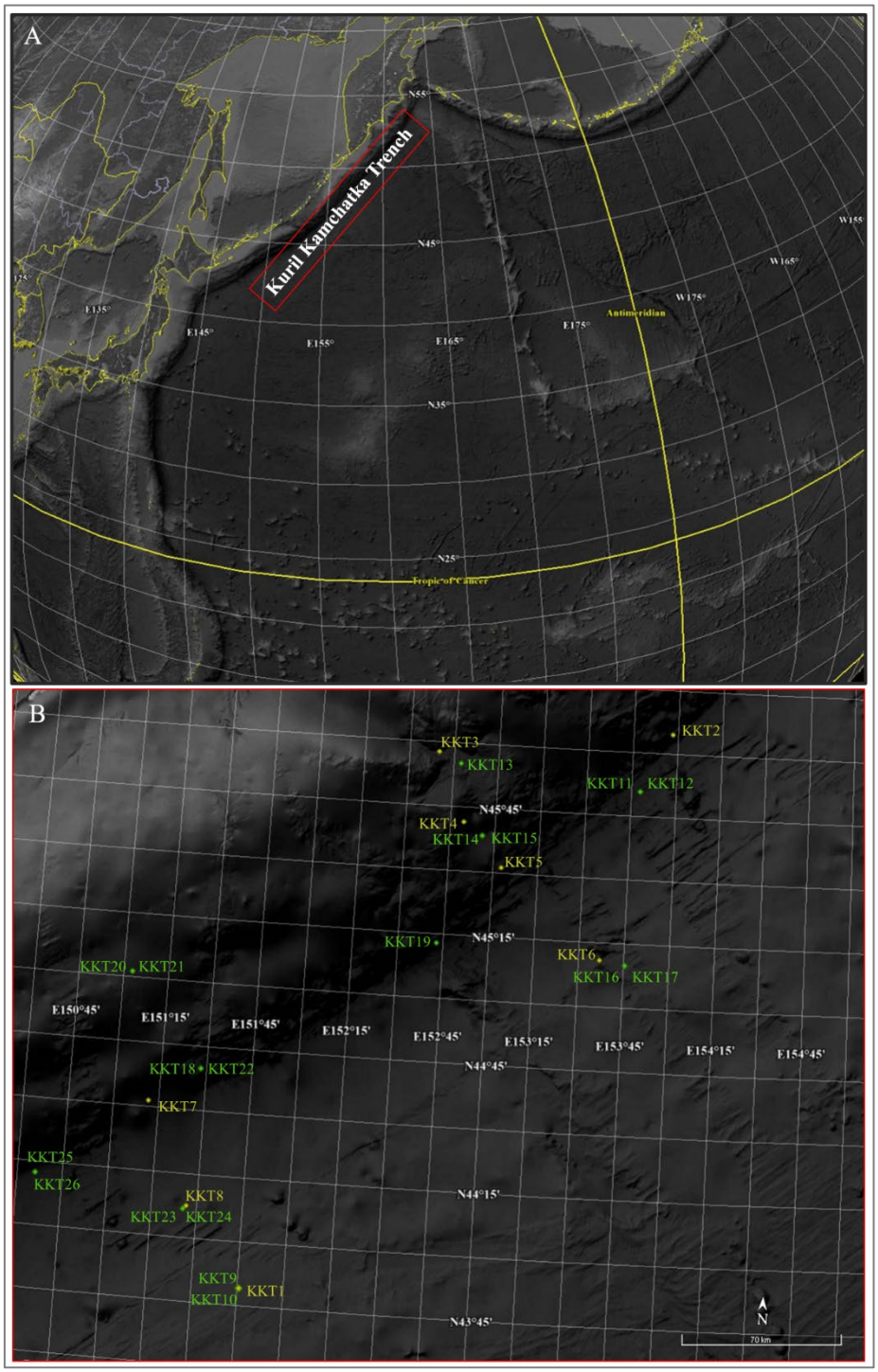

Fig. 4.3 Location of the sampling sites in the Kuril Kamtchatka Trench. An overview of the Kuril Kamtchatka Trench region (A) and a detailed map of the sampling area with indication of the sampling points (B) are shown. Yellow and green colors indicate bathyal and abysso-hadal samples, respectively. Maps were generated using Google Earth Pro version 7.3.1. and graphically edited.

Twenty-six samples were collected in various areas within the Kuril Kamchatka Trench region (Northwest Pacific Ocean) between 16 August-26 September 2016, aboard the deep-ocean Research Vessel (RV) *Sonne*, during the German-Russian expedition KuramBio II (Kurile-Kamchatka Biodiversity Studies II) (Fig. 4.3). Eight samples (KKT1-KKT8) were collected in the water column within the bathyal zone (at 1000-2000 m depth) using a rosette gear holding 24 Niskin bottles and bearing a CTD probe for the measurement of temperature, conductivity and oxygen saturation (Fig. 4.4A). The eighteen interface water-sediment samples (KKT9-KKT26) were obtained from the water over the cores collected by a multicorer (MUC) equipped with 12 transparent acryl-glass-cylinders (tubes) (Fig. 4.4B), within the abysso-hadal zone (depth range 5000-9500 m). In Table 4.3, for each KKT sample, the sampling sites and the recorded environmental parameters are reported.

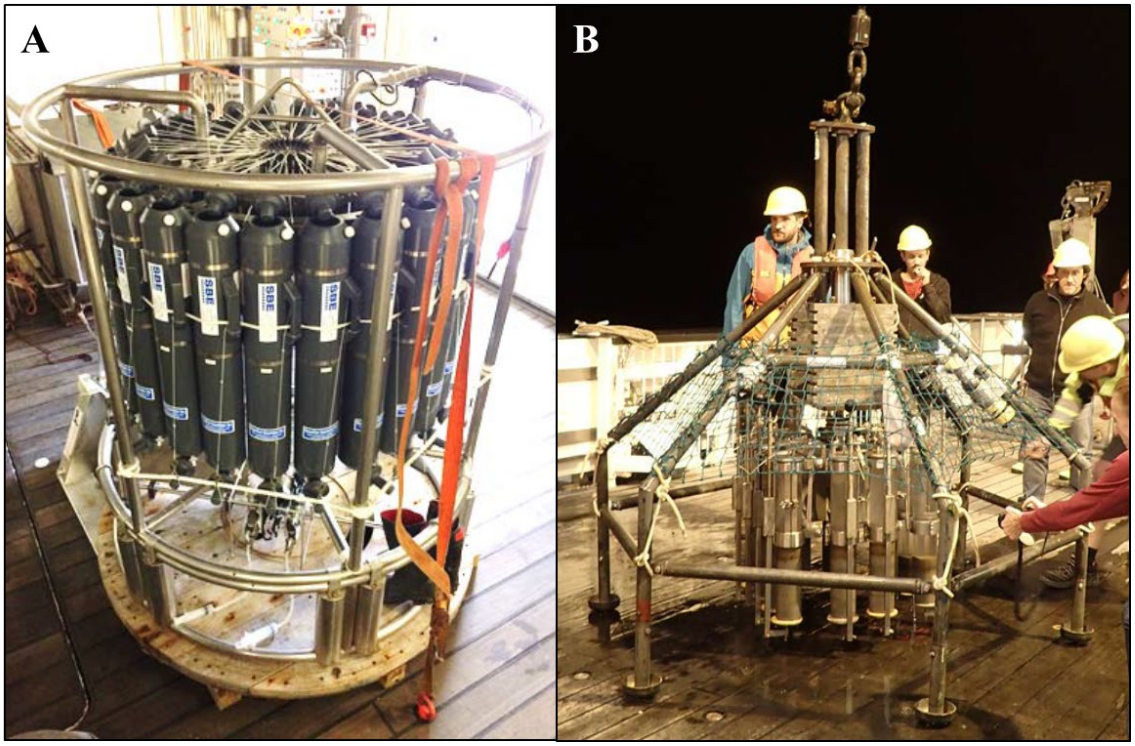

Fig. 4.4 Gears employed for the sampling in the Kuril Kamtchatka Trench region. (A) CTD (Conductivity, Temperature, Density) rosette; (B) Multicorer.

Table 4.3 GPS coordinates of the sampling sites in the Kuril Kamtchatka Trench and environmental parameters recorded during sampling.

Sample	Sampling site coordinates (Latitude - Longitude)	Depth (m)	Temperature (°C)	Conductivity (S/m)	Oxygen (V)
KKT1	43° 49.20' N; 151° 45.60' E	2000	1.70	3.12	1.89
KKT2	46° 04.62' N; 153° 58.20' E	1000	2.72	3.14	1.04
KKT3	45° 57.73' N; 152° 39.91' E	1000	2.79	3.14	0.99
KKT4	45° 41.58' N; 152° 49.49' E	1000	2.94	3.15	1.01
KKT5	45° 31.35' N; 153° 02.82' E	1500	2.33	3.14	1.12
KKT6	45° 11.00' N; 153° 36.99' E	1000	2.72	3.14	0.92
KKT7	44° 31.50' N; 151° 11.59' E	1000	2.54	3.13	1.03
KKT8	44° 07.59' N; 151° 26.58' E	1000	2.54	3.13	1.04
KKT9	43° 49.19' N; 151° 45.59' E	5146.6	NA	NA	NA
KKT10	43° 49.19' N; 151° 45.59' E	5146.7	NA	NA	NA
KKT11	45° 50.87' N; 153° 47.99' E	8254.7	NA	NA	NA
KKT12	45° 50.87' N; 153° 47.99' E	8255.2	NA	NA	NA
KKT13	45° 55.23' N; 152° 47.46' E	6065.0	NA	NA	NA
KKT14	45° 38.60' N; 152° 55.91' E	7136.7	NA	NA	NA
KKT15	45° 38.60' N; 152° 55.92' E	7134.6	NA	NA	NA
KKT16	45° 09.99' N; 153° 45.41' E	5741.2	NA	NA	NA
KKT17	45° 10.00' N; 153° 45.43' E	5739.4	NA	NA	NA
KKT18	44° 39.89' N; 151° 28.11' E	8221.1	NA	NA	NA
KKT19	45° 12.94' N; 152° 42.83' E	9498.9	NA	NA	NA
KKT20	45° 01.36' N; 151° 02.89' E	5211.3	NA	NA	NA
KKT21	45° 01.36' N; 151° 02.89' E	5217.4	NA	NA	NA
KKT22	44° 39.90' N; 151° 28.10' E	8224.0	NA	NA	NA
KKT23	44° 06.85' N; 151° 25.54' E	6517.5	NA	NA	NA
KKT24	44° 06.84' N; 151° 25.55' E	6515.8	NA	NA	NA
KKT25	44° 12.40' N; 150° 36.02' E	9538.6	NA	NA	NA
KKT26	44° 12.39' N; 150° 36.01' E	9540.2	NA	NA	NA

NA: Not Available

Then, 1 L of water was vacuum-filtered on sterile membranes that were maintained frozen until DNA extraction.

4.2 DNA extraction

Kandalaksha Bay samples

Membranes were processed for total DNA extraction as reported by Pesciaroli et al. (2015a). Each membrane was transferred to a 15 mL sterile tube, containing 1.5 mL of sterile distilled water, and finely ground. Tubes were vortexed, in order to allow cell re-suspension. Suspensions were transferred to sterile microcentrifuge tubes, and cells pelleted by centrifugation (7500 ×g, 20 min.); supernatants were removed, leaving ca. 25 µL of liquid. The cell pellets were re-suspended by Vortex and processed using the MasterPureTM purification Kit (Epicentre® Biotechnologies, USA) accordingly to the manufacturer's instruction

"Saline di Tarquinia" marine saltern samples

Membranes were processed for total DNA extraction as reported by Pesciaroli et al. (2015a) and Aquilanti (2015). Each membrane was transferred to a 15 ml sterile tube, containing 1,5 mL of sterile distilled water, and finely ground. Tubes were vortexed, in order to allow cell re-suspension. Suspensions were transferred to sterile microcentrifuge tubes, and cells pelleted by centrifugation (7,500 x g, 20 min.); supernatants were removed, leaving ca. 25 µl of liquid. The cell pellets were re-suspended by Vortex and processed using GeneMATRIX Bacterial & Yeast Genomic DNA Purification Kit (EURx Ltd., Poland) were accordingly to the manufacturer's instructions.

Kuril Kamtchatka Trench samples

DNA was extracted from the membranes using ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research Corp., Irvine, CA, USA), according to manufacturer's instructions.

4.3 16S rDNA amplicon libraries and sequencing

Kandalaksha Bay amplicon libraries

The PCR amplification of target region and the subsequent pyrosequencing, carried out as reported by De Filippis et al. (2013), were performed at the Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy. Amplification of bacterial 16S rRNA V1-V3 hypervariable regions was carried out using the universal primers Gray28F 59-TTTGATCNTGGCTCAG and Gray519r 59-GTNTTACNGCGGCKGCTG (obtaining 520 bp-fragments) (Ercolini et al., 2011). To allow sample multiplexing, the 454-adaptors were included in the forward primer followed by a 10 bp sample-specific Multiplex Identifier (MID). PCR reactions were performed in 50 μL of final volume containing 50 ng of template DNA, 0.4 μM of each primer, 0.50 mmol/L of each deoxynucleoside triphosphate, 2.5 mmol/L MgCl2, 5 mL of 10 PCR buffer and 2.5 U of Taq polymerase (Invitrogen, Milano, Italy). PCR conditions were: 94 °C for 2 min, 35 cycles of 95 °C for 20 s, 56 °C for 45 s and 72 °C for 5 min, and a final extension at 72 °C for 7 min. Amplicons were purified twice by the Agencourt AMPure kit (Beckman Coulter, Milano, Italy) and quantified using the QuantiFluorTM (Promega, Milano, Italy). The equimolar pool of PCR products was sequenced on a GS Junior platform (454 Life Sciences, Roche Diagnostics, Italy) according to the manufacturer's instructions by using a Titanium chemistry.

"Saline di Tarquinia" marine saltern and Kuril Kamtchatka Trench amplicon libraries

The V5-V6 hypervariable regions of 16S rRNA gene were sequenced by Illumina MiSeq (Illumina Inc., San Diego, CA, USA) using a 2 × 250 bp paired-end protocol as reported by Gandolfi et al. (2017) and Pittino et al. (2018). The multiplexed libraries were prepared using a dual PCR amplification protocol. The first PCR was carried out in 3 × 80 μL volume reactions using the GoTaq Green Master Mix (Promega Corporation, Madison, WI) and 1 μM of each primer. The primers used, 783F and 1046R (Huber et al., 2007; Wang and Qian, 2009), were modified by adding external barcodes to allow the parallel processing of multiple samples. The PCR cycling conditions were: 98°C for 30 s; 20 cycles at 98°C for 10 s, 47°C for 30 s, and 72°C for 5 s and a final extension at 72°C for 2 min. The amplicons were purified by Wizard SV Gel and PCR Clean-up System

(Promega Corporation, Madison, WI) and quantified using Qubit 2.0 (Life Technologies, Carlsbad, CA). Then, the purified amplicons were pooled to obtain a single library (each library contained nine samples, identifiable by different external barcode pairs). Further library preparation (second PCR) with the addition of standard Nextera indexes (Illumina, Inc., San Diego, CA, USA) and sequencing were carried out at Nuova Genetica Italiana SRL (Monza-Brianza, Italy).

4.4 Sequence processing and Data Analysis

Kandalaksha Bay amplicon libraries

Raw reads were preliminary filtered according to the 454 processing pipeline and subsequently processed using QIIME version 1.6.0 (Caporaso et al., 2010). After demultiplexing, in order to increase the accuracy of OTUs detection, sequences were submitted to a quality filtering step: reads shorter than 300 bp, with an average quality score lower than 25 and ambiguous base calls, were removed. The filtered sequences were denoised (Reeder and Knight, 2010) and singletons were excluded. Taxonomic annotation was carried out as follows: reads were clustered into OTUs, defined by a 97% of similarity, using the uclust method (Edgar, 2010); the representative sequences were submitted to the RDPII classifier (Wang et al., 2007) using the Greengenes 16S rRNA gene database (McDonald et al., 2012), obtaining the taxonomical assignment and the relative abundance of each OTU.

"Saline di Tarquinia" marine saltern and Kuril Kamtchatka Trench amplicon libraries

Reads from sequencing were demultiplexed according to the indices and internal barcodes. Forward and reverse reads were merged with perfect overlapping and quality filtered with default parameters using Uparse pipeline (Edgar, 2013). Suspected chimeras and singleton sequences (i.e. sequences appearing only once in the whole data set) were removed. OTUs were defined on the whole data set clustering the sequences at a 97% of similarity and defining a representative sequence for each cluster. A subset of 10,000 random sequences was chosen from each sample, and the abundance of each OTU was

estimated by mapping the sequences of each sample against the representative sequence of each OTU at 97% of similarity. Taxonomic classification of the OTU representative sequences was obtained by RDP classifier using 50% confidence cutoff as suggested for sequences shorter than 200 bp (Claesson et al., 2009).

4.5 Statistical methods

Kandalaksha Bay amplicon libraries

Alpha diversity analyses were performed using QIIME to calculate Observed OTUs, Good's coverage(Good 1953), Chao1 richness (Chao and Bunge, 2002), and Shannon diversity indices (Shannon, 1948), and to generate rarefaction curves. Chao1 is a richness estimator, which calculate expected OTUs based on observed OTUs (Chao and Bunge, 2002). The Shannon index takes into account the uniformity of species and its abundance. It ranges from 0 to $+\infty$ and increases as both the richness and the evenness of the community increase (Shannon, 1948).

Shared phylotypes among the samples were visualised by a six-way Venn diagram, generated using the JVenn tool (http://bioinfo.genotoul.fr/jvenn) (Bardou et al., 2014).

β-Diversity was analysed by clustering analysis and non-metric multidimensional scaling (nMDS) performed using the software package PRIMER-E v.6.1.18 (Plymouth, UK) (Clarke and Gorley, 2005). The Bray-Curtis dissimilarity matrix was calculated from square root-transformed data of taxa relative abundance, and used for nMDS analysis (25 restarts; minimum Kruskal stress = 0.01). Clustering analysis (group average) was carried out to identify similarities among samples and used to generate the similarity contours superimposed on the nMDS ordination plot.

The environmental drivers of community structure were analysed by Redundancy Analysis (RDA) using the software Canoco v. 5.0 (Microcomputer Power, Ithaca, NY, USA) (Ter Braak and Šmilauer, 2012), considering the following parameters: salinity, water temperature, and depth. Collinear variables were removed before the RDA analysis; parameters were tested by SYSTAT v. 8.0 (SPSS Inc., Chicago, IL, USA) for potential collinearity. OTU relative abundances were log-transformed for RDA analysis. The

Monte Carlo permutation test was used to assess the statistical significance of the canonical axes.

"Saline di Tarquinia" marine saltern and Kuril Kamtchatka Trench amplicon libraries

The coverage of each sample was evaluated by the Good's method (Good 1953).

Before subsequent analyses, global singletons (i.e. sequences occurring only once in the whole dataset) were removed, since they may represent sequencing errors.

The Shannon index and Gini index were used to describe alpha diversity. The Gini index (Gini, 1912), developed as a measure of inequality of income in economics, is used in ecology to measure community evenness. Its values range from 0 to 1 and increasing values indicate a decrease in evenness (Wittebolle et al., 2009). The Shannon and the Gini indices were calculated on rarefied samples. Analyses of alpha diversity indices were performed using linear models after checking that no relevant deviation from model assumption has occurred by visual inspection of residuals plots (details not shown).

Shared taxa among bathyal and abysso-hadal samples (KKT) were visualised by a Venn diagram, generated using the JVenn tool (http://bioinfo.genotoul.fr/jvenn) (Bardou et al., 2014).

Beta diversity analyses were run on the non-rarefied samples (McMurdie and Holmes, 2014) using the number of sequences as abundance estimation of each OTU in a sample. OTU abundances per sample were transformed using the Hellinger method (Legendre and Legendre, 2012). As for ST samples, the analyses were performed using the Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA) to investigate the environmental parameters driving the community structure. Salinity, water temperature, pH, rainfall, chlorophylls, BOD_5, sampling month and sampling year were used as predictors. Sampling month data were entered as Fourier series transformed data to account for seasonality (Legendre and Legendre, 2012). The forward selection with double-stopping criterion was used to identify the variables that significantly affected bacterial community structures, without inflating type I error rate (Blanchet et al. 2008). As for KKT samples collected, analyses were performed using the Principal Component Analysis (PCA) to visualise data distribution and Redundancy Analysis (RDA) to investigate variation of community structure in relation to the depth (the only significantly

variable parameter in KKT samples). Significance of CCA, RDA and PCA was assessed through 9999 permutations.

The abundance variation of the most abundant genera (arbitrarily selected for their abundance that was notably above the average) in relation to the parameters driving bacterial communities structure was investigated by Generalized Linear Models (GLMs). GLMs were performed assuming a negative binomial distribution and with a log-link function for BSB data, whereas were performed assuming a Poisson distribution and corrected for overdispersion for ST data of samples collected from the ponds and the neighbouring sea and for KKT data. To account for taxa seasonal variation (ST samples collected from the ponds and the neighbouring sea) we used periodic regression based on Fourier series transformation of month (Legendre and Legendre, 2012). The post-hoc test was used to assess pairwise differences in taxa abundance variation among the sampling sites/groups of samples. In GLMs analyses, we accounted for multiple statistical tests by correcting GLM significance (P-value) according to the false discovery rate (FDR) procedure (Benjamini and Yekutieli, 2001).

All the statistical analyses were performed in R environment (R 3.4.2, R Core Team, 2018) using VEGAN, BIODIVERSITYR, MULTTEST, and MULTCOMP packages.

Chapter 5

RESULTS

5.1 Metagenetic profiling of the bacterial communities of Kandalaksha Bay

5.1.1 Composition and diversity of the bacterial communities

A total of 53,870 validated reads were obtained, with an averaged value of 8978 reads/sample. Alpha diversity was estimated by Observed OTUs, Chao1, Shannon, and Good's coverage indices (Table 5.1). The Estimated Sample Coverage (ESC) was satisfactory (≥98%) for all samples. The number of observed OTUs and the estimated OTU richness (Chao1), ranged from 296 to 610 and from 411.02 to 669.40, respectively; the Shannon diversity index ranged from 2.82 to 6.65. The highest and lowest values for the all indices were obtained for the intertidal pool sample (S1) and the deepest sample (S6), respectively.

Table 5.1 Alpha-diversity indices of the six amplicon libraries.

Sample	Reads	Observed OTUs	Chao1	Shannon	ESC
S1	8448	610	669.40	6.65	99%
S2	9067	539	623.24	6.56	98%
S3	7135	568	640.57	5.80	98%
S4	8899	521	627.56	5.97	98%
S5	10664	437	613.55	5.97	98%
S6	9657	296	411.02	2.82	99%

OTU = operational taxonomic unit; ESC = estimated sample coverage.

Chao1, Shannon and ESC were calculated with Qiime at the 3% distance level.

The taxonomical analysis allowed assigning reads to 20 phyla, accounted for ≥94% of the sequences across all samples; reads not classified ("Unassigned") at the phylum level were detected in all samples ranging from 0.8 to 6% (Fig. 5.1). *Proteobacteria* was the predominant phylum (67–96%); *Bacteroidetes* were also rather abundant (5–12%) in all samples except S6 (−70 m), while *Cyanobacteria* were mainly present on the surface waters collected offshore (11 and 15% for S3 and S5, respectively).

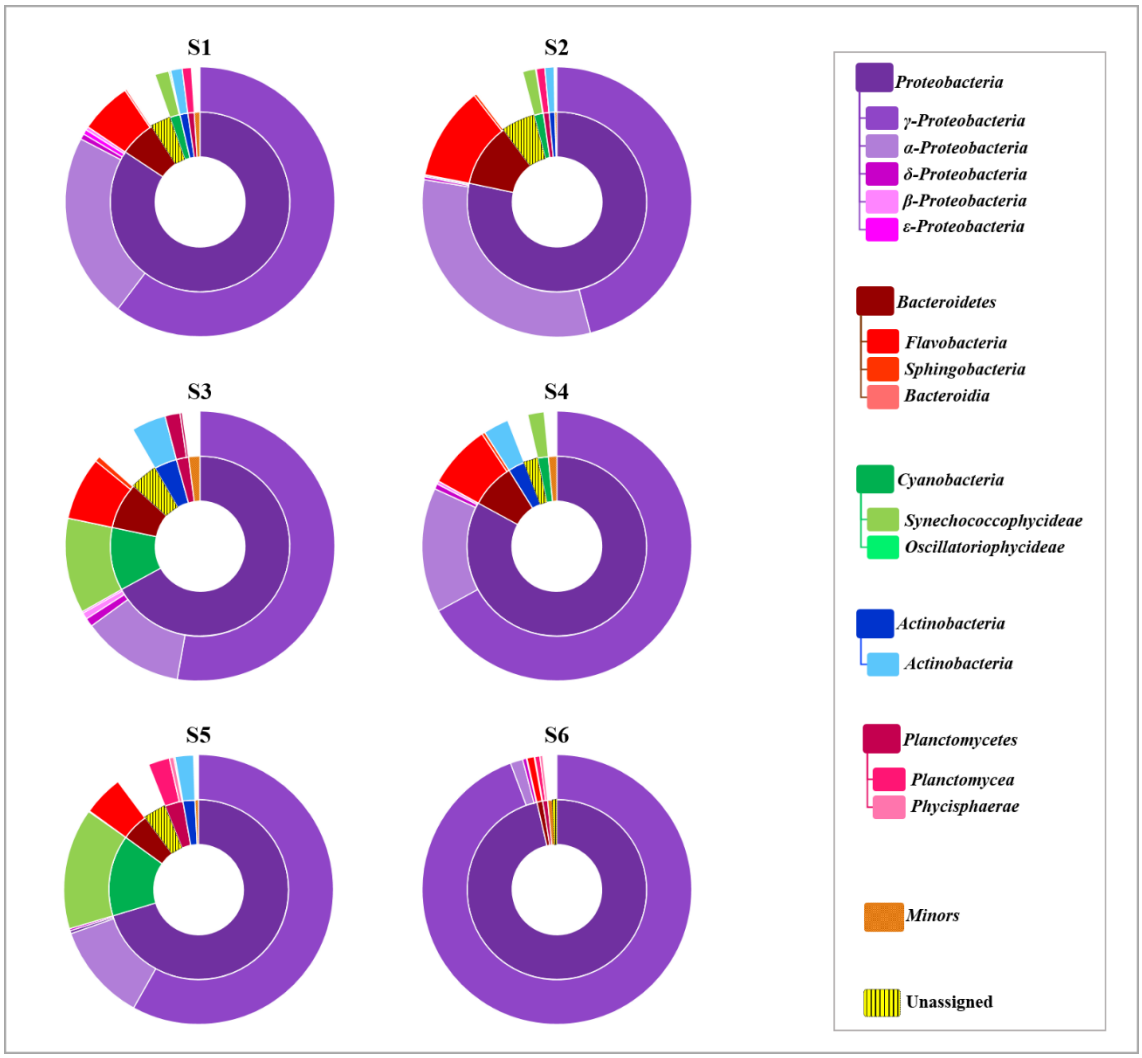

Fig. 5.1 Distribution of Phyla and relative Classes among the various KB samples. Phyla (inner rings) with Ra < 1% were gathered in "Minors".

The class *γ-Proteobacteria* represented the main fraction (46–94%) in all samples, while *α-Proteobacteria* were rather abundant (11–31%) in S1–S5, which showed also presence of *Flavobacteria* (5–11%). The sub-class of *Synechococcophycideae* was relatively abundant in S3 and S5 (11 and 15%, respectively).

Overall, at the genus level, reads were assigned to 217 taxa, ranging from 93 to 159 across samples. Fig. 5.2 shows the pattern of major genera in the various samples, evidencing those having relative abundance (Ra) ≥ 1% at least in one sample; minor genera (Ra < 1%) were gathered in "Others". Major genera were only few in each sample, but they accounted for the main fraction of the whole bacterial communities. In S1-S5 a

total of 12–15 major genera were recorded, representing the 75–82% of the community. S1, S2, S4 and S5 communities were not characterised by the marked dominance of a single genus, but several majority taxa were present with similar abundances. In S3, predominance of *Psychrobacter* (37%) was quite evident. In S6, only 4 major genera were present, accounting for ca. 92% of the community, but the sole genus *Halomonas* constituted the 65% of the community. The other abundant genera were *Pseudoalteromonas* (18%), *Vibrio* (6%) and *Marinomonas* (3%). However, in all samples, *Pseudoalteromonas* represented one of the most abundant taxon (5–23%).

The number of shared phylotypes among the samples (Venn diagram) is reported in Fig.5.3. Overall, 63 phylotypes (ca. 27% of the annotated phylotypes) were in common among all samples, representing an important moiety of the communities (77–97%). Various phylotypes were found only in one community.

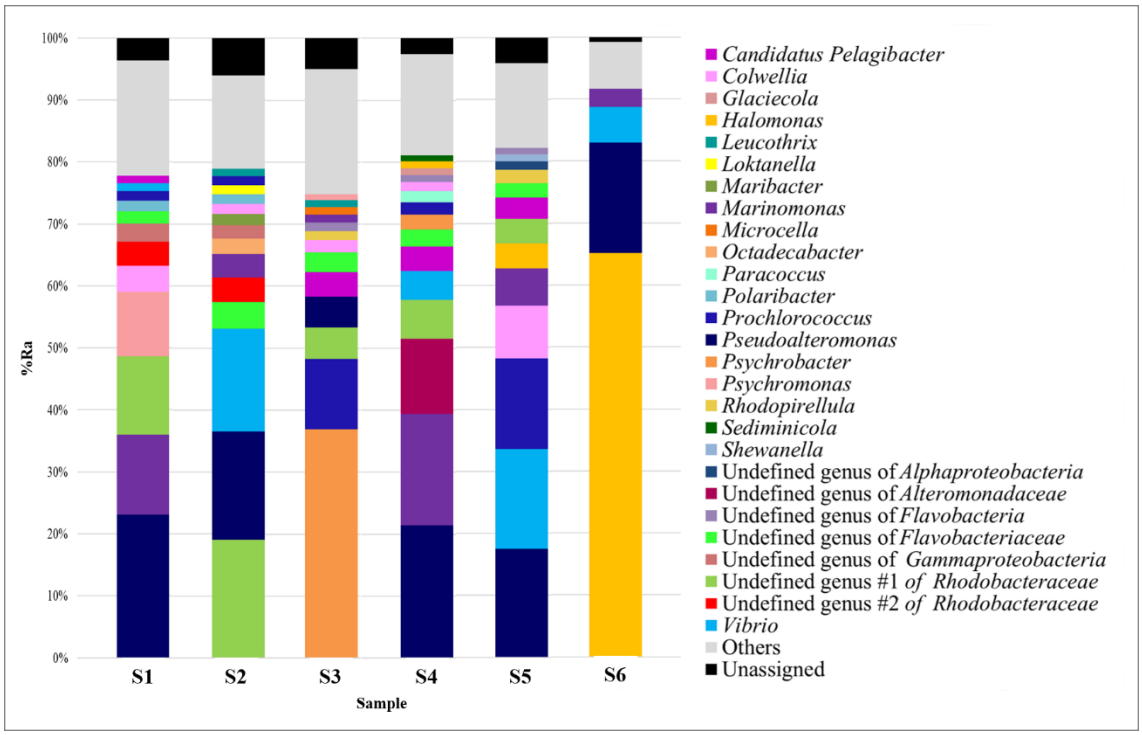

Fig. 5.2 Genus-level bacterial composition of the KB samples. Distribution of the major genera (Ra > 1% in at least one sample); genera with Ra < 1% were gathered in "Others"

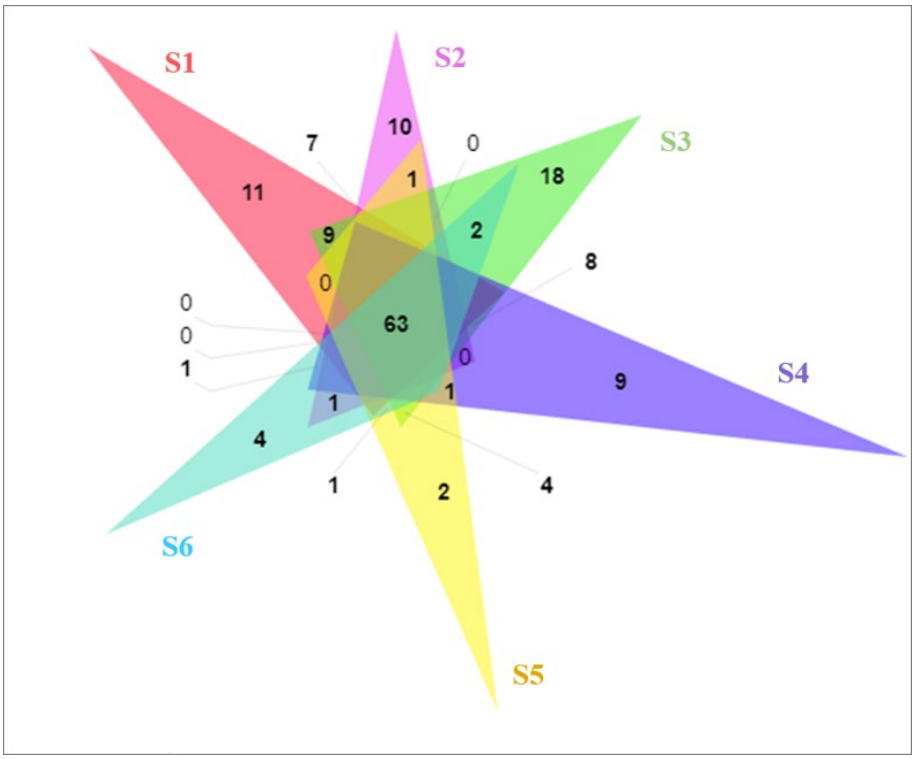

Fig. 5.3 Six-way Venn diagram depicting the number of exclusive and shared phylotypes among the KB samples.

5.1.2 Similarity among KB communities (nMDS) and environmental drivers of their structure (RDA)

The nMDS ordering, with superimposed similarity contours reflecting the cluster analysis, was used to display patterns of similarity among communities (Fig. 5.4). The group comprising samples S1 and S2, showed the most similar communities (71% similarity); S3, S4 and S5, grouped together (65% similarity), while S6 revealed the most different community (47% similarity with other samples).

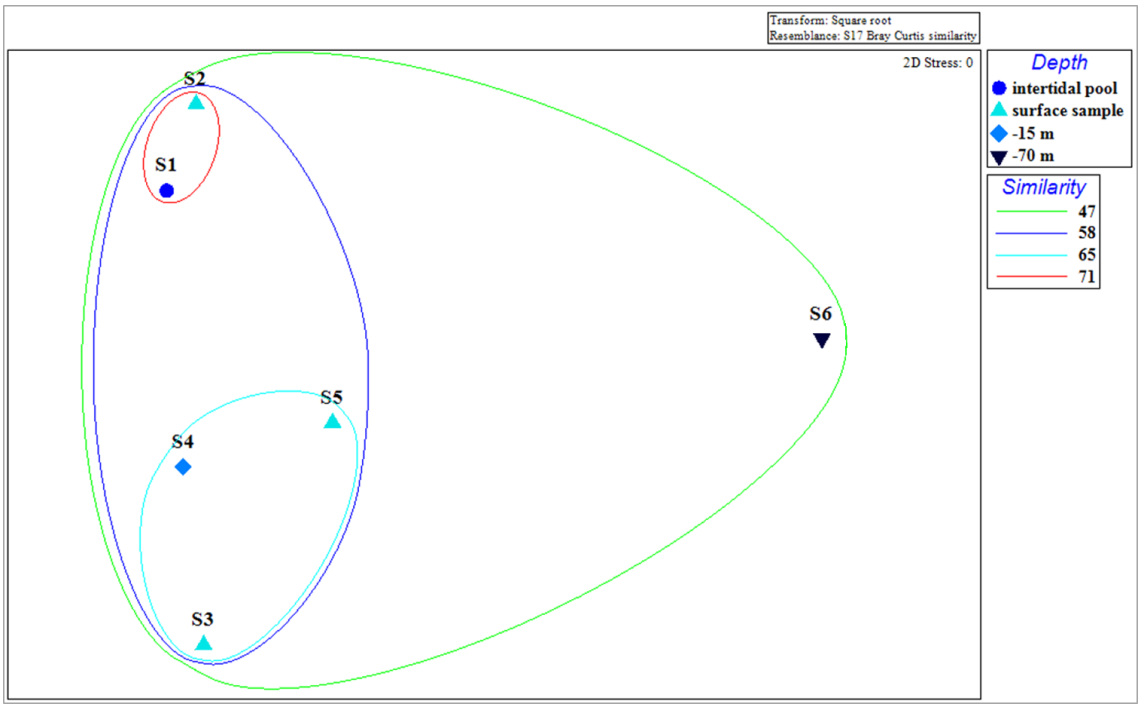

Fig. 5.4 Bray-Curtis based non-metric multidimensional scaling (nMDS) ordination of KB samples. The similarity contours superimposed on the nMDS plot derived from the Cluster Analysis calculated on Bray-Curtis distance matrix.

The environmental parameters included in the RDA analysis were water temperature and salinity; depth was excluded due to its high collinearity (−0.996) with water temperature. The RDA (Fig. 5.5) explained 58.5% of the total variability ($F = 2.1$; $P = 0.01$) found in the relationship between environmental parameters (factors) and samples (variables). The two ordination axes accounted for 31.7% and 26.8% of the explained variability, respectively. The first axis was mainly correlated to water temperature, which describes 31.7% of total variance. As for the association between factors and variables, it is noteworthy that S6 showed the strongest relations with low water temperature and S3 with low salinity.

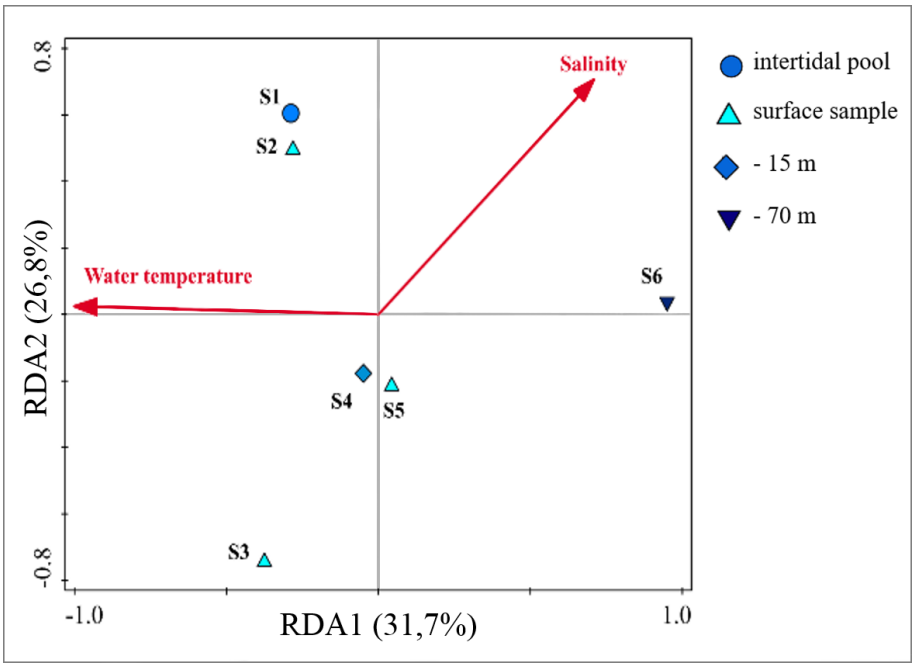

Fig. 5.5 Redundancy analysis (RDA) ordination diagram (biplot) showing samples and environmental parameters. Arrows indicate significant ($p < 0.05$) environmental parameters.

5.2 Metagenetic profiling of the bacterial communities of the "Saline di Tarquinia" marine salterns

5.2.1 Bacterial communities found along the salinity gradient

5.2.1.1 Composition of the bacterial communities

A total of 2,539,445 mapping sequences was obtained from the monthly survey of the bacterial communities along the salinity gradient, over the period May 2012-April 2014. Overall, Good's index ranged from 90.2 to 99.6, indicating a good coverage for the libraries. The clustering sequence process defined 13942 bacterial OTUs.

OTUs retrieved were assigned to 38 phyla, 78 classes, 148 orders, 335 families and 1052 genera. At the phylum level, unassigned OTUs ranged from 0.6 to 4.2% in the sea, from 0.3 to 3.5% in P5, from 0.3 to 13% in P24 and from 0.1 to 20.4% in P37. At the class level, unassigned OTUs ranged from 1.4 to 14.4% in the sea, from 0.7 to 11.6% in

P5, from 0.4 to 13.8% in P24 and from 0.3 to 20.8% in P37. At the order level, unassigned OTUs ranged from 3.6 to 27.7% in the sea, from 3.3 to 23.4% in P5, from 1.2 to 19.6% in P24 and from 1.2 to 27.6% in P37. At the family level, unassigned OTUs ranged from 6.1 to 31.1% in the sea, from 4.1 to 33.5% in P5, from 2.1 to 22.6% in P24 and from 1.7 to 33.6% in P37. At the genus level, unassigned OTUs ranged from 15.8 to 48.5% in the sea, from 16 to 45.9% in P5, from 5.3 to 45.9% in P24 and from 6.7 to 45.2% in P37.

According to the majority of literature we will present the results (and discussion) of the taxonomical analysis at the phylum and genus levels, only.

At the phylum level, the major taxa (Ra ≥ 1% in at least one sample) found in ST were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Verrucomicrobia*, *Planctomycetes*, *Fusobacteria*, *Candidatus Saccharibacteria* and *Deinococcus-Thermus* (Figs. 5.6-5.9).

Among them, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were the most represented and always recorded with a Ra ≥ 1%, in all sampling sites over the two-year campaign.

In the sea samples (Fig. 5.6), except for Jun13 in which *Actinobacteria* predominated (Ra = 43.5%), *Proteobacteria* was always the most abundant phylum (range 40.8-77.4%, maxima Ra in April 2014). *Bacteroidetes* represented most often the second abundant taxon (range 6.5-29.8%, maxima Ra in October 2012), except in the periods June-August 2013 and November 2013-January 2014, characterised by a higher abundance of *Actinobacteria*.

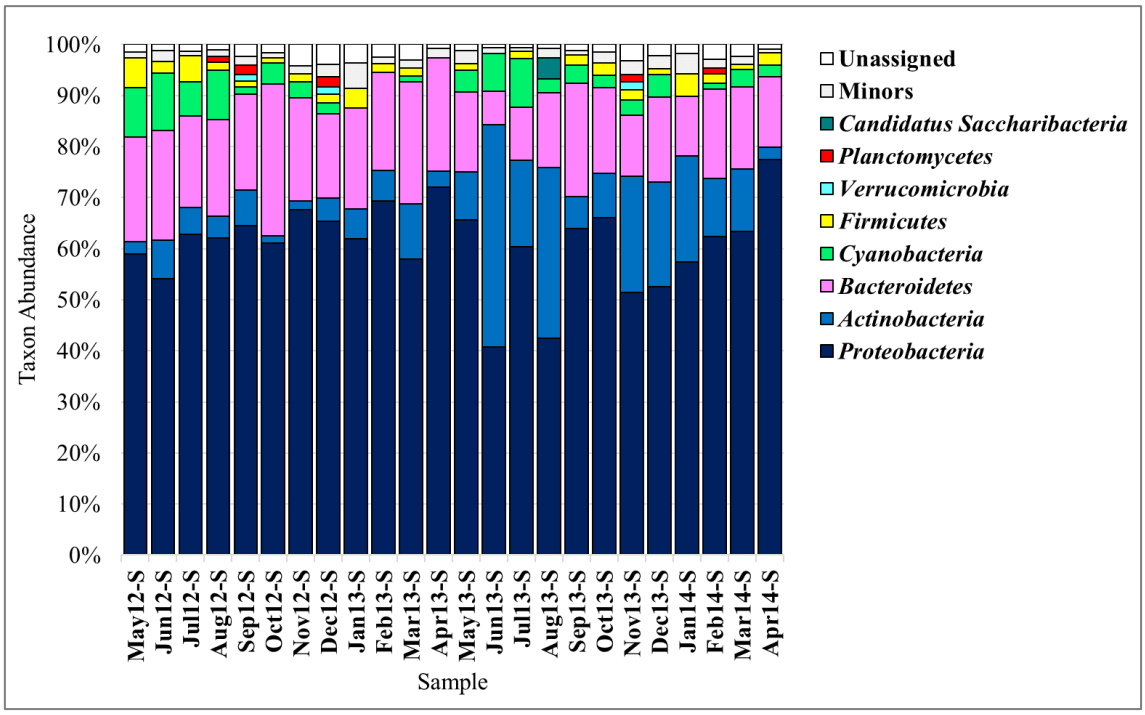

Fig. 5.6 Phylum-level bacterial composition of the sea samples. Distribution of the major phyla (Ra > 1% in at least one sample); phyla with Ra < 1% were gathered in "Others"

In P5 (Fig. 5.7), the bacterial communities were dominated by *Proteobacteria* and *Actinobacteria*; the first represented the most abundant phylum in 67% of samples and the latter in the remaining samples. In particular, *Proteobacteria* represented the predominant phylum (Ra range 25.1-60.1%, maximum in May 2013) in the periods May 2012-January 2013, April-May 2013, July 2013, December 2013 and February-April 2014. It was generally followed by *Actinobacteria*; the only exception was in December 2012, where a peak of *Bacteroidetes* (Ra = 29.3%) was recorded. In the other months, the most abundant taxon was *Actinobacteria* (Ra range 13.3-69.5%, maximum in September 2013), with peaks (Ra > 60%) in February, June and September 2013, and January 2014.

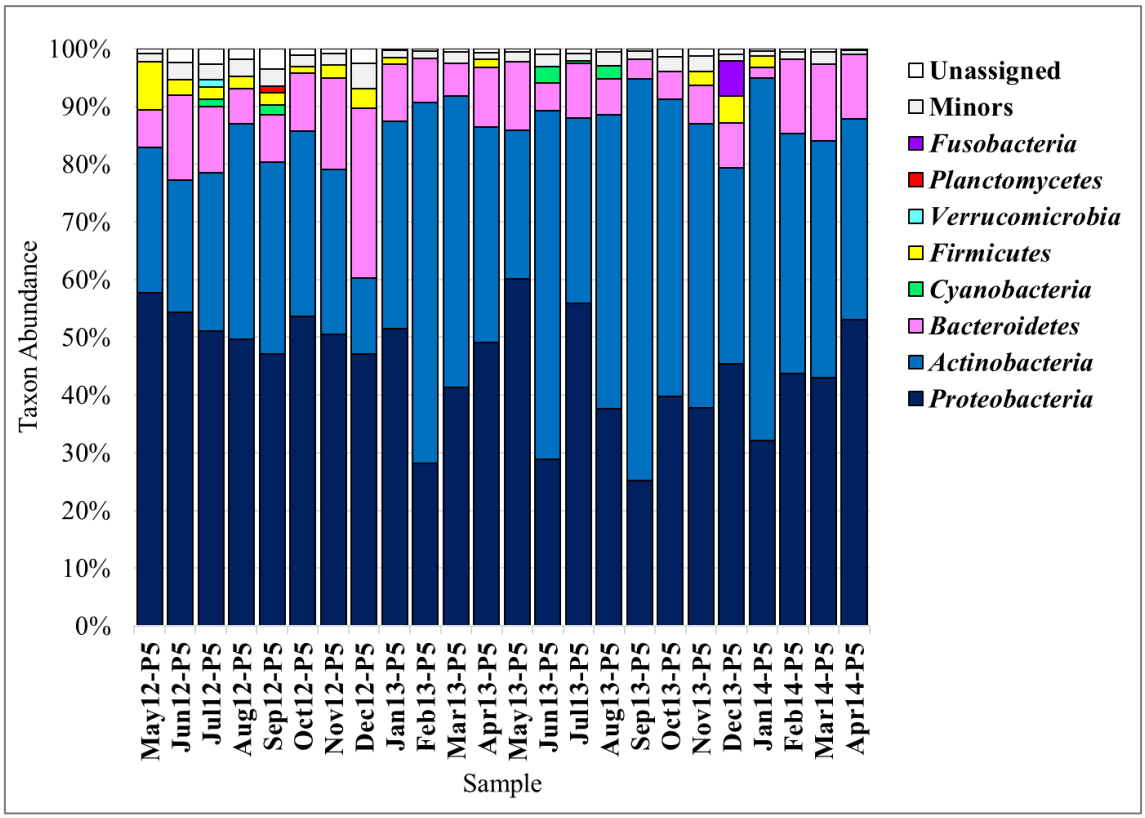

Fig. 5.7 Phylum-level bacterial composition of the P5 samples. Distribution of the major phyla (Ra > 1% in at least one sample); phyla with Ra < 1% were gathered in "Others"

P24 bacterial communities were dominated by *Proteobacteria* or *Actinobacteria* (Fig. 5.8). *Proteobacteria* represented the most abundant phylum in the periods May-June 2012, August 2012-April 2013, and December 2013 (Ra range 9.7-86.5%, maximum in April 2013), whereas *Actinobacteria* (Ra range 10.2-87.4%, maximum in January 2014) represented the predominant phylum in the remaining months. In general, in months characterised by *Proteobacteria* preponderance, *Actinobacteria* was the second most abundant taxon, and vice versa. The only exception was in December 2012, when *Proteobacteria* represented the predominant phylum, followed by *Cyanobacteria* (Ra = ~27%).

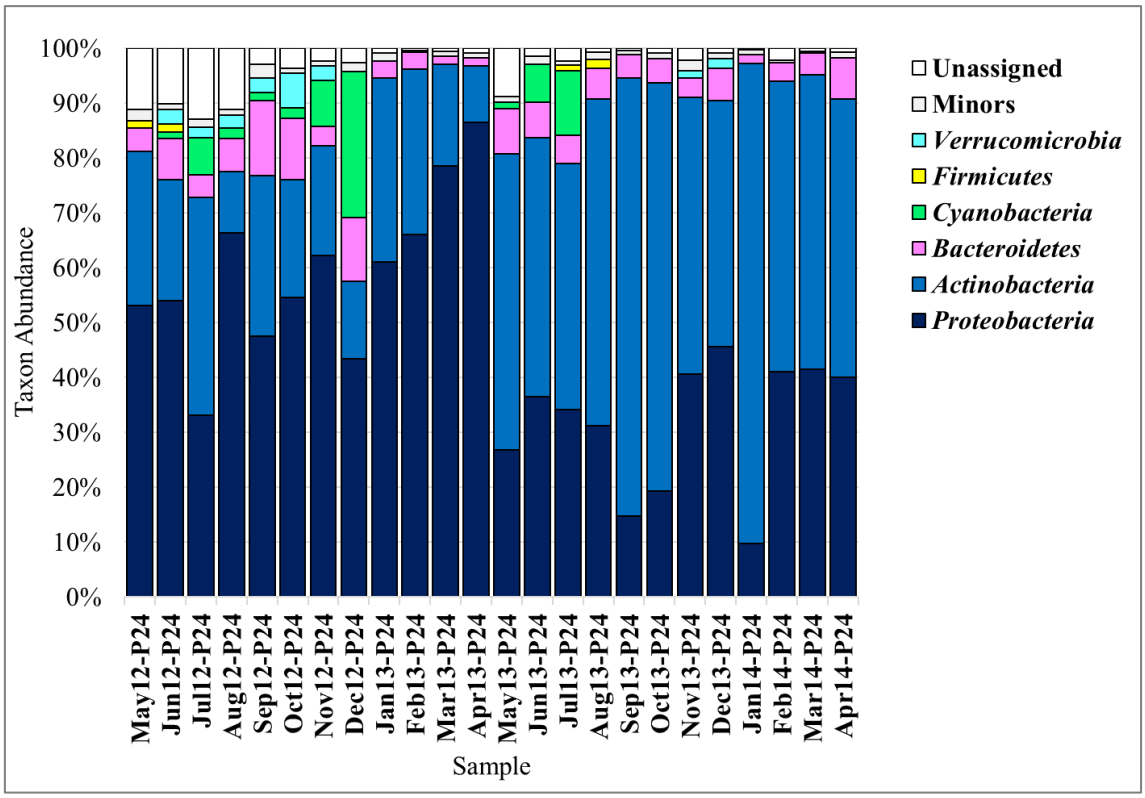

Fig. 5.8 Phylum-level bacterial composition of the P24 samples. Distribution of the major phyla (Ra > 1% in at least one sample); phyla with Ra < 1% were gathered in "Others"

Also P37 communities were dominated by *Proteobacteria* and *Actinobacteria*, predominating in 83% and 17% of samples, respectively (Fig. 5.9). *Proteobacteria* (Ra range 28.3-91.3%, maximum in February 2013) was the most abundant phylum during the first sampling year (May 2012-April 2013), in July 2013 and in the periods August-September 2013 and February-March 2014. *Actinobacteria* (Ra range 1.3-67.7%, maximum in January 2014) predominated in all remaining months (but in August 2013, its abundance was only slightly higher than that of *Proteobacteria*), and mostly represented the second abundant taxon (mainly in the second sampling year). *Bacteroidetes* represented the second phylum in summer and autumn times (Jun 2012, August-October 2012, and July-October 2013). Although *Proteobateria*, *Actinobacteria* and *Bacteroidetes* were generally the most represented taxa, a strong peak of *Cyanobacteria* (Ra = ~30%) was detected in November 2012 (when it was the second most abundant phylum).

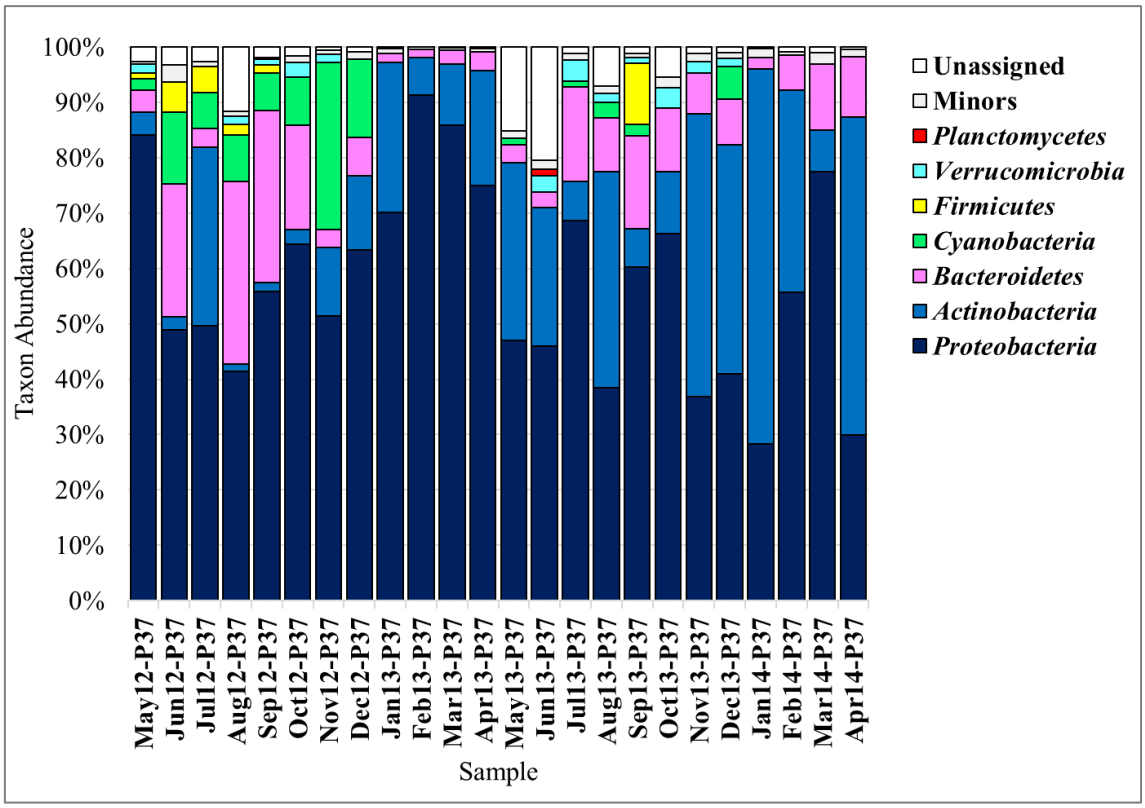

Fig. 5.9. Phylum-level bacterial composition of the P37 samples. Distribution of the major phyla (Ra > 1% in at least one sample); phyla with Ra < 1% were gathered in "Others"

Overall, considering all sampling sites, *Proteobacteria* was high in the sea to decrease in P5 and P24 (pools characterised by low and intermediate salinities, respectively), to increase again in P37 (crystallisation pond). (Figs. 5.6-5.9). However, the abundance variations of this taxon, quite limited in the sea, were rather evident in the ST pools (they were much broader in P24 and P37).

Proteobacteria, with some exceptions, seemed to be more abundant at low-intermediate salinities (26-180‰) (Fig. 5.10).

On the contrary, *Actinobacteria* was higher in P5 and P24 than in the sea and P37, although showing the broadest abundance variations in P24 and P37 (Figs. 5.6-5.9). Also *Actinobacteria* seemed to be higher mainly at low-intermediate salinities (26-146‰) (Fig. 5.11).

In general, in all sites it was observed an increase of *Actinobacteria* in the second sampling year (May 2013-April 2014), accompanied by the concomitant decrease of

Proteobacteria, and sometimes of *Bacteroidetes* (although to a lesser extent) (Figs. 5.6-5.9). This was particularly evident in P24 (dominated in the first 12 months by *Proteobacteria*) where in the second sampling year a strong increase of *Actinobacteria* and consequently a marked reduction of *Proteobacteria* were evidenced. This was particularly manifest in P24 where, starting from May-14 a shift of the community was very evident and domination of *Proteobacteria* was replaced by that of *Actinobacteria*.

Compared to *Proteobacteria* and *Actinobacteria*, *Bacteroidetes* showed more limited abundance variations within the ST communities (Figs. 5.6-5.9). However, this phylum was generally higher in the sea than in the pools, with abundances steadily around 15-20%. In P37, *Bacteroidetes* showed the broadest abundance variations. Moreover, peaks of its abundance were recorded in some months characterised by highest salinities (Fig. 5.12).

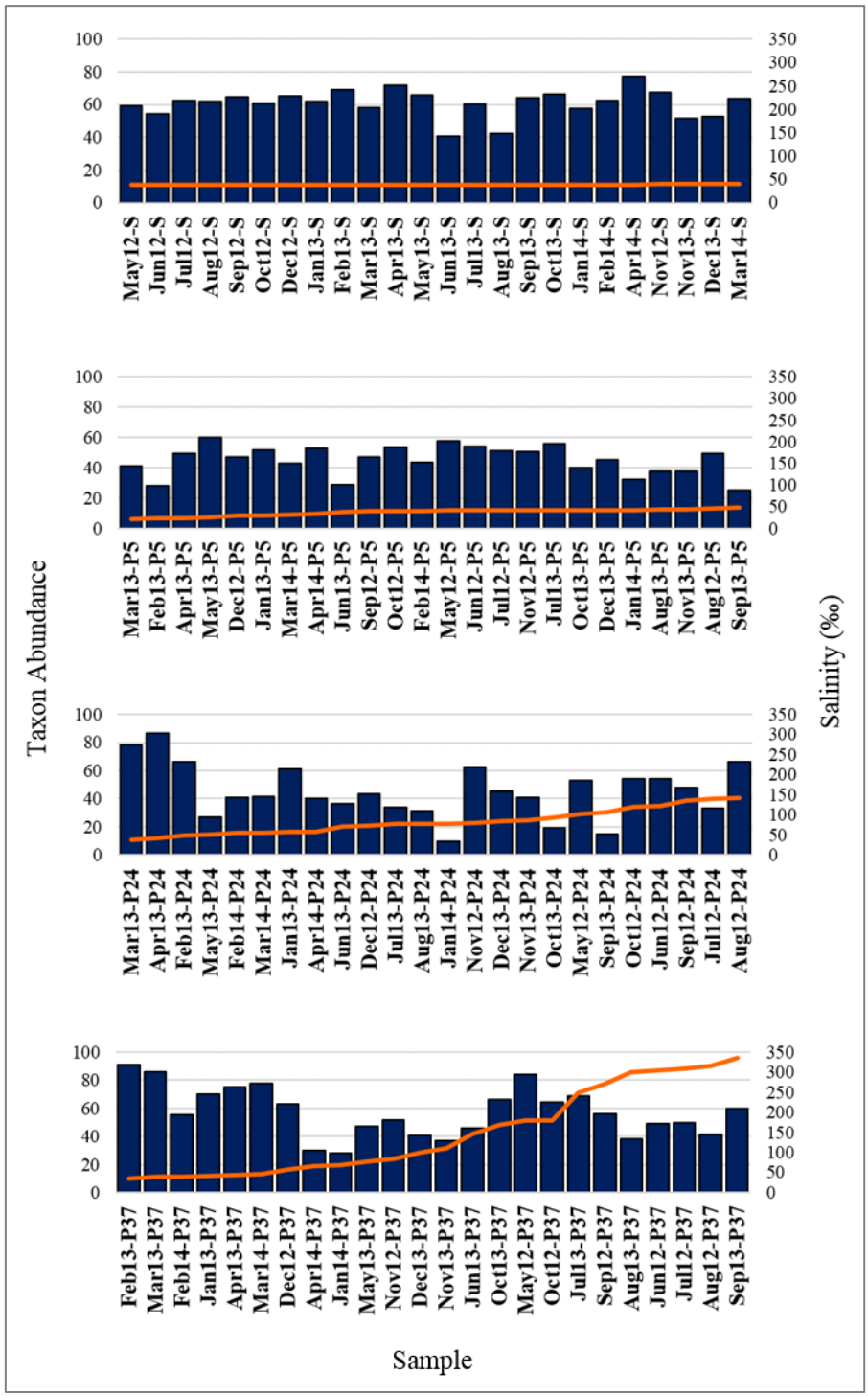

Fig. 5.10. *Proteobacteria* distribution in the sampling sites according to the increasing salinities recorded in each site over the two-year survey

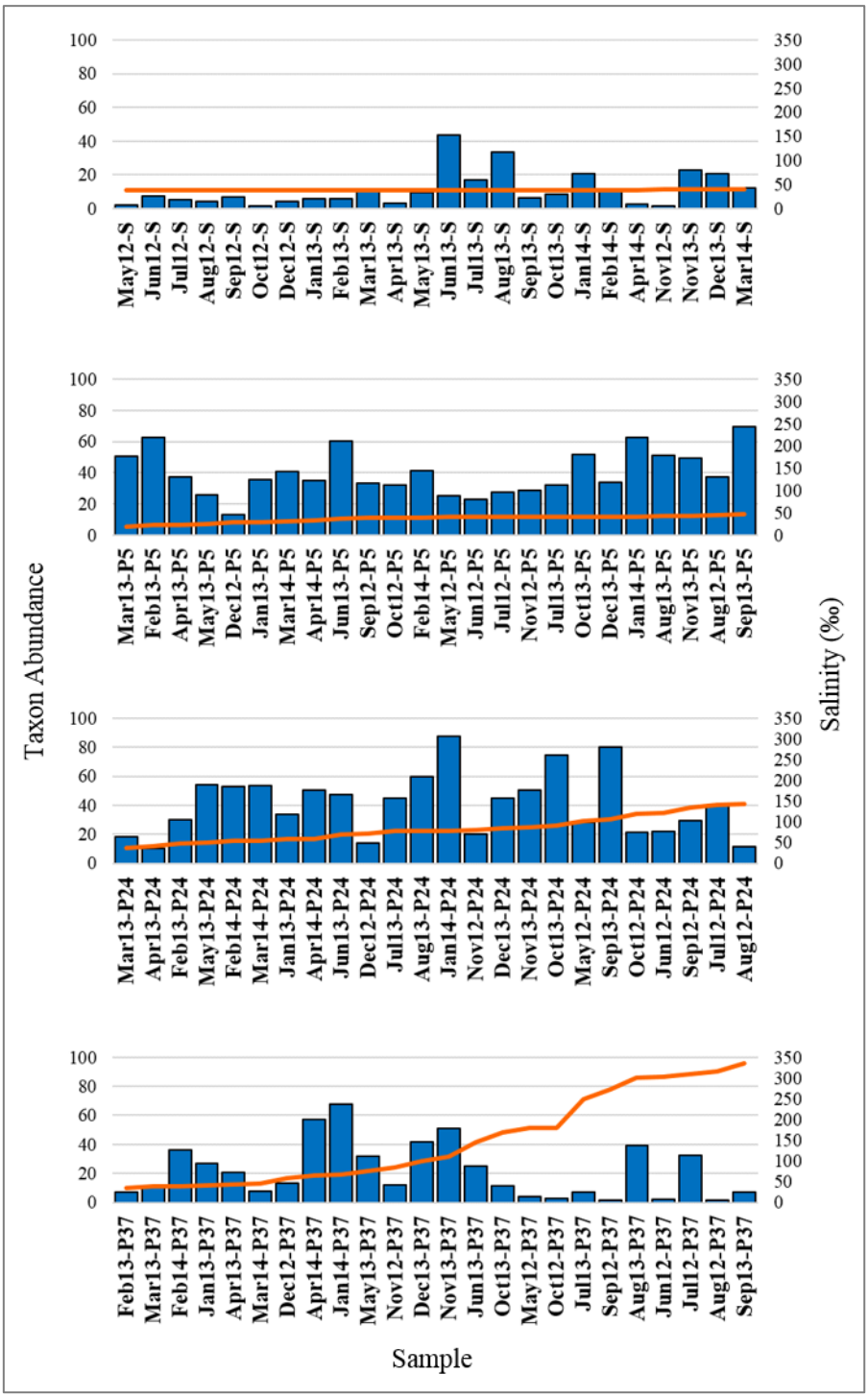

Fig. 5.11 *Actinobacteria* distribution in the sampling sites according to the increasing salinities recorded in each site over the two-year survey

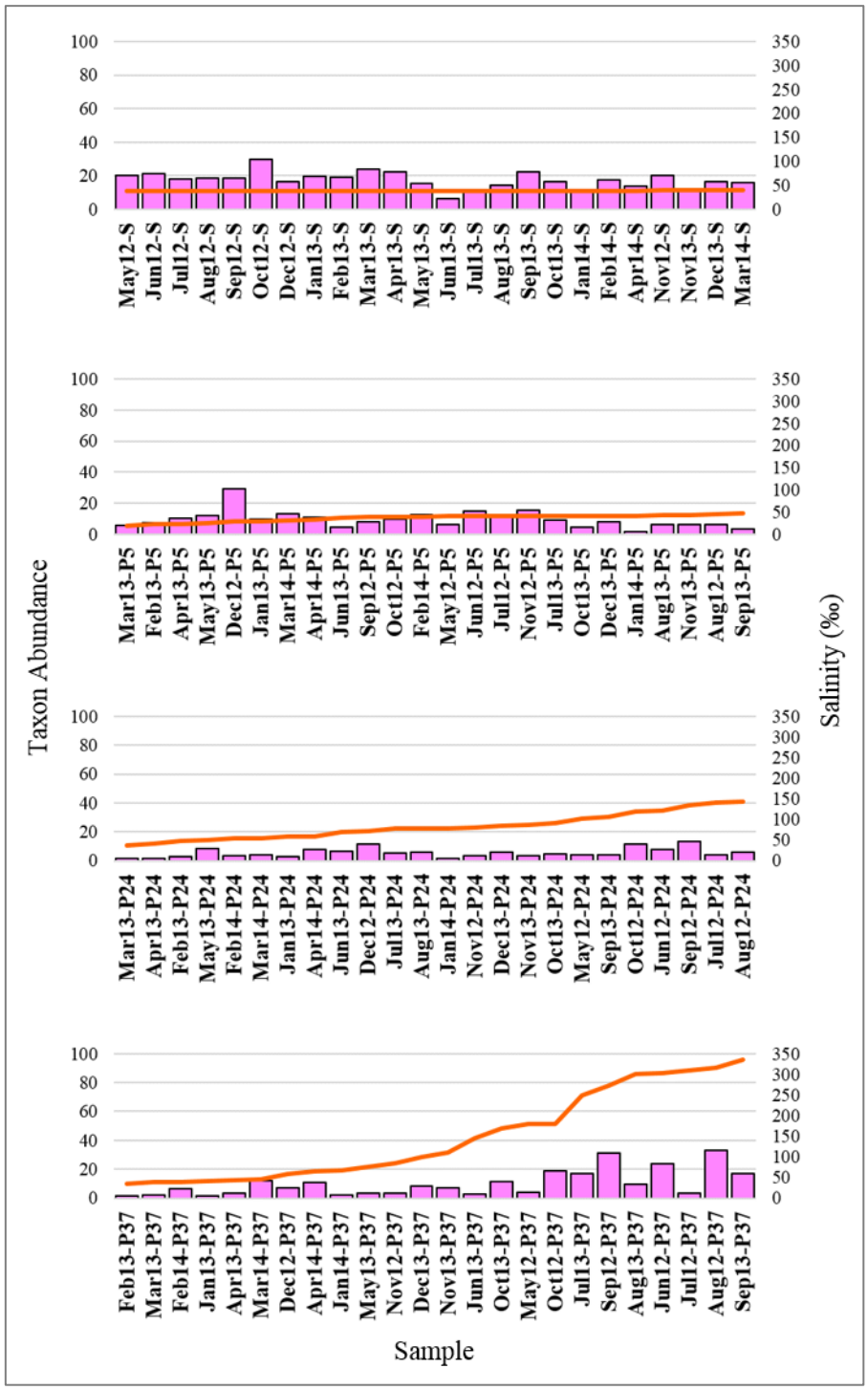

Fig. 5.12 *Bacteroidetes* distribution in the sampling sites according to the increasing salinities recorded in each site over the two-year survey

With the sole exception of February 2013 and April 2014 in P24, *Cyanobacteria* was always detected (Figs. 5.6-5.9). In the sea, *Cyanobacteria* (range 0.3-11.2%; average Ra = ~4%) represented a major taxon in 19 out 24 months (showing Ra ≥ 3% in 13 months), being more abundant during the period from late spring to early autumn (the highest abundances (Ra > 6%) were recorded in the periods May-August 2012 and May-July 2013). P5 definitely revealed the lowest occurrence of this phylum: it was detected as a rare taxon (Ra <1%) in all samples excluding July 2012, September 2012, June 2013 and August 2013, where its abundances were in the range 1.3-2.8%. In P24 and P37 *Cyanobacteria* was recorded with some notable peaks in some months only.

In P24, except for July 2012, November-December 2012 and June-July 2013, in which abundances were greater than 6% (highest abundances in December 2012, Ra = ~27% and November 2012, Ra = ~30% for P24 and P37, respectively), *Cyanobacteria* were always rather low or a minor taxon. However, in P37 it was always major taxon at salinities higher than 180‰ (with Ras in the range 1.5-11.2%).

Firmicutes was on average higher in the sea and P5 than P24 and P37 (Figs. 5.6-5.9). In the sea, it ranged from ~0.6 to ~5.8%, having an average abundance of about 2%. It represented a rare taxon only in three months (April, June and August 2013), and its highest Ras (> 3.8%) were recorded in May 2012, July 2012, January 2013 and January 2014. In P5, it ranged from ~0.1 to ~8.2%, with an average abundance of about 1.8%. In this pool, *Firmicutes* represented a rare taxon in 11 out 24 months, and its highest Ras (> 3.4%) were recorded in May 2012, December 2013 and December 2014. In P24, it was definitely lower than in the other three sampling sites. It represented usually a rare taxon, except for May-June 2012 (Ras ~1.5%) and July-August 2013 (Ras of ~1 and ~1.6%, respectively). Also in P37, *Firmicutes* was mostly a rare taxon; it was recorded among major phyla only in the first 5 months (May-September 2012, with Ras in the range 1.5-5.4%), and in September 2013 (when a peak was detected; Ra = ~11%).

Verrucomicrobia was found in all samples, but often it represented a rare taxon (Figs. 5.6-5.9). This is particularly true for the sea and P5 (the only exceptions were Sep12-S and Nov13-S, in which this phylum had an abundance of about 1.3 and 1.6%, respectively). *Verrucomicrobia* was detected as major taxon mainly in P24 and P37 pools. In P24, during the semester June-November 2012, it was constantly found among the major phyla with a peak of ~6.4% in October 2012. In P37 it was detected as major

phylum in May 2012 (Ra = ~1.6%), and in the periods August-November 2012 (Ras in the range 1-2.6%) and June-December 2013 (Ras in the range 1-4%).

With the only exception of Sep12-P5, *Planctomycetes* represented always a rare taxon in the pools, whereas in the sea it represented a major taxon (with Ras in the range 1-2%) in August-September 2012, December 2012, November 2013 and February 2014 (Figs. 5.6-5.9). .

Fusobacteria and *Candidatus Saccharibacteria* represented always a rare taxon, excluding the peaks recorded for *Fusobacteria* in P5 (December 2013, Ra = 6%) and for *Candidatus Saccharibacteria* in the sea (August 2013, Ra = 4%) (Figs. 5.6-5.9).

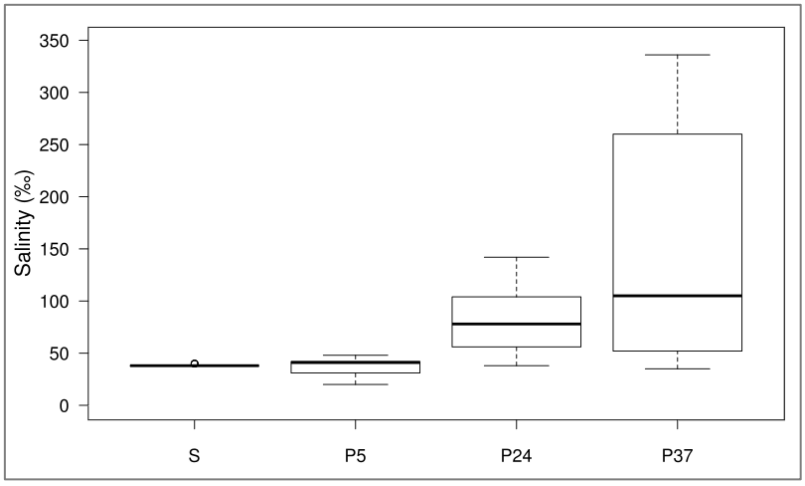

Fig. 5.13 Box-whisker plot showing salinity variation across the sampling sites. Lower and upper whiskers indicate minimum and maximum abundance values, box limits indicate the 25th and 75th percentiles, center line represents the median and dots are the outliers.

Among the 1052 genera globally retrieved, 111 had Ra ≥ 1% in at least one sample.

Although the following considerations are based on abundance data calculated only from the OTUs assigned at genus level, it is rather evident that, the sea samples did not show a marked predominance of some taxa as occurred in the ponds. In any case the sea samples showed the highest diversity of major genera (Fig 5.14). *Pontimonas*, *Marivita*, *Escherichia*/*Shigella*, and *GpIIa* were constantly found with rather high abundances (Figs. 5.14-5.17).

In P5 (Fig. 5.15), *Pontimonas* always notably dominated the bacterial communities, ranging from 18.7 to 61.7%. Except for *Lentibacter* and *Marivita* in two samples, all the other taxa were detected with Ras of about 10% or even less.

In P24 (Fig. 5.16), *Pontimonas* showed broader abundances variations (Ra 8.4-85.8%). It dominated mainly in the period May 2013-April 2014, but in some months, other genera predominated. For instance, *Thiohalocapsa* was always a rare taxon except in August 2012 when it showed the notable abundance of 35%. *Marivita* and *Bordetella* were particularly abundant from January to July 2013, and *Spiribacter* from May to November 2012. *GpVII* was present as major genus in same months, in particular in December 2012 (Ra = 26.4%).

In P37 (Fig. 5.17), except for *Thiohalocapsa*, all the other taxa mentioned above increased their abundance and they were present as major genus in a higher number of samples. Moreover, *Salinibacter* that in the other sampling sites was usually a rare taxon, in this pond was among the major taxa in various months, with abundances in the range 1.3-25.4%. By contrast, a strong reduction of *Pontimonas* was observed mainly in the first sampling year.

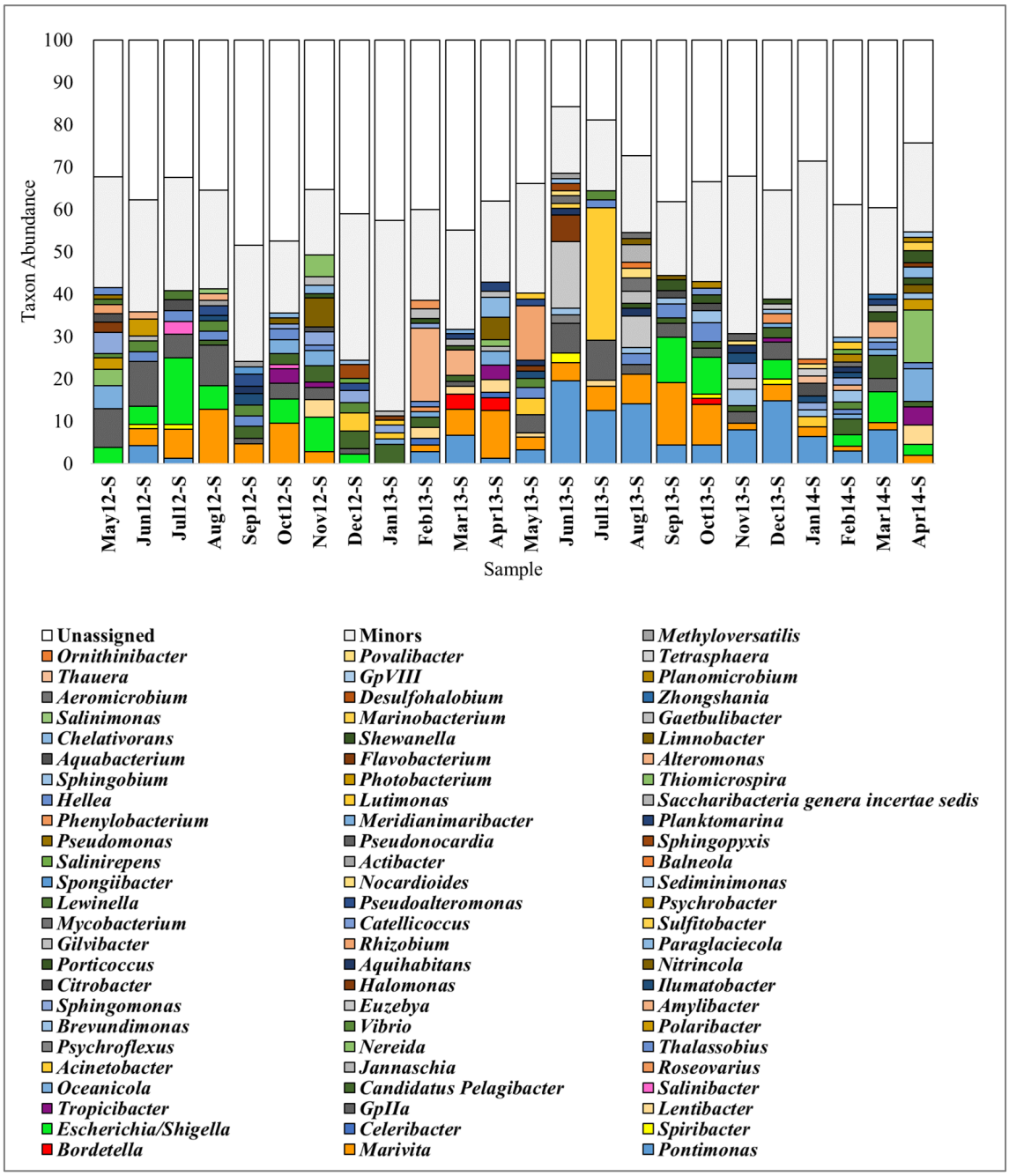

Fig. 5.14 Genus-level bacterial composition of the sea samples. Distribution of the major genera (Ra > 1% in at least one sample); genera with Ra < 1% were gathered in "Others

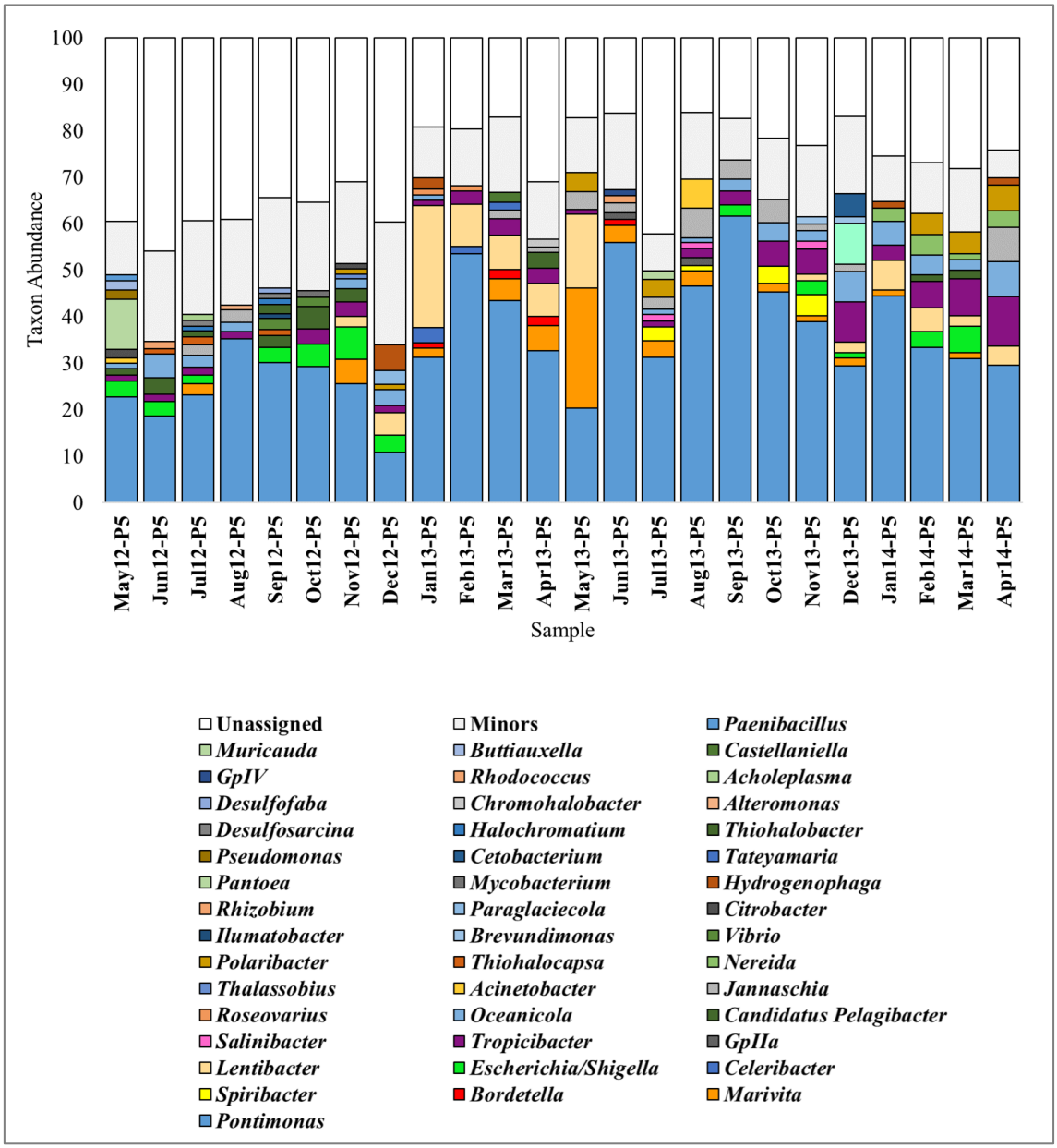

Fig. 5.15. Genus-level bacterial composition of the P5 samples. Distribution of the major genera (Ra > 1% in at least one sample); genera with Ra < 1% were gathered in "Others"

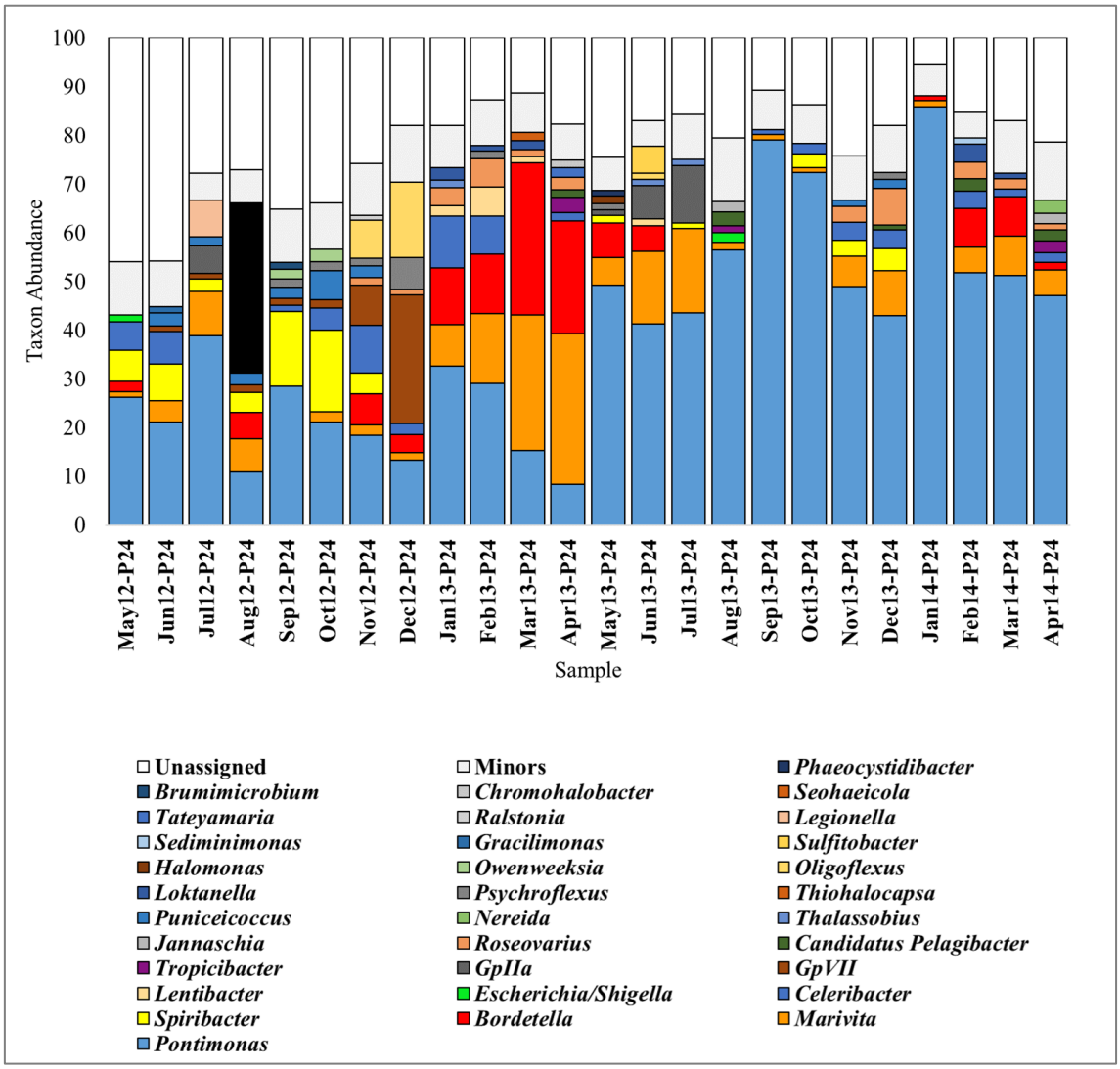

Fig. 5.16 Genus-level bacterial composition of the P24 samples. Distribution of the major genera (Ra > 1% in at least one sample); genera with Ra < 1% were gathered in "Others"

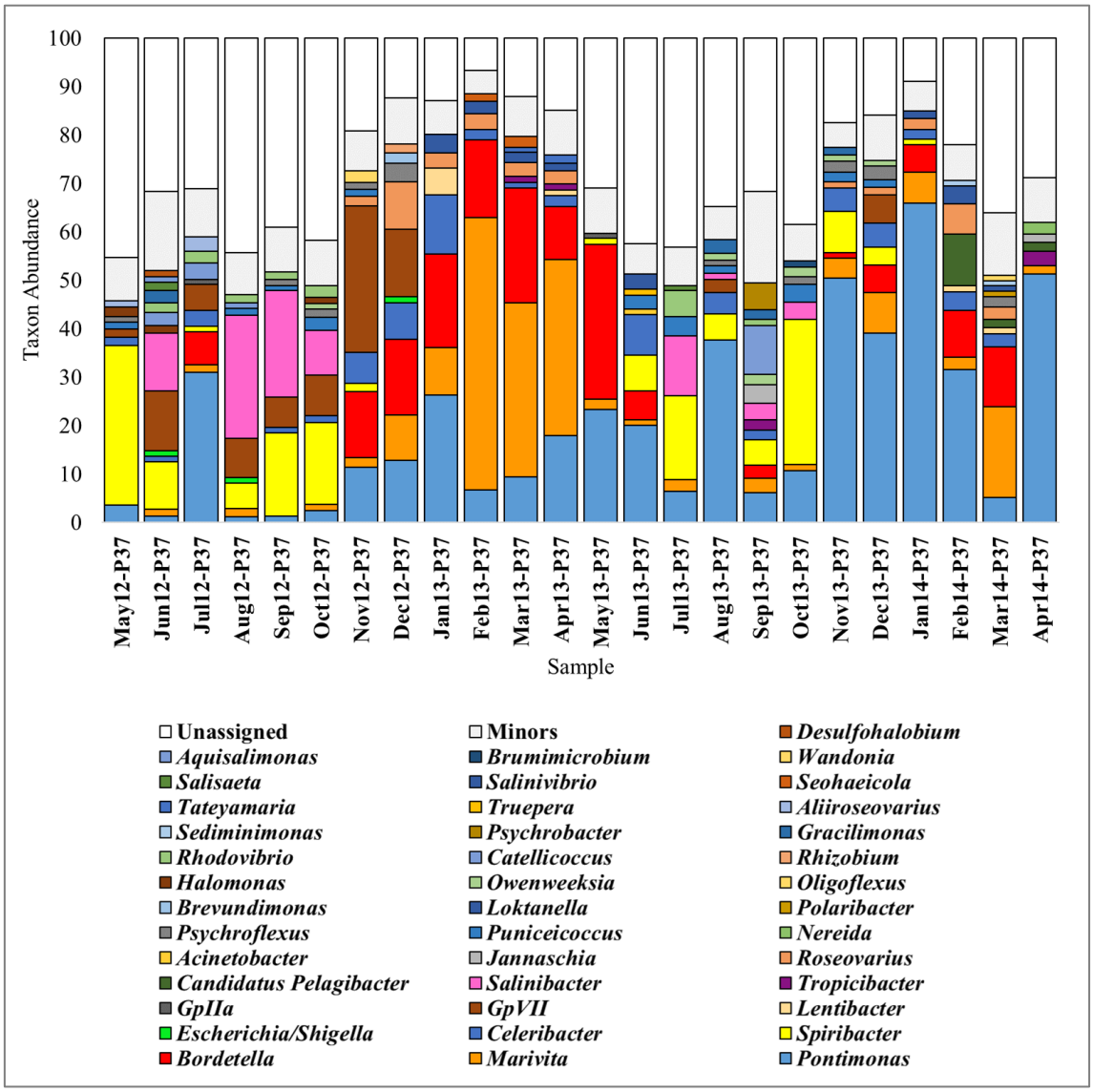

Fig. 5.17. Genus-level bacterial composition of the P37 samples. Distribution of the major genera (Ra > 1% in at least one sample); genera with Ra < 1% were gathered in "Others"

Unlike the samples collected from the sea, those taken from the ponds, showed a constant presence of *Pontimonas* as major taxon, with very high abundance (mainly in P5 and P24) (Figs. 5.14-5.17). However, this taxon seemed to be more abundant at low-intermediate salinities (20-142‰) (Fig. 5.18).

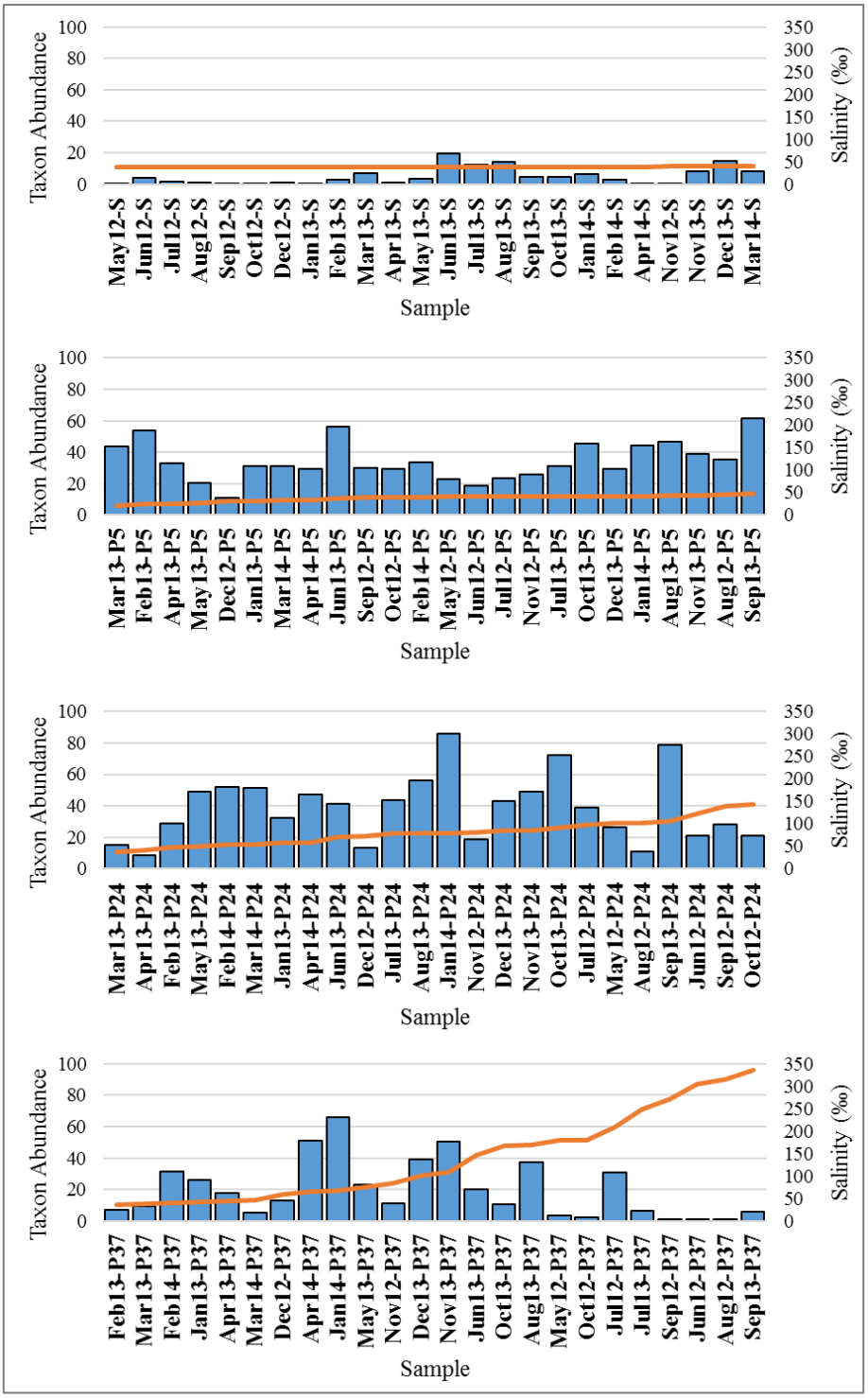

Fig. 5.18 *Pontimonas* distribution in the sampling sites according to the increasing salinities recorded in each site over the two-year survey

5.2.1.2 Alpha- and Beta-Diversity of the bacterial communities

Table 5.2 reports the α-diversity indices of the bacterial communities of ST samples collected from the sea and the selected pools along the salinity gradient. The number of OTUs ranged from 86 to 397, from 112 to 254, from 253 to 712 and from 129 to 455 for the sea, P5, P24 and P37, respectively. The Shannon index ranged from 1.871 to 4.660, from 1.480 to 3.857, from 3.518 to 5.843 and 2.565 to 4.578 for the sea, P5, P24 and P37, respectively. The Gini inequality index ranged from 0.991 to 0.993, from 0.996 to 0.999, from 0.976 to 0.996 and from 0.990 to 0.999 for the sea, P5, P24 and P37, respectively.

Overall, P24 bacterial communities showed higher diversity (higher Number of OTUs and Shannon Index) than those of sea, P5 and P37, and were the least dominated (lower Gini Index)

Table 5.2 Alpha-diversity indices of the ST amplicon libraries (Sea, P5, P24 and P37 samples)

Sample	Number of OTUs	Shannon Index	Gini Index
May12-S	311	4.512	0.994
Jun12-S	180	2.796	0.998
Jul12-S	126	2.526	0.999
Aug12-S	208	3.479	0.997
Sep12-S	215	3.605	0.997
Oct12-S	121	2.300	0.999
Nov12-S	164	2.965	0.998
Dec12-S	171	3.469	0.998
Jan13-S	113	2.721	0.999
Feb13-S	122	3.098	0.999
Mar13-S	148	2.992	0.998
Apr13-S	190	3.066	0.998
May13-S	124	3.261	0.998
Jun13-S	162	3.258	0.998
Jul13-S	167	2.944	0.998
Aug13-S	163	3.505	0.998
Sep13-S	138	2.816	0.999
Oct13-S	144	3.205	0.998
Nov13-S	188	3.255	0.998
Dec13-S	196	3.830	0.997

Table 5.2 (Continued)

Sample	Number of OTUs	Shannon Index	Gini Index
Jan14-S	86	1.871	0.999
Feb14-S	187	3.344	0.998
Mar14-S	154	3.374	0.998
Apr14-S	397	4.660	0.991
May12-P5	144	2.037	0.999
Jun12-P5	139	3.032	0.999
Jul12-P5	142	2.556	0.999
Aug12-P5	156	3.018	0.998
Sep12-P5	142	2.686	0.999
Oct12-P5	112	1.480	0.999
Nov12-P5	200	3.164	0.998
Dec12-P5	254	3.697	0.996
Jan13-P5	189	3.166	0.998
Feb13-P5	164	3.591	0.998
Mar13-P5	150	2.836	0.998
Apr13-P5	165	3.198	0.998
May13-P5	139	2.315	0.999
Jun13-P5	116	2.824	0.999
Jul13-P5	141	2.736	0.999
Aug13-P5	205	3.149	0.998
Sep13-P5	178	3.182	0.998
Oct13-P5	125	2.704	0.999
Nov13-P5	150	3.123	0.998
Dec13-P5	230	3.857	0.997
Jan14-P5	130	2.931	0.999
Feb14-P5	220	3.365	0.997
Mar14-P5	251	3.281	0.997
Apr14-P5	198	3.445	0.998
May12-P24	378	4.405	0.992
Jun12-P24	511	5.096	0.987
Jul12-P24	432	4.884	0.990
Aug12-P24	432	4.787	0.990
Sep12-P24	354	4.670	0.993
Oct12-P24	672	5.791	0.978
Nov12-P24	648	5.751	0.978
Dec12-P24	593	5.602	0.982
Jan13-P24	712	5.843	0.976
Feb13-P24	395	4.690	0.992
Mar13-P24	307	3.518	0.995
Apr13-P24	417	4.909	0.990
May13-P24	386	4.865	0.991

Table 5.2 (Continued)

Sample	Number of OTUs	Shannon Index	Gini Index
Jun13-P24	253	4.037	0.996
Jul13-P24	313	4.511	0.994
Aug13-P24	503	4.952	0.988
Sep13-P24	332	4.499	0.994
Oct13-P24	660	5.775	0.979
Nov13-P24	426	4.951	0.990
Dec13-P24	356	4.541	0.993
Jan14-P24	402	4.528	0.992
Feb14-P24	706	5.809	0.977
Mar14-P24	314	4.605	0.993
Apr14-P24	462	4.972	0.989
May12-P37	171	2.684	0.998
Jun12-P37	185	3.001	0.998
Jul12-P37	238	3.314	0.997
Aug12-P37	372	4.376	0.992
Sep12-P37	260	3.592	0.997
Oct12-P37	150	2.608	0.999
Nov12-P37	305	3.747	0.995
Dec12-P37	455	4.525	0.990
Jan13-P37	174	2.949	0.998
Feb13-P37	272	3.933	0.996
Mar13-P37	152	2.938	0.998
Apr13-P37	246	3.738	0.996
May13-P37	276	3.463	0.996
Jun13-P37	295	3.358	0.996
Jul13-P37	231	3.537	0.997
Aug13-P37	393	3.931	0.993
Sep13-P37	129	3.013	0.999
Oct13-P37	192	3.190	0.998
Nov13-P37	248	3.441	0.997
Dec13-P37	306	3.801	0.995
Jan14-P37	168	2.565	0.998
Feb14-P37	450	4.578	0.991
Mar14-P37	244	3.344	0.997
Apr14-P37	375	4.190	0.993

Among the environmental variables taken into account (salinity, water temperature, pH, rainfall, chlorophyll pigments, BOD_5, sampling month, sampling year and sampling site), pH was excluded due to its high collinearity with salinity. The forward selection with double-stopping criterion in CCA analysis showed that the community structure changed significantly with sampling site, sampling year, salinity and sampling month (Sin(Month), Cos(Month) and Sin(Month)*Cos(Month)), whereas all the other variables were discarded (Table 5.3).

Table 5.3 CCA of variation of the OTU abundance of sea, P5, P24 and P37 according to the parameters

Variable	**df**	**Variance**	**F**	**P**
Sin(Month)	1	0.1699	2.64	0.001
Cos(Month)	1	0.172	2.682	0.001
Sin(Month)*Cos(Month)	1	0.137	2.148	0.001
Year	2	0.315	2.46	0.001
Sampling site	3	0.821	4.28	0.001
Salinity	1	0.289	4.256	0.001
Chlorophyll pigments	1	0.08	1.244	0.259
Residuals	85	5.435		

$F_{10,85} = 3.813$, P = 0.001, adjusted $R^2 = 0.229$

The first axis of CCA plot (Fig. 5.19) correlated with sampling site. Based on this parameter, samples collected from the pools were ordered to form overlapping clusters, from P5 to P37, reflecting the ST salinity gradient, whereas sea samples clustered apart. In addition, the bacterial communities principally affected by the salinity were those of P37 and P5; most P37 samples were positively related to this parameter, whereas all P5 samples were negatively related with salinity.

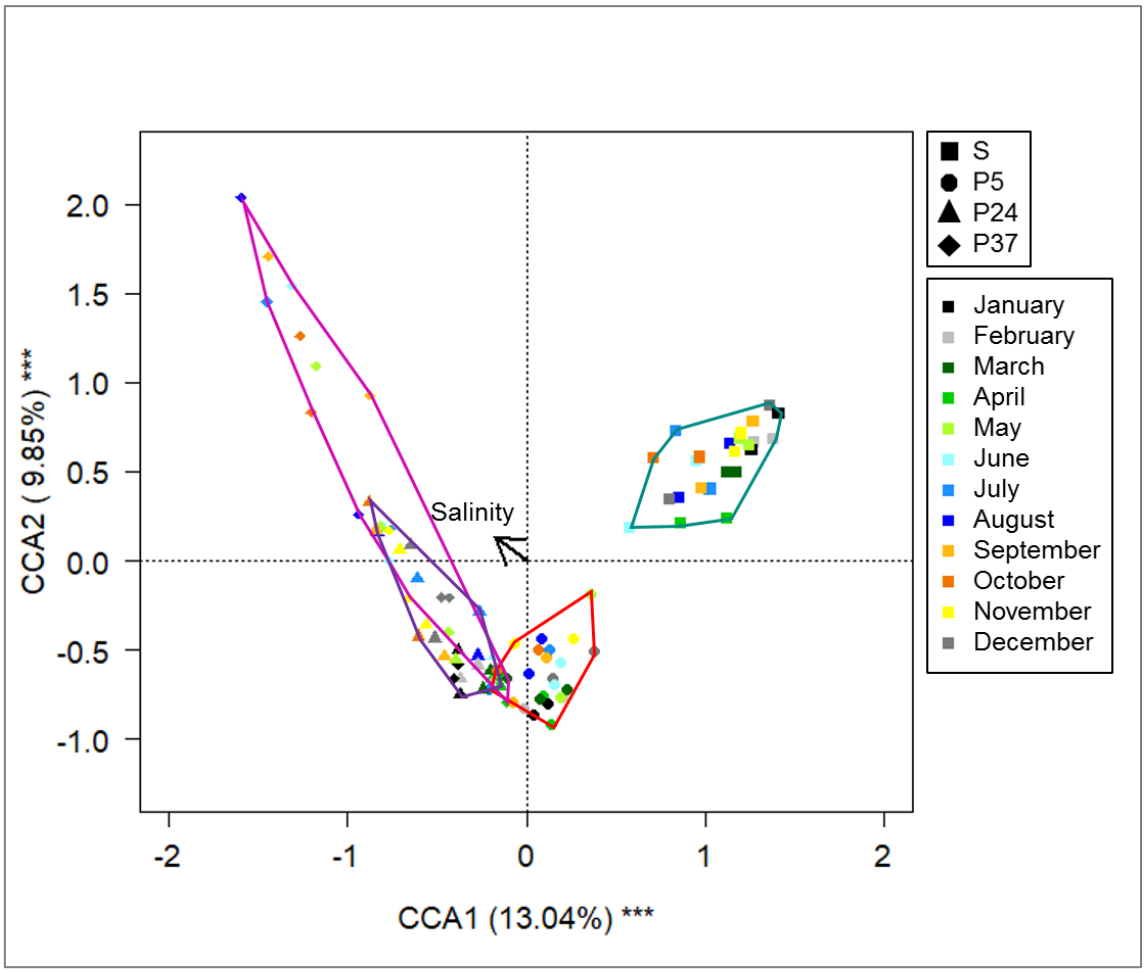

Fig. 5.19 Canonical Correspondence Analysis (CCA) ordination biplot showing samples according to the parameters driving the bacterial community structure. OTU abundance were Hellinger-transformed. Arrow represent constraining variable. Variance explained by the two axes and axes significance (***: $P < 0.001$) is reported.

5.2.1.3 Variation of α-diversity indices and genera abundance in relation to the sampling sites, the salinity and the seasonality

The GLM analyses were run to analyse whether α-diversity indices and taxa (most abundant genera) abundance variations among the sampling sites, in relation to the salinity, as well as the seasonality were significant.

The α-diversity indices did not show significant variations ($P_{FDR} > 0.05$), whereas some taxa changed significantly according to the tested parameters.

Among the most abundant genera (*Pontimonas*, *Marivita*, *Spiribacter*, *Bordetella*, *GpVII* and *Lentibacter*), except for *Lentibacter*, the other taxa showed significant variations among the sampling sites (Fig. 5.20). *Pontimonas* ($F_{3,85}$ = 57.063, P_{FDR} < 0.0001) showed the lowest abundances in the sea, increased in P5 and P24, and decreased again in P37; no statistically significant differences were recorded between the sea and P37 and between P5 and P24. *Marivita* ($F_{3,85}$ = 11.703, P_{FDR} < 0.0001), *Spiribacter* ($F_{3,85}$ = 14.670, P_{FDR} < 0.0001) and *GpVII* ($F_{3,85}$ = 25.173, P_{FDR} < 0.0001) showed similar trend among sampling sites, being lower in the sea and P5 and higher in P24 and P37 (with no statistically significant differences between the sea and P5 and between P24 and P37). *Bordetella* ($F_{3,85}$ = 24.538, P_{FDR} < 0.0001) was very low in the sea and P5 (with no statistically significant differences between these sites) to increase in P24 and even more in P37.

Pontimonas, *Marivita*, *Bordetella* and *GpVII* showed significant variation in relation to the salinity (Fig. 5.21). *Pontimonas* (z = 13.434, P_{FDR} < 0.001), *Marivita* (z = 30.694, P_{FDR} < 0.0001) and *Bordetella* (z = 14.761, P_{FDR} < 0.001) decreased with increasing salinity, whereas *GpVII* (z = 16.290, P_{FDR} < 0.001) increased.

Taxa seasonal variation was also investigated (Fig. 5.22). *Pontimonas* showed a maximum in January, then decreased reaching a minimum in spring, and increased slightly in autumn (Sin(Month): $F_{1,85}$ = 26.755, P_{FDR} < 0.0001). *Marivita* had similar abundances from June to January (from the late summer to the winter) and reached a maximum in spring (Cos(Month): $F_{1,85}$ = 14.794, P_{FDR} = 0.001). *Spiribacter* showed a minimum in winter to increase until June (early summer), then it experienced a slight decrease to rise again and reach a maximum in autumn (Sin(Month): $F_{1,85}$ = 13.075, P_{FDR} = 0.002; Cos(Month): $F_{1,85}$ = 9.921, P_{FDR} = 0.009; Sin(Month)*Cos(Month): $F_{1,85}$ = 38.416, P_{FDR} < 0.0001). *Bordetella* had a maximum in spring and a minimum in autumn (Sin(Month): $F_{1,85}$ = 7.268, P_{FDR} = 0.024). *GpVII* was usually quite low to increase from September and reach a maximum in December (it increased from the early autumn to reach the maximum in early winter) (Sin(Month): $F_{1,85}$ = 13.770, P_{FDR} = 0.002; Cos(Month): $F_{1,85}$ = 70.592, P_{FDR} < 0.0001).

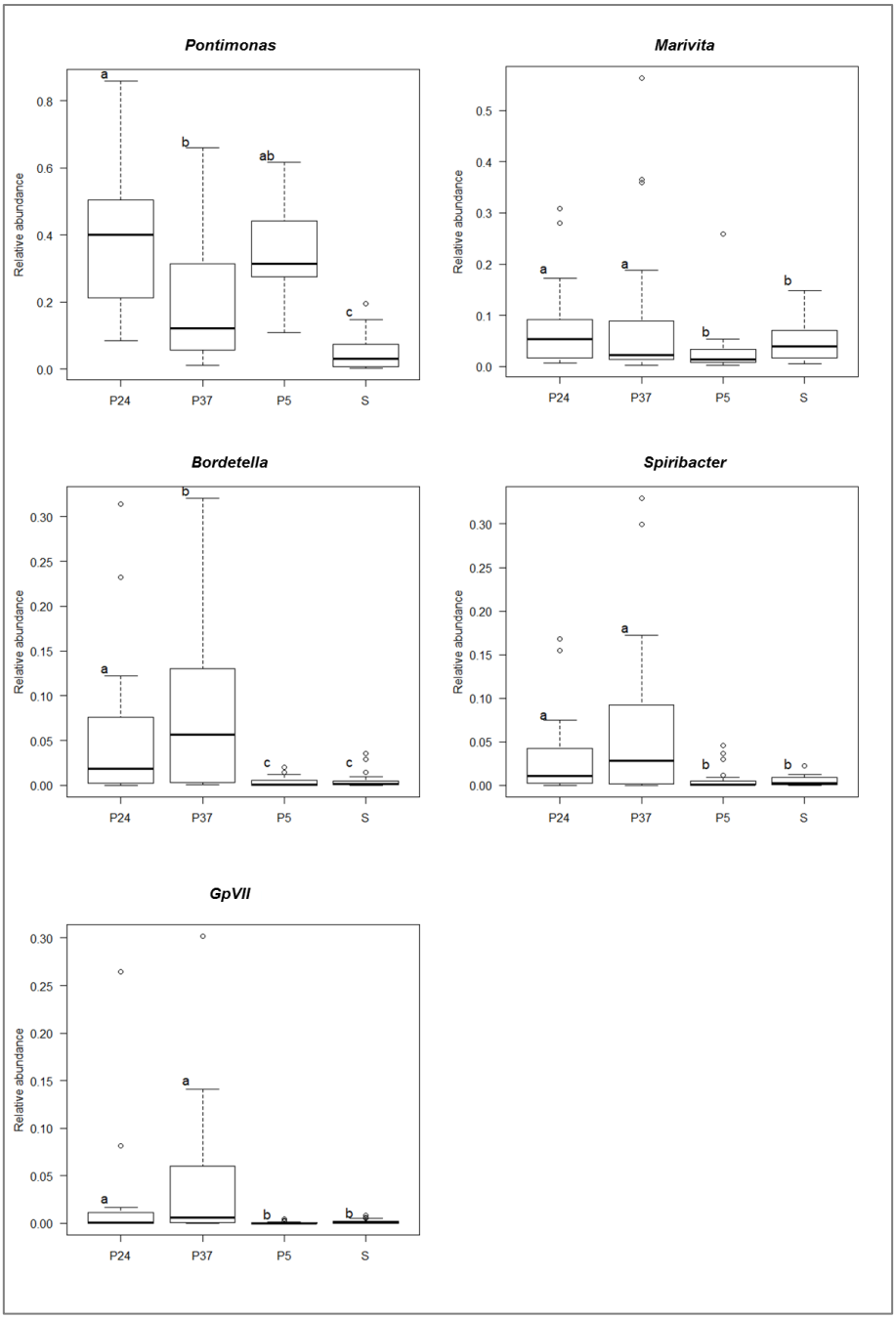

Fig. 5.20. Box-whisker plot (GLM analysis) showing abundance variation of *Pontimonas*, *Marivita*, *Bordetella*, *Spiribacter* and *GpVII* across the sampling sites. Lower and upper whiskers indicate minimum and maximum abundance values, box limits indicate the 25th and 75th percentiles, center line represents the median and dots are the outliers. Differences were analysed by Post Hoc test. Same lower case letters indicate no significant differences in taxa abundances variation between the sampling sites.

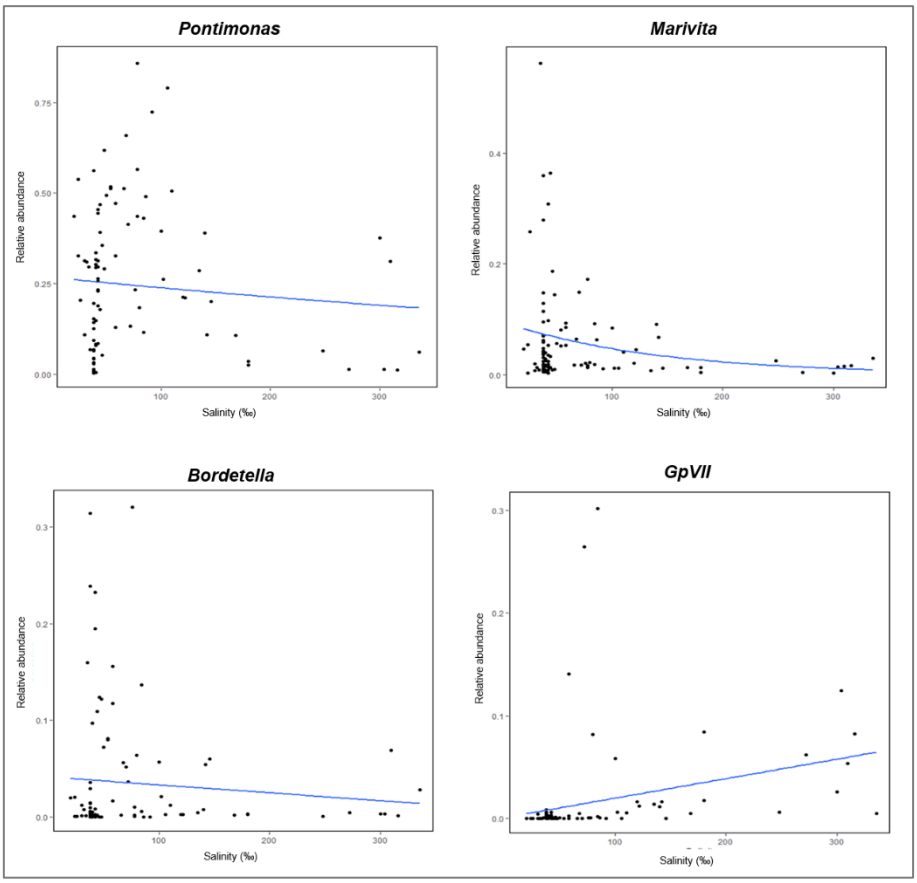

Fig. 5.21 GLM plots showing abundance variation of *Pontimonas*, *Marivita*, *Bordetella* and *GpVII* in relation to the salinity

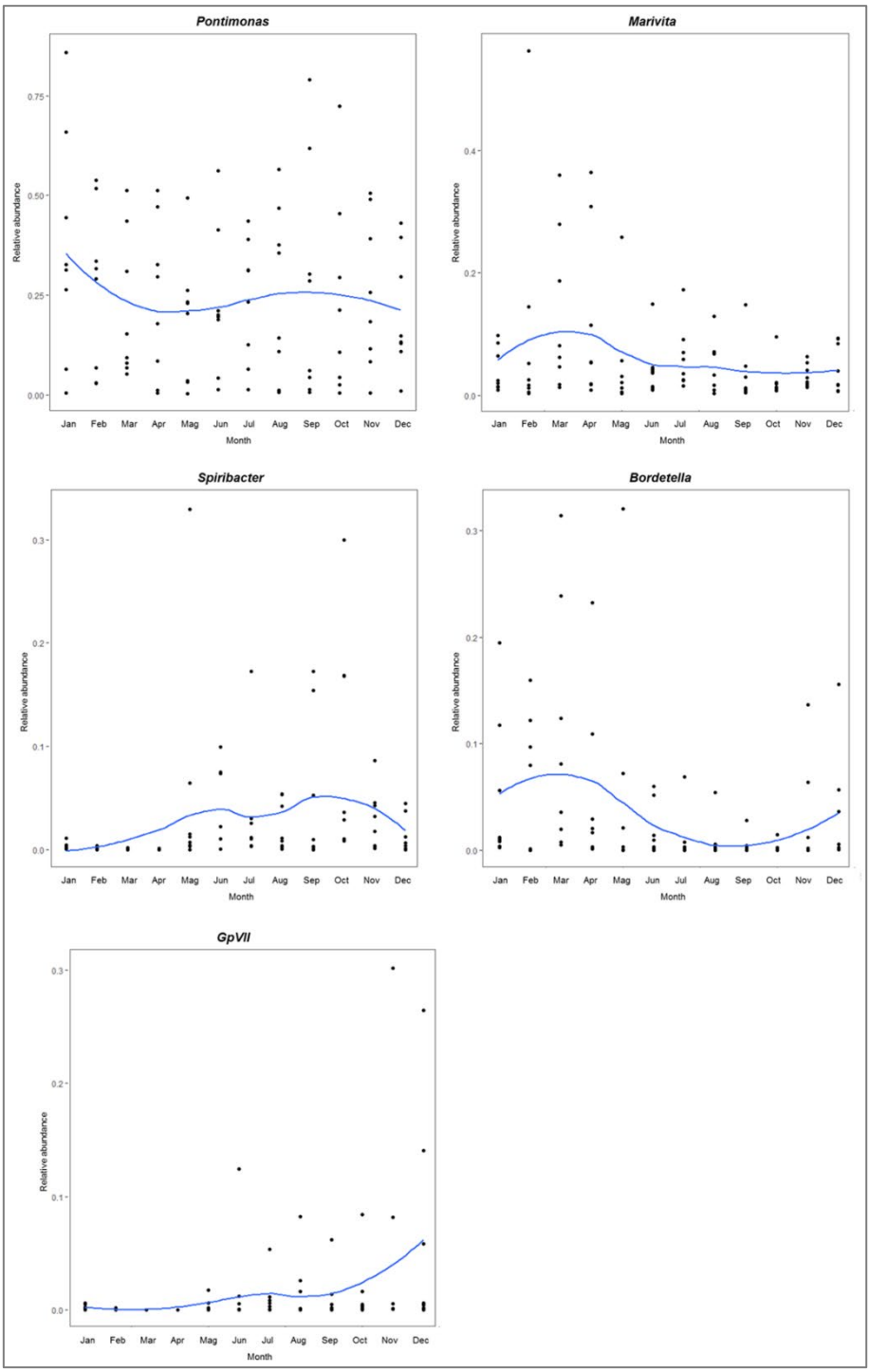

Fig. 5.22 GLM plots showing abundance seasonal variation of *Pontimonas*, *Marivita*, Spiribacter, *Bordetella* and *GpVII*

5.2.2 Bacterial communities harboured by the Brine Storage Basin

5.2.2.1 Composition of the bacterial communities

A total of 710,030 mapping sequences was obtained from the monthly surveys of the bacterial communities of BSB over the period May 2012-April 2014. Good's index was > 94.6% in each sample, indicating a good coverage for the libraries. The overall OTUs retrieved in the BSB were assigned to 27 phyla, 51 classes, 103 orders, 256 families and 733 genera. Unassigned OTUs ranged from 0.01-7.3% at phylum level, from 0.2 to 9% at class level, from 0.4 to 24.2% at order level, from 0.6 to 26.7% at family level and from 7.2 to 80.9% at genus level.

Proteobacteria, *Bacteroidetes* and *Actinobacteria* were the most abundant phyla (Fig. 5.23). *Proteobacteria* represented the predominant phylum in 17 months out of 24 (67% of the samples), accounting from 12% (Oct12) to 92% (Mar13) of the total bacterial community. *Bacteroidetes* prevailed in the remaining samples, especially in some months of 2012 (May, June, September and October), accounting from 5% (Mar13) to 84% (Oct12) of the total bacterial community. Excepting for February 2013, *Cyanobacteria* were always detected and, in 14 months, their Ra was > 1%. In general, this taxon showed the greatest abundances during the summer months, reaching a maximum (16%) in July 2013.

Among the 733 genera globally detected, 54 had Ra ≥ 1% in at least one sample. *Pontimonas*, *Salinibacter*, *Psychroflexus*, *Celeribacter*, *Sediminimonas*, *Spiribacter* and *Halomonas* were the most abundant genera (Fig. 5.24). Among them, *Pontimonas* was constantly found from January 2013, showing the greatest abundances during May-December 2013 and April 2014 (with a peak in June 2013 when its Ra was ~34%). *Spiribacter* was found as major genus mainly in 2013, over a period spanning from the end of the winter to the end of the subsequent autumn, being almost absent in the winter months; however, it reached a notable abundance (Ra = ~35%) in April 2014. *Psychroflexus*, *Celeribacter* and *Sediminimonas* (highest abundances in September 2012, March 2014 and February 2014, Ra_s ~36%, ~34% and ~38%, respectively) had a rather similar pattern, they were recorded as major taxa in the periods August 2012-July 2013

and January-April 2014. *Halomonas* was recorded with Ra ≥ 1% mainly from the end of the autumn up to the end of the spring (November 2012-May 2013, November 2013-January 2014 and in April 2014), showing its maximum (Ra = ~30%) in April 2013. *Salinibacter* was recorded as abundant taxon from May 2012 to October 2012, in January 2013 and from July 13 to January 2014 (reaching its highest abundance in June 2012; Ra = ~27%).

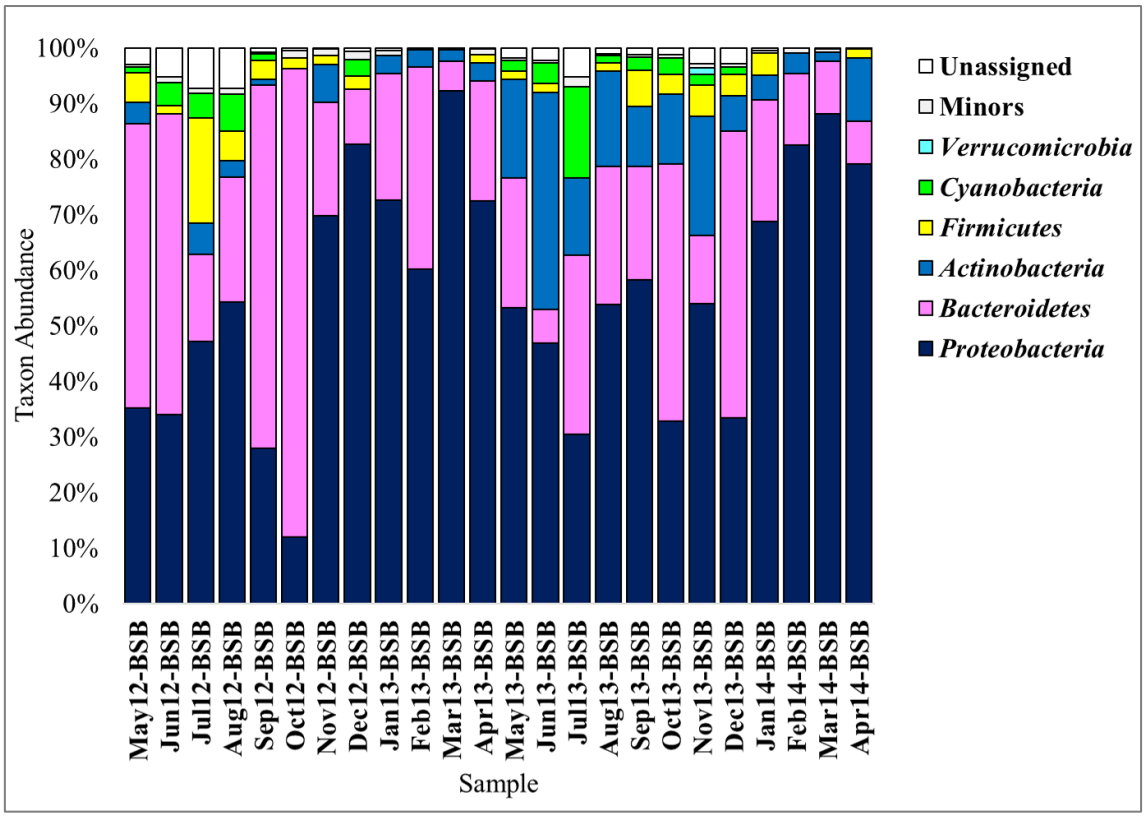

Fig. 5.23 Phylum-level bacterial composition of the BSB samples. Distribution of the major phyla (Ra > 1% in at least one sample); phyla with Ra < 1% were gathered in "Others"

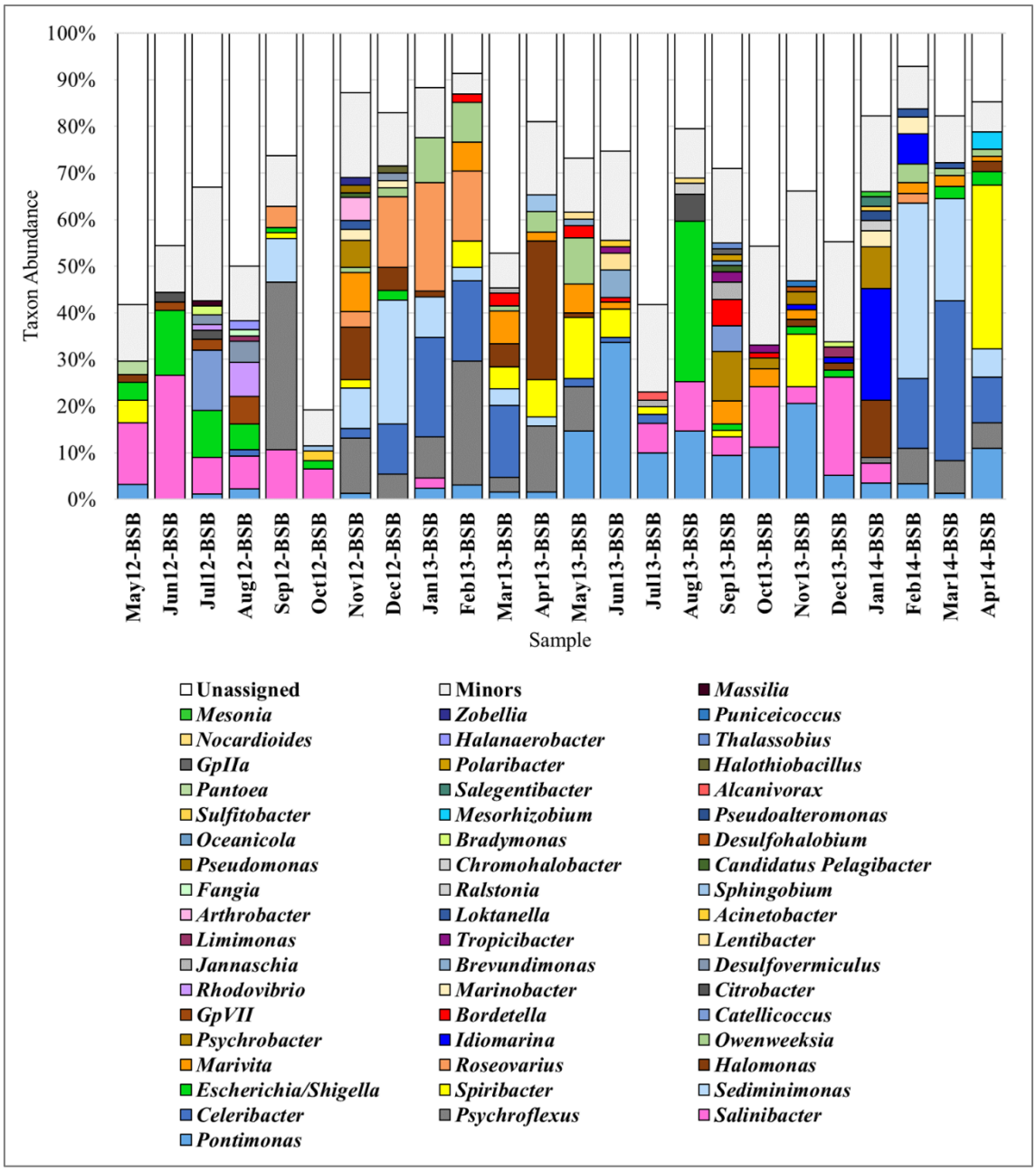

Fig. 5.24 Genus-level bacterial composition of the BSB samples. Average distribution of the most abundant genera; taxa with Ra < 1% were gathered in "Others".

5.2.2.2 Alpha- and Beta-Diversity of the bacterial communities of BSB

Table 5.4 reports α-diversity indices of the bacterial communities of BSB. The number of OTUs ranged from 78 (Feb13) to 377 (Jul12). The Shannon diversity and the Gini inequality indices ranged from 1.504 (Oct12) to 4.569 (Jul12) and from 0.929 (Jul12) to 0.993 (Feb13), respectively.

Table 5.4 Alpha-diversity indices of the BSB amplicon libraries.

Sample	Number of OTUs	Shannon Index	Gini Index
May12-BSB	172	3.091	0.981
Jun12-BSB	190	3.120	0.980
Jul12-BSB	377	4.569	0.929
Aug12-BSB	210	4.144	0.967
Sep12-BSB	150	2.694	0.987
Oct12-BSB	145	1.504	0.991
Nov12-BSB	254	4.170	0.960
Dec12-BSB	179	3.099	0.982
Jan13-BSB	157	2.875	0.986
Feb13-BSB	78	2.723	0.993
Mar13-BSB	126	2.727	0.989
Apr13-BSB	206	3.606	0.975
May13-BSB	196	3.768	0.974
Jun13-BSB	258	3.836	0.961
Jul13-BSB	217	3.770	0.967
Aug13-BSB	153	2.816	0.986
Sep13-BSB	252	4.282	0.958
Oct13-BSB	281	3.916	0.956
Nov13-BSB	256	4.273	0.955
Dec13-BSB	268	3.723	0.958
Jan14-BSB	251	3.692	0.966
Feb14-BSB	121	2.578	0.990
Mar14-BSB	116	2.516	0.991
Apr14-BSB	111	2.692	0.991

Among the environmental variables taken into account (salinity, water temperature, pH, rainfall, chlorophylls, BOD_5, sampling month and sampling year), only salinity significantly affected the structure of the communities. The forward selection

with double-stopping criterion in the RDA analysis showed that the community structure changed significantly with salinity only ($z = 6.450$, $P < 0.001$, adjusted-$R^2 = 0.192$), whereas all the other entered variables were discarded (details not shown). In particular, Jun12 and Feb14 communities showed the strongest relation with this parameter (Jun12 was positively related to salinity, whereas Feb14 was negatively related to the parameter) (Fig. 5.25)

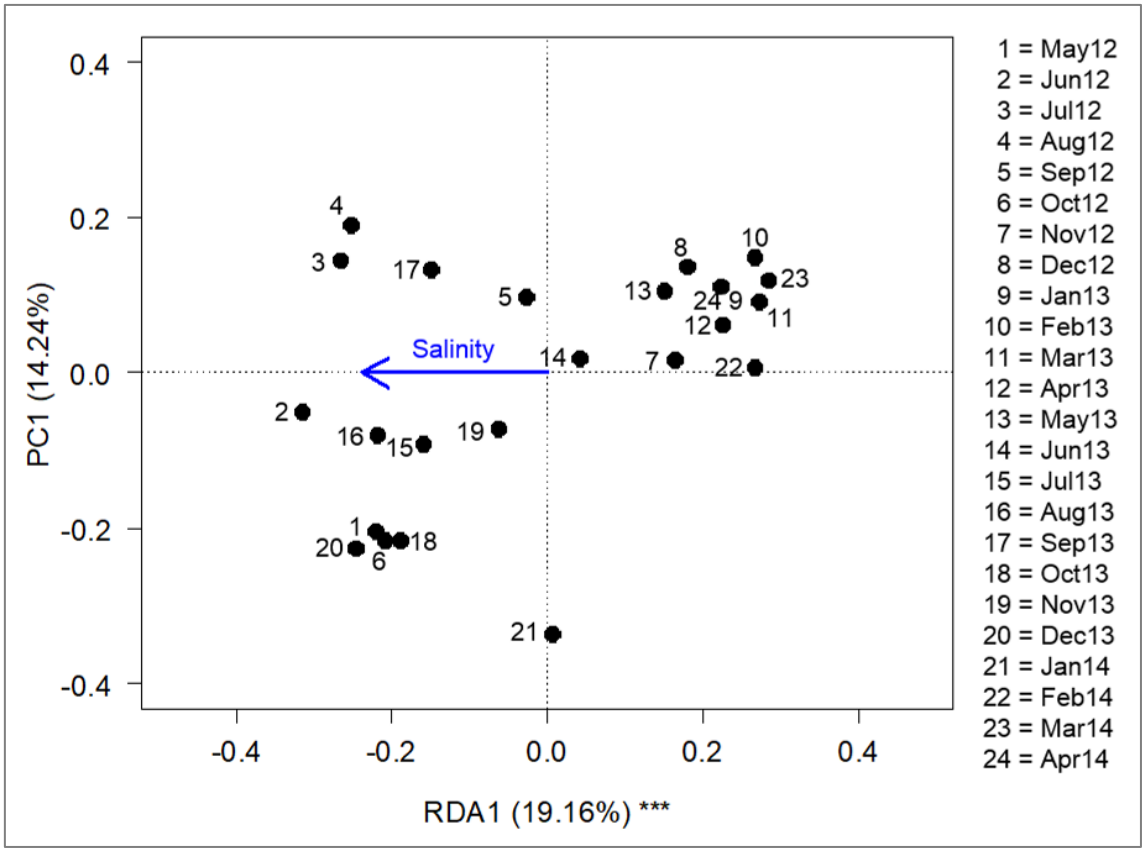

Fig. 5.25 Redundancy analysis (RDA) ordination biplot showing BSB samples according to salinity. Bacterial OTU abundance were Hellinger-transformed. Arrow represent constraining variable. Variance explained by the first axis and axis significance (***: $P < 0.001$) is reported

5.2.2.3 Variation of the α-diversity and genera abundance in relation to the salinity

The GLM analyses were run to investigate the significance of the variations recorded for α-diversity and genera abundance (only for the most abundant taxa) in relation to the driving parameter.

The Shannon diversity index showed a weak tendency toward increasing values at increasing salinity, but this trend was marginally non-significant ($t_{22} = 1.881$. $P = 0.073$) (Fig. 5.26A). In contrast, Gini index decreased significantly with salinity ($t_{22} = -2.590$. $P = 0.017$), indicating increasing community evenness (Fig. 5.26B).

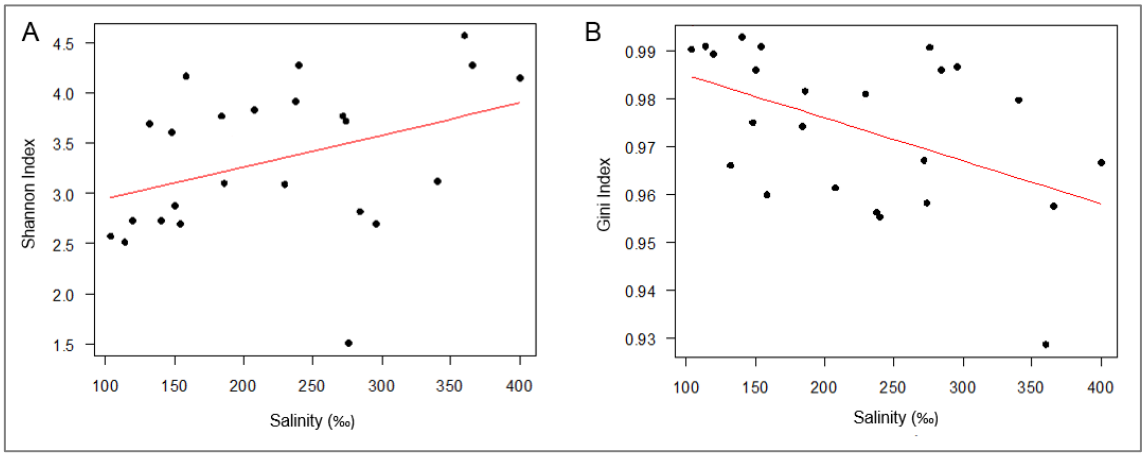

Fig. 5.26 GLMs showing Shannon diversity and Gini inequality indices variation in relation to the salinity.

Among the most abundant genera (*Pontimonas*, *Salinibacter*, *Psychroflexus*, *Celeribacter*, *Sediminimonas*, *Spiribacter, Halomonas, Roseovarius* and *Idiomarina*), *Salinibacter* increased significantly with salinity ($z = 4.516$. $P_{FDR} \leq 0.001$), whereas others decresed ($z \leq -3.309$, $P_{FDR} \leq 0.034$) (Fig. 5.27).

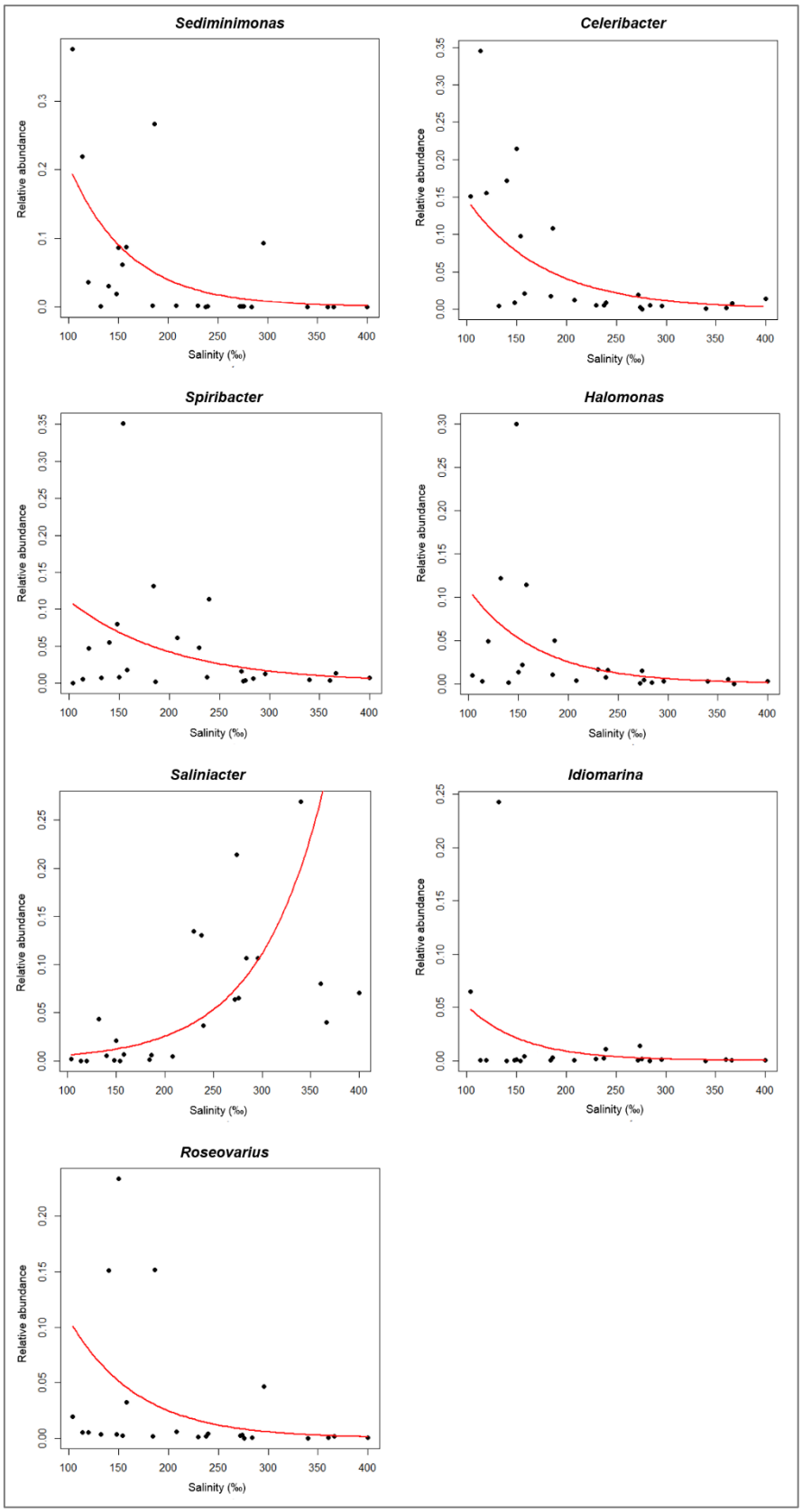

Fig. 5.27 GLM plots showing abundance variation of *Sediminimonas, Celeribacter, Spiribacter, Halomonas, Salinibacter, Idiomarina* and *Roseovarius* in relation to the salinity

5.3 Metagenetic profiling of the bacterial communities of the Kuril Kamchatka Trench

5.3.1 Composition of the bacterial communities

A total of 1,616,630 mapping sequences was obtained; Good's index calculated for all KKT samples ranged from 97.9 to 99.7, indicating a good coverage for the libraries. Overall, through the clustering sequence process 1515 and 4628 bacterial OTUs were defined for the bathyal (BS) and the abysso-hadal (AHS) samples, respectively.

Bathyal OTUs were assigned to 20 phyla, 38 classes, 74 orders, 159 families and 276 genera. Unassigned OTUs ranged from 0.9 to 3.1%, from 3.4 to 12.2%, from 7.8 to 28.6%, from 9.1 to 33.8% and from 11.4 to 43.1% at phylum, class, order, family and genus level, respectively.

Abysso-hadal OTUs were assigned to 27 phyla, 58 classes, 108 orders, 234 families and 453 genera. Unassigned OTUs ranged from 2.4 to 9.1%, from 5.6 to 14.1%, from 24.9 to 41.7%, from 33.9 to 50.7% and from 44.9 to 68.5% at phylum, class, order, family and genus level, respectively.

As showed by the Venn diagram (Fig. 5.28), among the phyla globally retrieved in KKT samples, 1 and 8 taxa were exclusively found in BS and AHS, respectively; however, they were rare taxa.

Among the 19 shared phyla, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Marinimicrobia*, *Firmicutes* and *Planctomycetes* represented major taxa in both BS and AHS, whereas *Verrucomicrobia* and *Acidobacteria* were detected with Ra ≥ 1 % in BS and AHS, respectively (Figs. 5.27-5.28).

Proteobacteria, *Bacteroidetes* and *Actinobacteria* were the most represented taxa over all the KKT samples, and except for *Actinobacteria* in KKT13, they were always recorded with Ra ≥ 1 %.

Proteobacteria notably dominated the KKT bacterial communities, followed by *Bacteroidetes* and *Actinobacteria*. In BS, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* ranged from 65.5 to 90.6%, from 2.4 to 10.7% and from 2.5 to 9.6%, respectively. In AHS, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* ranged from 56.1 to 74.5%, from 6.5 to 19.1% and from 0.9 to 16.1%, respectively. In general, in the

KKT samples, characterised by lesser abundances of *Proteobacteria*, slightly higher abundances of *Bacteroidetes* and *Actinobacteria* were recorded.

Firmicutes that was usually rather low in BS (Ras ~1-2%), except for KKT2 (Ra = 4.4%), showed Ra ≥ 2% in most of AHS and was ~10% in KKT26 and KKT9.

In BS, *Planctomycetes* and *Verrucomicrobia* in some cases were higher than *Firmicutes*.

Marinimicrobia was detected almost always as major taxon in BS (except in KKT8), but only in 2 samples (KKT14 and KKT26) in AHS.

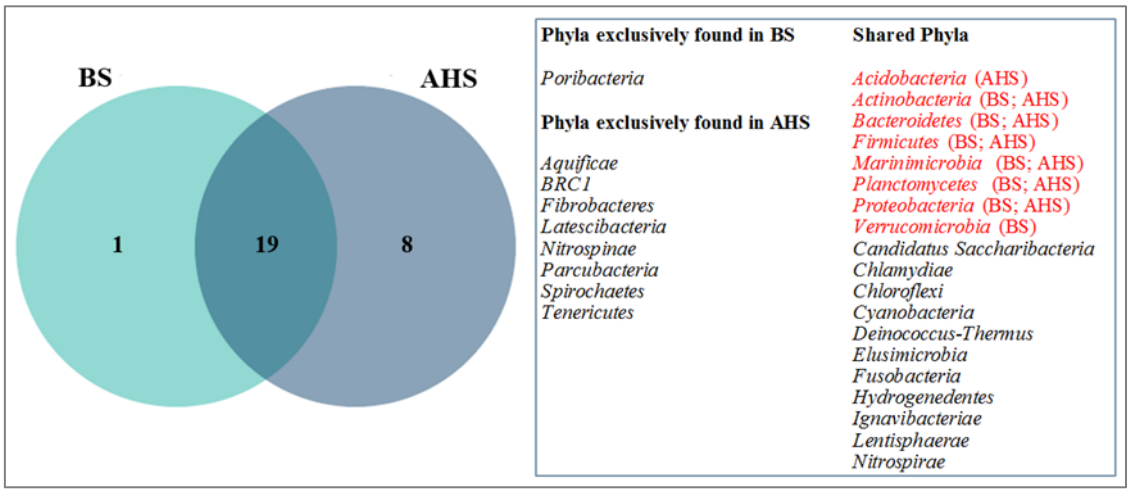

Fig. 5.28 Venn diagram showing the number of exclusive and shared phyla between bathyal (BS) and abysso-hadal (AHS) samples. Exclusive taxa of each sample group and those shared are also reported. The sample group where the major phyla (in red) were found with Ra ≥ 1% are reported in brackets.

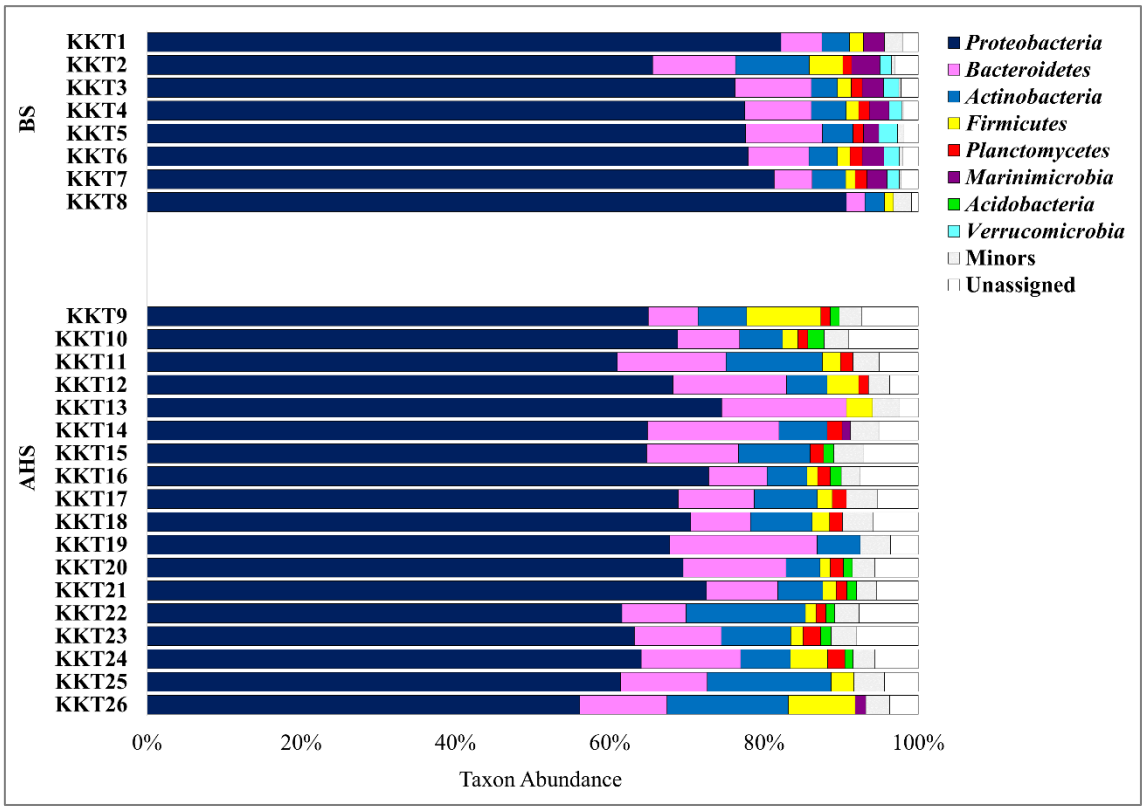

Fig. 5.29 Phylum-level bacterial composition of the KKT samples. Distribution of the major phyla (Ra > 1% in at least one sample); phyla with Ra < 1% were gathered in "Others"

BS and AHS revealed 15 and 26 major genera out of 276 and 453, respectively (Fig. 5.30).

Acinetobacter was the sole genus detected in all KKT samples with Ra ≥ 1 %. This taxon was higher in BS (Ra 21.5-62.5%, maximum in KKT8) than AHS (Ra 5.4-27.3%, maximum in KKT9).

Also *Colwellia* and *Pseudomonas* were detected in all KKT samples, but they showed Ra ≥ 1% in more than 50% and 70% of them, respectively. These two taxa were detected with abundances slightly higher in BS (*Colwellia*: 0.4-14%; *Pseudomonas*: 1.4-11.4%) than in AHS (*Colwellia*: 0.4-5.5%; *Pseudomonas*: 0.3-2.4%).

Candidatus Pelagibacter, *Aquihabitans* and *Marinimicrobia genera incertae sedis*, which were quite well represented in BS, were usually found as rare taxa in AHS.

They were always major genera in BS (*Candidatus Pelagibacter*: Ra 3.3-7.6%; *Aquihabitans*: Ra 1.3-4.2%; *Marinimicrobia genera incertae sedis*: Ra 1-3.6%), whereas in AHS they were detected with Ra ≥ 1% only in a couple of samples.

On the contrary, *Zhongshania* was quite well represented in AHS (Ra 0.1-11.7%; Ra ≥ 1% in 11 out of 18 samples), but it was always a rare taxon in BS (Ra ≤ 0.04%).

At the genus level, a high abundance of unassigned OTUs occurred, particularly in AHS bacterial communities. Unassigned OTUs ranged from 11.4 to 43.1% and from 44.9 to 68.5% in BS and AHS, respectively (Fig. 5.30).

In all KKT samples, the main proportion of unassigned OTUs was represented by those belonging to the phyla *Proteobacteria* and *Bacteroidetes* (Fig. 5.31). Unassigned OTUs of *Proteobacteria* accounted from 8.2 to 30.9% in BS and from 23.3 to 50% in AHS, whereas those of *Bacteroidetes* accounted from 1.6 to 7.3% in BS and from 4.4 to 12.6% in AHS.

In addition, in AHS also *Actinobacteria* had a high proportion of OTUs not assigned at the genus level (accounting from 0.4 to 14.3%).

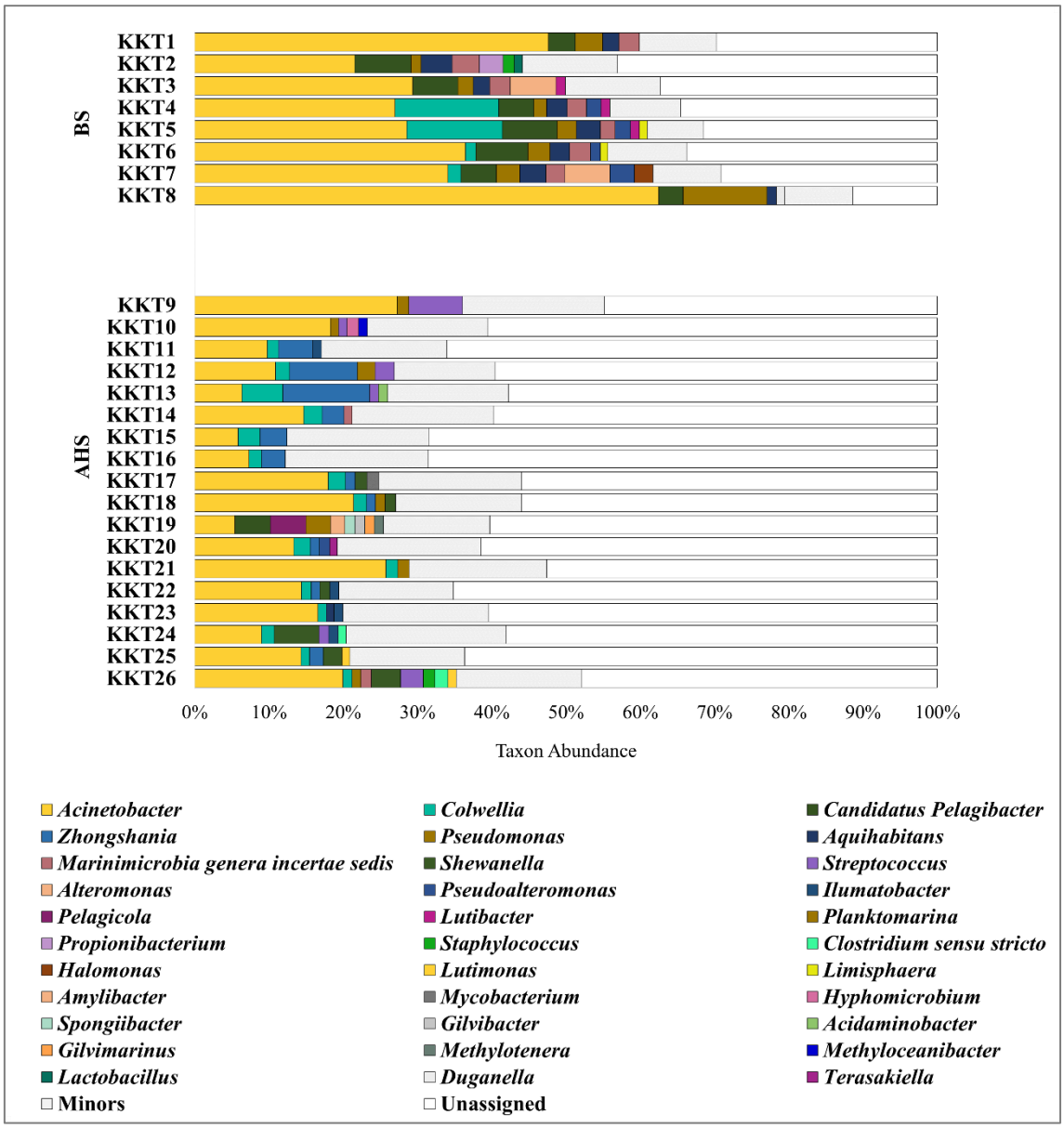

Fig. 5.30 Genus-level bacterial composition of the P5 samples. Distribution of the major genera (Ra > 1% in at least one sample); genera with Ra < 1% were gathered in "Others"

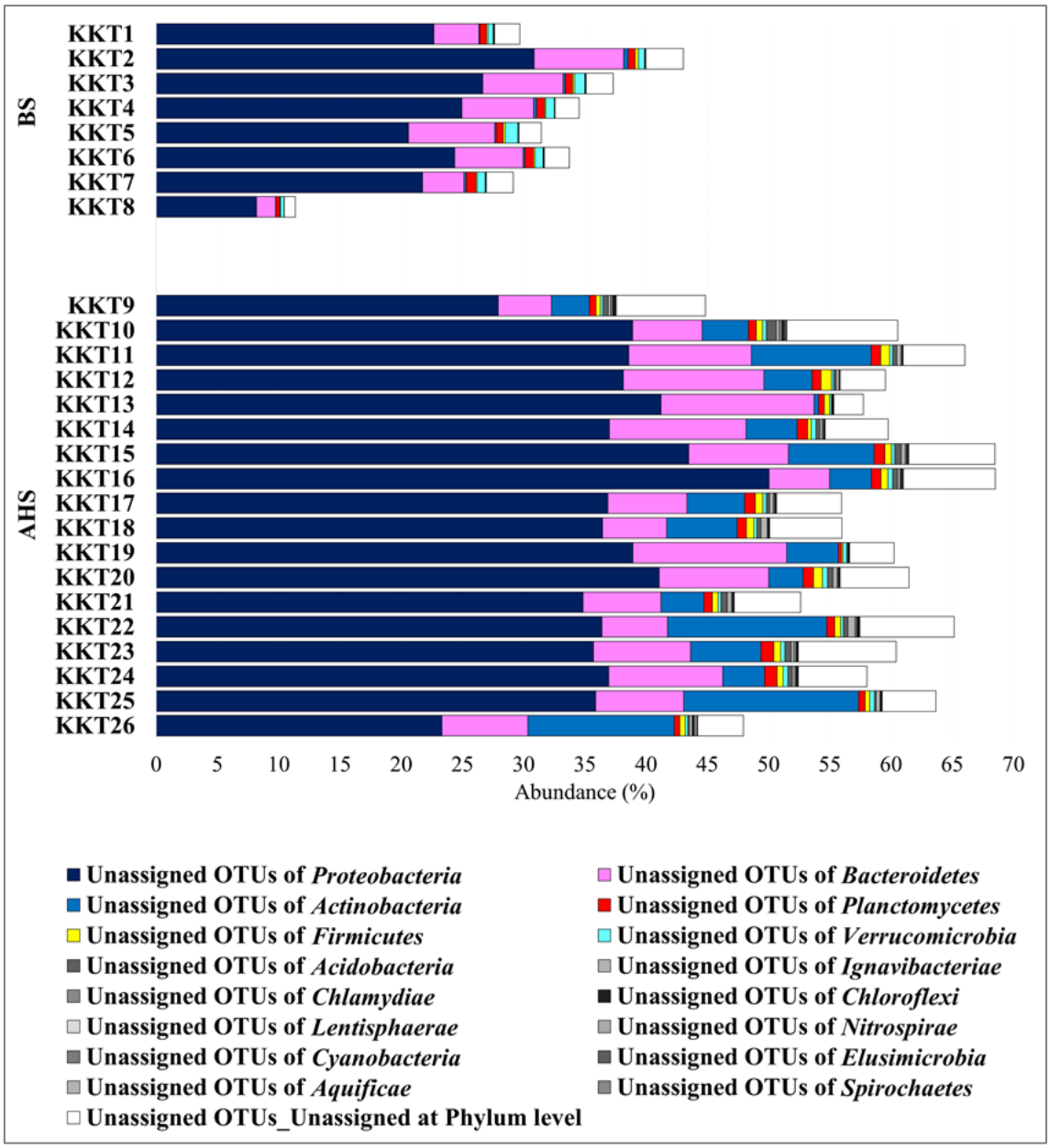

Fig. 5.31 Distribution of Unassigned OTUs at the genus level in KKT samples with respect to the phylum of their affiliation

5.3.2 Alpha- and Beta-Diversity of the bacterial communities

Table 5.5 reports α-diversity indices of the bacterial communities of KKT samples.

Overall, the Number of OTUs ranged from 716 (KKT16) to 2386 (KKT3). The Shannon diversity and the Gini inequality indices ranged from 2.686 (KKT16) to 5.838 (KKT3) and from 0.913 (KKT3) to 0.992 (KKT16), respectively.

KKT12, KKT13, KKT15 and KKT16 (AHS) and KKT1 (BS) were characterised by the lowest diversity (lowest values of Number of OTUs and Shannon Index) and the most dominated (highest Gini Indices). On the contrary, KKT3, KKT6 and KKT7 (BS) were characterised by the highest diversity and lowest evenness (showing the highest Number of OTUs, among the highest Shannon Index values and the lowest Gini Index values).

Table 5.5 Alpha-diversity indices of the KKT amplicon libraries

Sample	Number of OTUs	Shannon Index	Gini Index
KKT1	979	3.464	0.982
KKT2	1563	5.007	0.959
KKT3	2386	5.838	0.913
KKT4	2274	5.253	0.926
KKT5	1852	5.102	0.950
KKT6	2344	5.671	0.915
KKT7	2282	5.815	0.919
KKT8	1606	4.902	0.960
KKT9	1379	4.718	0.965
KKT10	1176	4.505	0.972
KKT11	1044	4.132	0.977
KKT12	946	3.958	0.982
KKT13	890	3.941	0.983
KKT14	1049	3.984	0.977
KKT15	979	3.925	0.981
KKT16	716	2.686	0.992
KKT17	1944	4.861	0.944
KKT18	1816	5.455	0.933
KKT19	1697	5.258	0.951
KKT20	1358	4.895	0.966
KKT21	1985	5.449	0.936
KKT22	2121	5.722	0.928
KKT23	1794	5.818	0.927
KKT24	2161	5.520	0.927
KKT25	2163	5.308	0.930
KKT26	1395	5.206	0.962

The PCA analysis, run to visualise KKT sample ordination, evidenced that BS and AHS clearly clustered in two distinct and well-separated groups (Fig. 5.32). Whereas all BS were very close together (showing very similar bacterial communities), some AHS samples were clearly parted (this was particularly evident for KKT13 and KKT19).

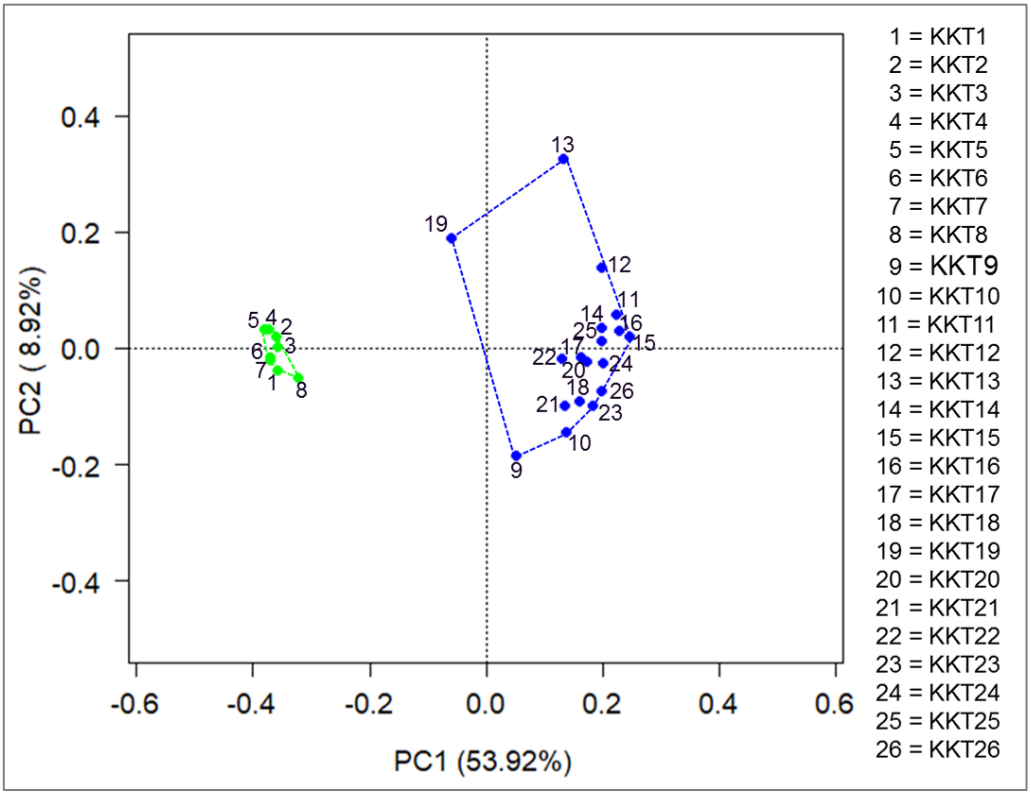

Fig. 5.32 PCA plot on Hellinger-transformed abundances of each OTU of KKT samples. Green and blue colours indicate bathyal and abysso-hadal samples, respectively.

The RDA analysis revealed that the structure of the KKT bacterial communities significantly differed ($F_{1,24}$=17.59, P=0.001, adjusted-R^2=0.122) according to the depth (Fig. 5.33). Globally, BS were negatively related to the depth, whereas opposite relation was observed for AHS.

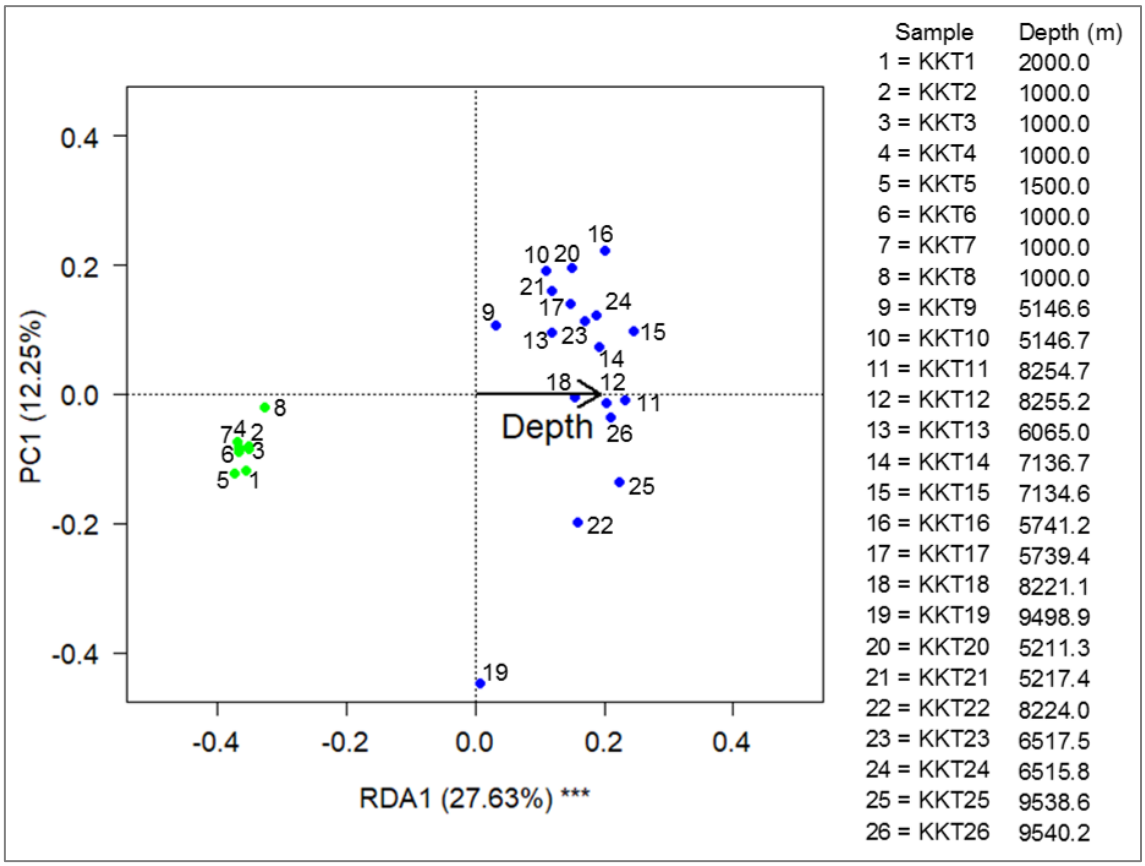

Fig. 5.33 Redundancy analysis (RDA) ordination biplot showing KKT samples according to depth. Bacterial OTU abundance were Hellinger-transformed. Arrow represent constraining variable. Variance explained by the first axis and axis significance (***: $P < 0.001$) is reported. For each sample, the depth was reported also.

Considering AHS only, the RDA showed how its community structure differed significantly ($z = 3.373$, $P = 0.001$, adjusted-$R^2 = 0.122$) according to the depth. In general, samples ordered along the vector direction according to their depths (Fig. 5.34).

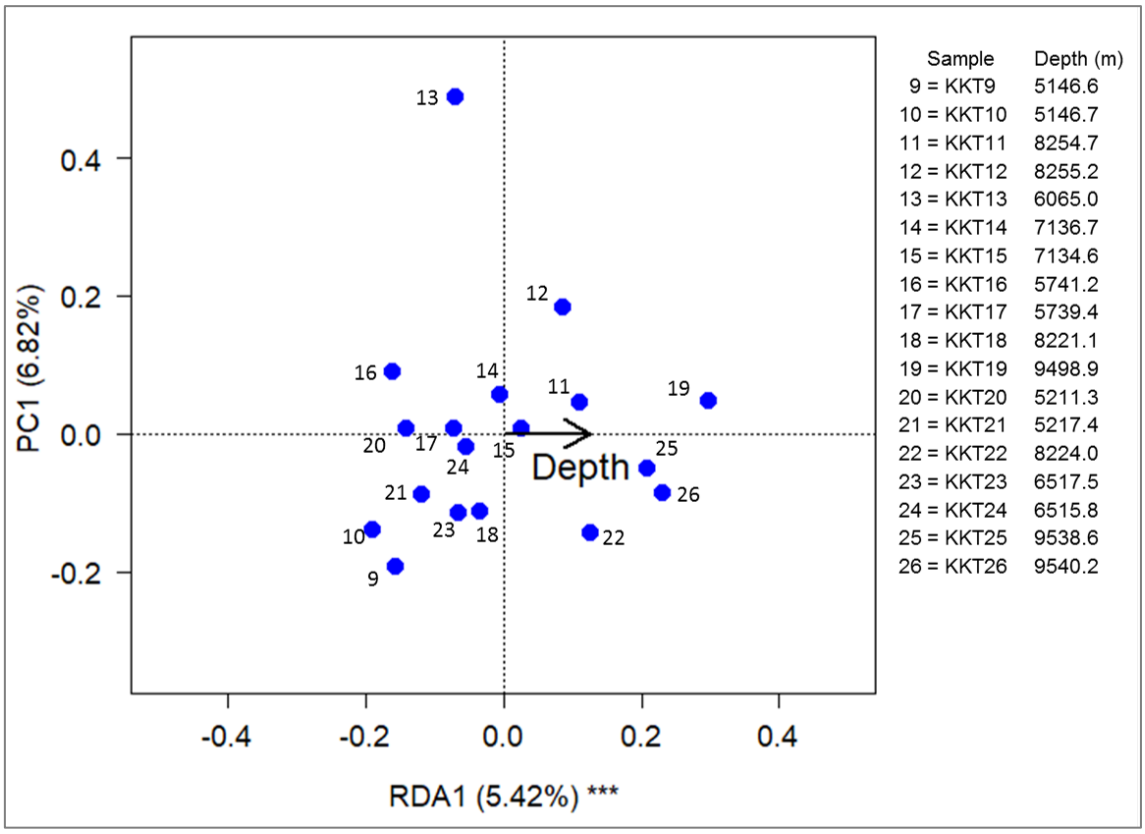

Fig. 5.34 Redundancy analysis (RDA) ordination biplot showing AHS samples according to depth. Bacterial OTU abundance were Hellinger-transformed. Arrow represent constraining variable. Variance explained by the first axis and axis significance (***: $P < 0.001$) is reported. For each sample, the depth was reported also.

5.3.3 Variation of α-diversity and genera abundance in relation to the sample group (BS and AHS) and the depth

The GLM analyses were run to investigate the significance of the variations recorded for α-diversity and genera abundance (only for most abundant taxa) in relation to the sample group and depth. As for depth, three different analyses were performed considering the whole depth range, the bathyal and the abysso-hadal zones separately.

The α-diversity indices did not show significant variations ($P_{FDR} > 0.05$), whereas some genera abundance changed significantly in relation to the considered parameters.

The abundance of all the considered taxa (*Acinetobacter*, *Colwellia*, *Pseudomonas*, *Candidatus Pelagibacter* and *Zhongshania*) changed significantly between BS and AHS (Fig. 5.35). Except for *Zhongshania* ($F_{1,24} = 6.258$, $P_{FDR} = 0.044$), that was higher in AHS than BS, all the other genera were more abundant in BS (*Acinetobacter*: $F_{1,24} = 23.018$, $P_{FDR} < 0.001$; *Colwellia*: $F_{1,24} = 8.953$, $P_{FDR} = 0.018$; *Pseudomonas*: $F_{1,24} = 18.487$, $P_{FDR} = 0.001$ and *Candidatus Pelagibacter*: $F_{1,24} = 41.196$, $P_{FDR} < 0.001$).

Considering the whole depth range, significant variation was recorded for *Acinetobacter* ($F_{1,16} = 12.680$, $P_{FDR} = 0.011$), *Pseudomonas* ($F_{1,16} = 12.127$, $P_{FDR} = 0.011$) and *Candidatus Pelagibacter* ($F_{1,16} = 9.932$, $P_{FDR} = 0.016$), which decreased at increasing depth (Fig. 5.36**)**. Instead, considering the bathyal and the abysso-hadal zones as separated areas, no significant genera abundance variation was evidenced in relation to the relative depth ranges.

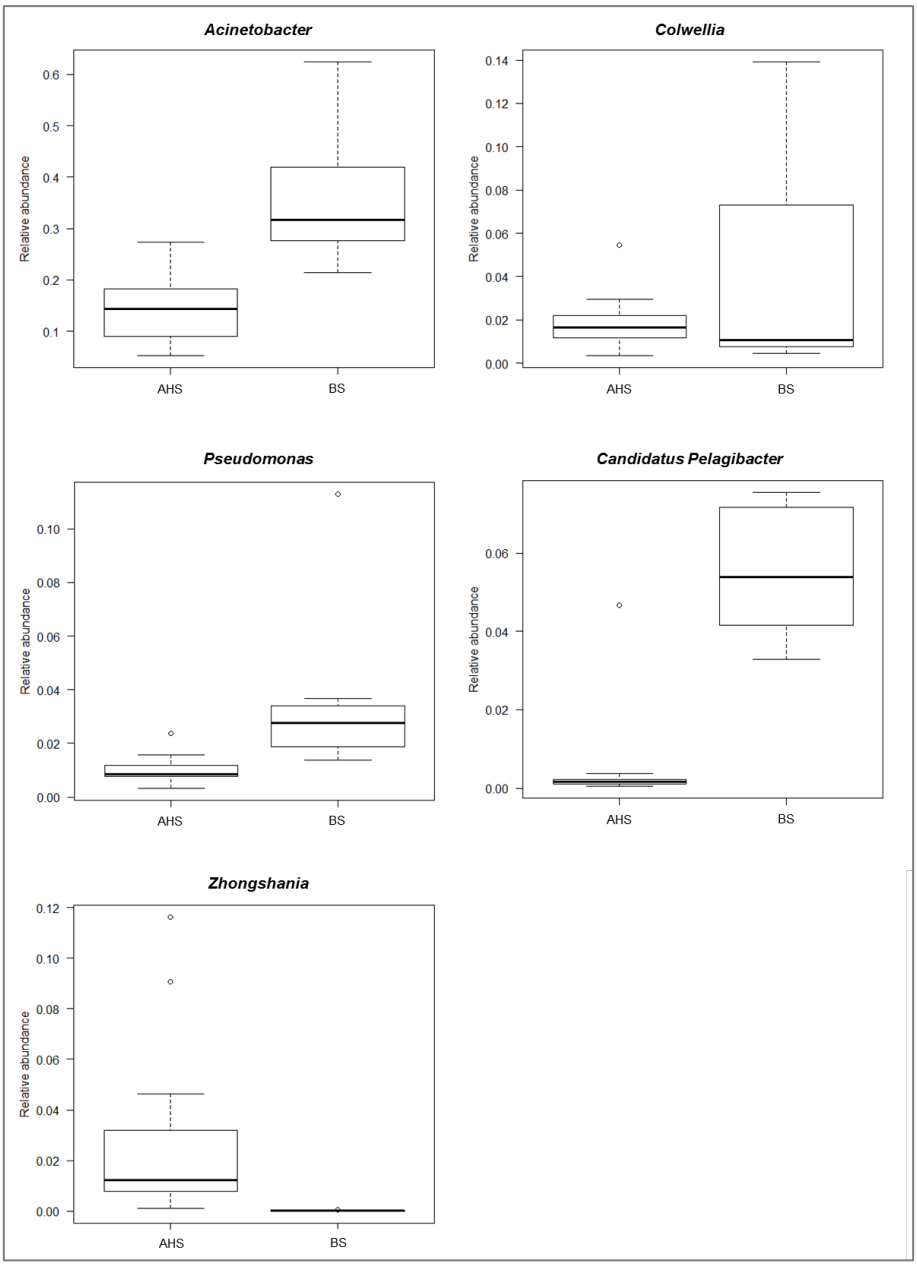

Fig. 5.35 Box-whisker plot (GLM analysis) showing abundance variation of *Acinetobacter, Colwellia, Pseudomonas, Candidatus Pelagibacter* and *Zhongshania* between the two sampling group (BS and AHS). Lower and upper whiskers indicate minimum and maximum abundance values, box limits indicate the 25th and 75th percentiles, center line represents the median and dots are the outliers.

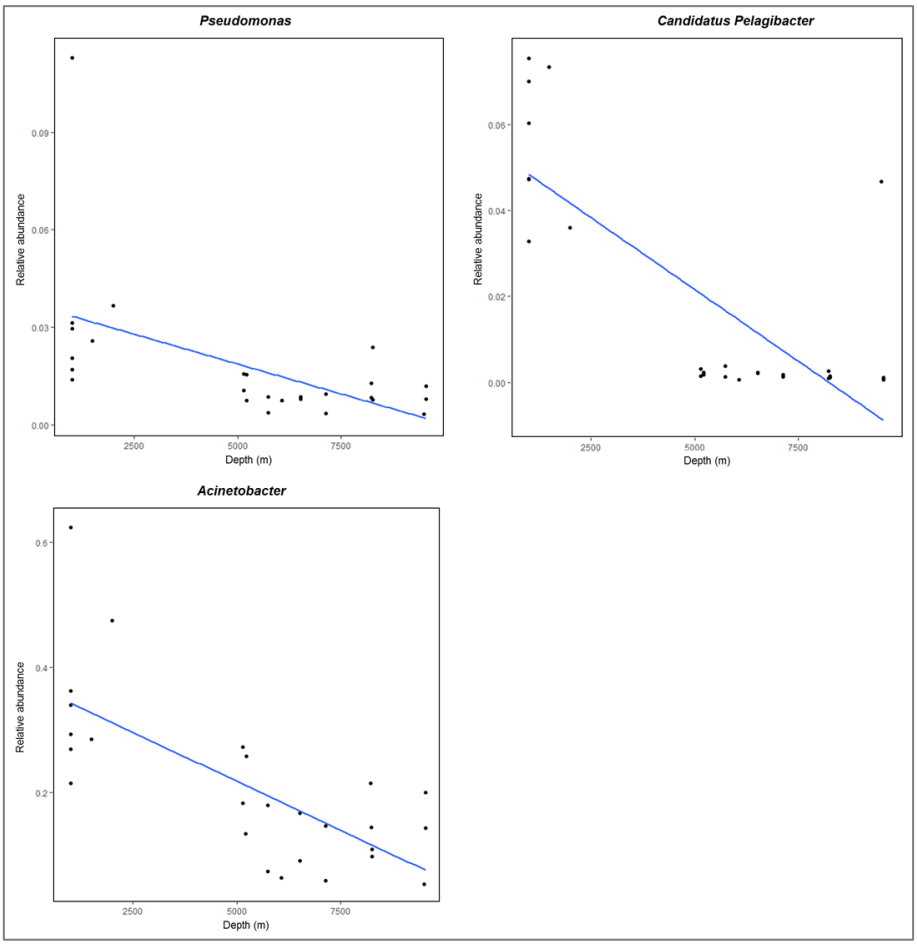

Fig. 5.36 GLM plots showing abundance variation of *Pseudomonas, Candidatus Pelagibacter* and *Acinetobacter* in relation to the depth (from 1000 to 9540.2 m)

Chapter 6

DISCUSSIONS

6.1 Metagenetic profiling of the bacterial communities of Kandalaksha Bay

The present study focused on producing a first high-resolution characterisation of the bacterial communities of some KB areas selected according to known flows of tidal currents. Although sampling did not involve the entire KB gulf and an extensive campaign, this work provides the first in-depth description of the bacterial communities in this region. Actually, the whole area has been overlooked from the microbiological point of view, and only a limited number of studies regarding the bacterial survey in KB gulf are available. Among them, some works dealt with phototrophs, others with invertebrate-associated bacteria (Gorlenko et al., 1985; Dul'tseva et al., 1996; Rabold et al., 2006; Gorelova et al., 2009) and the majority merely investigated the total number of bacterial cells in a given area (Savvichev et al., 2003, Savvichev et al., 2004, Savvichev et al., 2008; Kravchishina et al., 2008). However, no study on whole bacterial communities is available.

In 2008 Pesciaroli and co-workers started a deeper investigation of the bacterial microbiota of KB seawater, carried-out both using culture-dependent and culture-independent methods. A number of isolates were studied for their ability to grow at different temperatures and investigated under the taxonomic/phylogenetic and nutritional point of view (Pesciaroli et al., 2012, Pesciaroli et al., 2015a; Leyva-Díaz et al., 2017; Timperio et al., 2017). In addition, the total KB bacterial community was preliminary analysed by the PCR-TGGE fingerprinting method (Pesciaroli et al., 2015b), supplying only a partial information regarding the bacterial diversity. The NGS approach of the

present study allowed a deep survey and detailed profiling of the bacterial communities in this area, connoted by very interesting environmental features.

Since this work represents the first NGS investigation on the KB bacterial communities, the comparison of our results with those of others is rather difficult. In addition, no analogous data are available for the White Sea and the Barents Sea, which are closer to our area of study.

Comparable information is available only for other arctic or sub-arctic marine environments such as the Baltic Sea, Beaufort Sea, Chukchi Sea, and others. However, these Arctic areas are located quite far from KB and often show different environmental features (i.e. salinity, hydrodynamic regime and connection with the Arctic Ocean). In all these cold waters, evident preponderance of *Proteobacteria* (followed by *Bacteroidetes*) was detected (Kirchman et al., 2010; Koskinen et al., 2010; Comeau et al., 2011; Herlemann et al., 2011; Bowman et al., 2012; Ortega-Retuerta et al., 2013; Connelly et al., 2014; Dupont et al., 2014; Han et al., 2015; Pedrós-Alió et al., 2015; Rieck et al., 2015; Hu et al., 2016; Li et al., 2016; Wilson et al., 2017; Yergeau et al., 2017).

According to most of the cited works, both α- and γ-*Proteobacteria* are predominant, with particular relevance of the first class. Even if at the phylum level the composition of KB communities was coherent with the outcomes of the majority of the cited papers, at the class level an opposite ratio Alpha/Gamma *Proteobacteria* was recorded since γ-*Proteobacteria* accounted for the main fraction of KB *Proteobacteria*. However, similar results were obtained in other Arctic zones, located much more north than KB, by Kirchman et al. (2010) and Wilson et al. (2017) for the bacterial communities of coastal and epipelagic Arctic zones. It is difficult to identify a general trend regarding the distribution of the various *Proteobacteria* classes within the Arctic seas, since they

comprise heterogeneous environments. Nevertheless, dominance of γ-*Proteobacteria* in surface coastal waters and preponderance of α-*Proteobacteria* in offshore areas have been often demonstrated (Kirchman et al., 2010; Ortega-Retuerta et al., 2013; Connelly et al., 2014; Han et al., 2015; Li et al., 2016; Wilson et al., 2017).

In KB communities, among *Bacteroidetes*, the class *Flavobacteria* clearly represented the greatest proportion. In polar waters, *Bacteroidetes* (in particular *Flavobacteria*) were often found in correlation with the phytoplankton levels. *Flavobacteria* are abundant when light availability permits high phytoplankton growth (i.e. in epipelagic waters in summer) (Wilson et al., 2017). Our results showed a greater presence of this class in surface samples if compared to the deepest sample, but it is worth noting that similar amounts of *Flavobacteria* were found in samples S3 (surface water) and S4 (−15 m, where light penetration is very low or null). This was probably due to the mixing of waters from different layers caused by the aforementioned circular pattern of tidal currents.

Cyanobacteria were rather abundant in all samples except the deepest one (S6). Abundance of this phylum was particularly high in the surface samples (S3 and S5) collected in the main current stream. In S4 (−15 m), where light is almost absent (Bobrov et al., 1995; Kravchishina et al., 2013), the presence of this group with %Ra of ca. 2 could be due to a certain mixing with surface waters caused by the circular pattern of tidal currents.

The picocyanobacteria composition of the KB community appeared quite unusual for an Arctic region. Incidence of these photosynthetic prokaryotes, affected mainly by light, nutrients and temperature, generally decreases with latitude increase (Boeuf et al.,

2013; Celepli et al., 2017). *Prochlorococcus*, less ubiquitous than *Synechococcus* for its greater sensitivity to low temperatures, was usually found to be abundant at relatively low latitudes (between 40° S and 45° N) to sharply decline northward (Lee et al., 2014; Hess et al., 2016). Due to its temperature sensitivity, it is generally zoned in waters with temperatures above 15 °C; however, it has been found at 61°N in the north Atlantic (Buck et al., 1996) and it has also been recently detected in the Chukchi Sea (Lee et al., 2014) and the Gulf of Riga (Baltic Sea) (Tiirik et al., 2014). Nevertheless, it is often considered nearly absent in the polar oceans (Boeuf et al., 2013; Flombaum et al., 2013; Cadier et al., 2017). *Synechococcus* appears to be more widespread, showing a wider geographical distribution, ranging from tropical to polar waters (Hess et al., 2016; Cadier et al., 2017) including those from sub-Arctic and Artic regions (Michelou et al., 2007; Waleron et al., 2007; Zwirglmaier et al., 2008; Cottrell and Kirchman, 2009; Tremblay et al., 2009; Huang et al., 2012).

Although evidently in relation with depth and consequent light availability, presence of Prochlorococcus was observed in all KB samples; whereas *Synechococcus* was not detected at all.

This feature of KB waters, that has to be confirmed using other molecular targets (i.e. ITS) for better taxonomic resolution of the two genera, seems to be quite unusual for an estuarine Arctic system, since *Synechococcus* was often found in arctic rivers and lakes and freshwater runoff is thought to represent a source of these bacteria to the Arctic Ocean (Fuhrman et al., 2015; Paulsen et al., 2016).

The results of the present study did not reveal whether the distribution of *Cyanobacteria* in the KB waters can be related to the specific environment peculiarities or, as suggested for other Arctic environments (Lee et al., 2014), to possible global change effects that favoured the establishment of *Prochlorococcus* populations further north,

increasing its biogeographic distribution. In any case, this study supplies additional information about the presence of these taxa in cold waters and provides the bases for further investigations.

The taxonomic analysis at the genus level revealed that all samples appeared strongly dominated by few genera, accounting for ca. 50% of the community or more. Samples S3 and S6 were strongly dominated by a single genus, constituting a great proportion of the community (37% for *Psychrobacter* in S3) or even its main fraction (65% for *Halomonas* in S6). These outcomes are consistent with the α-diversity data; in fact, Shannon indices were low for all samples (and lowest for S6), indicating that KB bacterial communities were characterised by scarce diversity and equitability.

Another interesting issue evidenced by the present study is that tidal currents can somehow influence both distribution and composition of the KB bacterial communities. Overall, the results obtained from the taxonomical and α-diversity analyses together with those of the nMDS and RDA analyses seem to be explained by the strong water mixing caused by tidal currents, which affect in particular the surface and sub-surface layers (Pantyulin, 2003).

The nMDS and clustering analyses (Fig. 5.5) indicated that all surface samples, even those relatively far apart, showed rather similar communities. Such similarities could be explained by the strong mixing caused by the main flow of tidal currents, while the differences recorded might depend on the position of the various sampling points in relation to the main current stream. S1 and S2 showed the highest similarity; these samples were the closest to the shore and, being located slightly apart from the main current and somehow shielded by a small promontory, were probably less affected by the

tidal mixing. In addition, being in close relation with the intertidal zone, they might be more affected by inputs of microorganisms from this area.

Also S3, S4 and S5 were rather similar; the first two samples were collected from the same place but at different depths (surface and 15 m, respectively) and their similarity was probably due to the mixing caused by the circular pattern of the current recorded in that area (Fig. 4.1B). The similarity with S5, taken quite far away from S3 and S4 (ca. 2.5 km), could be attributed to the collection of the three samples within the main current stream (Fig. 4.1B). In addition, the diversity of S3 appeared to be influenced by the low salinity as shown by the RDA analysis (Fig. 5.5). Actually, the lowest salinity value (23.5‰) was recorded in S3. However, the differences in salinity among the various samples are too small (min 23.5‰ in S3, max 25.4‰ in S6) to justify possible differences in the communities. In general, deviations from the optimal conditions of few grams per litres of salinity do not cause any significant physiological stress. Thus, no substantial reduction of the microbial growth, and consequent replacement of some microorganisms with more adapted ones, would occur; it is known that environmental bacteria can cope with much wider variations of salinity (Barghini et al., 2018b). The variations of salinity recorded in the surface layers of the sampling area could be mainly ascribed to freshwater inputs, which are rather well mixed with seawater by the tidal currents. The circular pattern of the current in S3, attenuating the mixing action of the main tidal flow in this area, probably allows an increased persistence of freshwater and its microbial communities. Hence, all this could explain, from an ecological point of view, the results obtained for S3 by the RDA analysis.

S6 (−70 m) definitely showed the most dissimilar community (nMDS analysis), which was characterised by the lowest equitability (Shannon index), and strongly influenced by water temperature (RDA analysis), and consequently by depth (being water

temperature highly correlated with depth). The deepest cold layers represent a stable environment with strong dominance of adapted microorganisms, with scarce contacts with the surface.

It is worth noting that the temperature differences recorded between the surface and deepest layers are sufficient to select microorganisms having different adaptation abilities with regards to temperature. Sampling at KB was carried out at the end of the Arctic summer. In this period water temperature started to drop from the average summer values (ca. 15 °C) that could easily support the growth of mesophilic-psychrotolerant and/or psychrotolerant bacteria (Pesciaroli et al., 2012). On the bottom layers, temperature is constantly around 0 °C (Pantyulin, 2003), representing the optimal condition for psychrophilic bacteria, requiring stable low temperatures with very limited variations (Helmke and Weyland, 2004; Pesciaroli et al., 2012).

The effects of depth are related to various factors. First of all, with increasing depth, variation of water temperature and salinity occurs. Moreover, with increasing depth, increasing hydrostatic pressure and light diminution are recorded. However, the limited differences of depth recorded among our samples (just 70 m, 7 bar) would be insufficient to select communities with diverse adaptation to pressure (for example from piezosensitive to piezotolerant bacteria). It is a matter of general knowledge that the shift in communities with regards to pressure needs hundreds of bars. By contrast, intense light variations, that in this region occur within a few meters from the surface (Bobrov et al., 1995; Kravchishina et al., 2013), heavily affect the community composition due to the different occurrence of primary producers and consequent availability of nutrients.

6.2 Metagenetic profiling of the bacterial communities of the "Saline di Tarquinia" marine salterns

The current work was aimed to extend the knowledge on the ST environment, investigating the bacterial communities established along the salinity gradient, from the neighbouring sea (representing the saltern seawater source) and those harboured by BSB (representing a unique hypersaline ecosystem, having remained isolated from the saltpan system for decades).

Although some of the ST ponds and BSB have already been preliminarily studied by PCR-DGGE (Barghini et al., 2014, 2018a), the ST environment deserves more detailed investigations and this work represented the first in-depth characterisation (using a high-throughput metagenetic approach) of its bacterial communities. The value of the investigation is even broader since the study comprised a two-year survey.

This study evidenced that all the bacterial communities found along the ST salinity gradient were strongly dominated, as suggested by the high values (0.976-0.999) of Gini index (Table 5.2). The CCA analysis (Table 5.3, Fig. 5.19) showed that the community variation was related to the sampling site, salinity, sampling year and month (addressing a seasonality effect). Based on the combination of these factors, the communities were clearly ordered according to the salinity gradient. As expected, the highest differences were evidenced between the communities of the sea and P37.

The taxonomic analysis revealed that these bacterial communities were dominated by members of three phyla: *Proteobacteria*. *Bacteroidetes* and *Actinobacteria*. In general, they are retrieved as the most abundant bacterial taxa also in other solar salterns in the

world (Benlloch et al., 2002; Park et al., 2006; Tsiamis et al., 2008; Rodriguez-Brito et al., 2010; Ghai et al., 2011; Fernandez et al., 2014a,b,c; Gomariz et al., 2015; Kimbrel et al., 2018; Plominsky et al., 2018).

The development of bacterial community, which was established along the salinity gradient, from the neighbouring sea (representing the saltern seawater source) towards the crystallisation ponds, was evident already at high taxonomic level (Phylum) and was confirmed until the genus level.

Proteobacteria, that was the most abundant phylum in the sea, decreased in P5 where a codominance of *Proteobacteria* and *Actinobacteria* was observed. The increase of *Actinobacteria* in P5 was accompanied also by diminution of *Bacteroidetes*. This result was somehow surprising, being the salinities recorded in the sea and P5 quite similar; thus, a much similar community composition was expected.

In P24, *Proteobacteria* raised again in the first year, and even with more variations, its abundances were much similar with those of the sea. By contrast, in the second year, it was quite low and a high preponderance of *Actinobacteria* occurred. In P24, *Bacteroidetes* was always lower than in P5, but it increased in P37 (crystalliser pond), particularly in months with higher salinity.

In any case, in all sites both *Proteobacteria* and *Actinobacteria* were higher at low-intermediate salinities (26-180‰). Similar results were reported by other authors (Ghai et al., 2011; Fernandez et al., 2014b,c; Gomariz et al., 2015; Zhang et al., 2016; Kimbrel et al., 2018). However, a decrease of these phyla along the salinity gradient was often reported, and at salinities higher than 200‰ *Bacteroidetes* abundance highly increased (Baati et al. 2008; Pašić et al., 2009; Ghai et al., 2011; Boujelben et al., 2012; Fernandez et al., 2014a,b,c; Ventosa et al., 2014; Gomez-Villegas et al., 2018). Moreover, various studies evidenced that at the highest salinities (≥ 300‰), where diversity is low,

Euryarchaeota (Archaea) represented the greatest proportion of the community, but *Bacteroidetes*, was the most abundant phylum among Bacteria (often representing almost the entire bacterial community). (Benlloch et al., 2002; Pašić et al., 2009; Ghai et al., 2011; Fernandez et al., 2013; Zhaxybayeva et al., 2013; Fernandez et al., 2014b; Ventosa et al., 2014; Gómez-Villegas et al., 2018; Kimbrel et al., 2018; Ramos-Barbero et al., 2019; Couto-Rodríguez and Montalvo-Rodríguez, 2019). This was not confirmed by our work, since in ST, *Bacteroidetes* generally showed low abundances, excluding some peaks in months characterised by high salinities. However, it was lower than *Proteobacteria* and *Actinobacteria*, which dominated at high salinities also. Thus, our outcome was somehow incongruent with the majority of results from others, but most of them regarded the Santa Pola saltern (Spain), which was considered a study model for hypersaline environments.

Thus, our outcome appeared somehow incongruent with the majority of results from others; however, most of them regarded same hypersaline environment, the Santa Pola saltern (Spain). Actually, other authors reported quite different assemblages concerning the bacterial fraction of the prokaryotic communities of different solar salterns over the world, indicating possible bio-geographic patterns (Park et al., 2006; Tsiamis et al., 2008; Trigui et al., 2011; Dillon et al., 2013; Plominsky et al., 2014, 2018; Kambourova et al., 2017; Mora-Ruiz et al., 2018).

The taxonomic analysis at the genus level evidenced a relatively high number of major taxa in the sea, which showed in general low-medium abundances. Instead, along the salinity gradient, from the concentrator ponds to the crystalliser, the taxonomic pattern was simplified and an evident preponderance of some genera occurred.

An interesting outcome of this work regarded *Pontimonas* (Fig. 5.18); it was relatively rather scarce (< 20%) in the sea, but usually quite abundant in the ponds up to ca. 200‰ of salinity. In addition, also at the highest salinities of P37 it was always a major genus even if with much lower abundances (1.2-6.1%).

This genus includes only one species (*P. salivibrio*) and the only cultivable strain (CL-TW6) was isolated in Korea from a solar saltern pond showing salinity slightly higher than seawater. It was assumed to be a slightly halophilic marine bacterium (optimal growth at 30‰ of salinity) by the researchers that described the species (Jang et al., 2013; Cho et al.,2015), who supposed that it was drawn into the saltern from the coastal environment (Cho et al., 2018). However, molecular studies revealed presence of this bacterium also in continental hypersaline lakes at higher salinities (Selivanova et al., 2018; Shurigin et al., 2019).

In our case, the GLM analyses showed that *Pontimonas* abundance globally decreased with increasing salinities (Fig. 5.21); its strong presence was mainly observed in P5 and P24 (Figs. 5.15, 5.16, 5.20), whereas the lowest abundances were recorded in the sea. However, salinity ranges in the sea and P5 were very similar, thus the very high differences in *Pontimonas* abundance could be due to other variables (Figs. 5.14-5.15).

Due to its salinity preferences, this genus, even if with some exceptions, could be considered as slight and/or moderate halophile. In addition, the presence of this genus in P37 with abundances of 30-38% at high salinities (Fig. 5.17, Table 4.2), suggested also a possible behaviour as a borderline-extreme halophile. Since these results were obtained over two years, they strongly reinforce the hypotheses, which could be also substantiated by the presence in ST of bacterial strains with a marked euryhalinism, showing evident adaptation to broad salinity variations (Barghini et al., 2018b).

Also the presence of *Bordetella* in ST saltpan deserves to be discussed in details. *Bordetella* species were mainly isolated from warm-blooded animals (including humans) and, until recently, they were thought to be only animal pathogens (Sanden and Weyant, 2015; Hamidou Soumana et al., 2017). Nonetheless, recent studies revealed that *Bordetella* strains could also have an environmental origin (Badamchi and Papizadeh, 2017; Hamidou Soumana et al., 2017; Linz et al., 2019). It has been assumed that the *Bordetella* species related to animals evolved from ancestral environmental strains, and some animal-adapted species still maintain the ability to proliferate in the environment (Hamidou Soumana et al., 2017; Taylor-Mulneix et al., 2017). Among this genus, some members are known to be avian-associated microorganisms (Kersters et al., 1984; Raffel et al., 2002; Stenzel et al., 2017).

In our case, we have no definite information to explain the presence of this taxon, but since ST hosts a great avian biodiversity (Lanzuisi, 2009; Biondi, 2014), we can assume that the presence of *Bordetella* could have a primary avian origin with subsequent adaptation to the saltern environment, where it is quite diffused and not sporadic. In fact, this genus was constantly found in all samples; in the sea and P5 it represented often a rare taxon (Figs. 5.14-5.15), but in P24 and P37 it was frequently a major genus with some high abundances (Figs. 5.16-5.17; Table 4.2). Moreover, GLM analysis indicated also a main seasonal occurrence of this taxon, which increased in spring and decreased in autumn (Fig. 5.22).

The metagenetic survey confirmed the presence of *Spiribacter* and *Salinibacter* also in ST, like in other salterns worldwide (Pašić et al., 2009; Rodriguez-Brito et al. 2010; Ghai et al., 2011; Dillon et al., 2013; López-Pérez et al., 2013; León et al., 2014; Fernandez et al., 2014b,c; Zhang et al., 2016; Kambourova et al., 2017). However, they have a different distribution with respect to salinity: *Spiribacter* generally dominates in

the concentrator ponds with salinities of 100–250‰, while *Salinibacter* dominates in the crystallisers (Anton et al., 2002; Baati et al., 2008; Ghai et al., 2011; Fernandez et al., 2014b; León et al., 2014,2017). Presence of these genera at the mentioned specific conditions was confirmed also in our study, but they were not dominant. However, as already stated by other authors, changes in environmental and or climatic conditions may influence the saltern microbial communities; thus, in different geographic areas different microbial assemblages could be revealed (Pašić et al., 2007; Baati et al., 2008; Kambourova et al., 2017; Gómez-Villegas et al., 2018).

A particular attention within this work was given to BSB, which represents a unique ecosystem, having remained isolated from the saltpan from decades, when salt production was dismissed and the connection with the surrounding ponds was interrupted. Although no microbiological data are available, it is possible to presume that, during the saltern activity period, its bacterial diversity was somehow similar to that of the crystallisers, which served it. The strong inputs of microorganisms from the nearby ponds ceased many years ago and the halophilic community currently observed, although originated from the old inputs, is the result of selection events different than those occurred in the crystallisers. Nevertheless, minor external inputs of water, microorganisms and nutrients derive from precipitations, saline aerosol from the nearby sea (at ~500 m) and, above all, from both migratory and resident bird droppings (in some periods bird colonies in ST may include some hundreds individuals).

As observed for the bacterial communities found along the salinity gradient, also the BSB communities were highly dominated (Tab. 5.4), but in this environment the only parameter affecting both α- and β-diversity was the salinity (Figs 5.25-5.26). Salinity is a

key factor in ST hypersaline environment, but while for the gradient communities there were no significant variations of α-diversity according to this parameter, for the BSB a significant trend was evidenced. In fact, the GLM analysis showed that Gini index decreased significantly with salinity increase, suggesting a rise in the community evenness at increasing salinities. However, it is worth noting that, as mentioned before, all communities were highly dominated, thus the "evenness increase" actually should be considered as a slight decrease of dominance.

Also the BSB communities were dominated mainly by *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*. However, a different pattern was evidenced compared to the ST ponds. Considering that, P37 salinity very often had same values of those recorded in BSB (from 100‰ to saturation), and that in the past BSB was directly fed by P37, the comparison between these two hypersaline ecosystems is particularly worthwhile. Unlike P37, where *Proteobacteria* and *Actinobacteria* were the most predominant taxa, in BSB *Proteobacteria* and *Bacteroidetes* dominated (Fig. 5.9 and Fig. 5.23). Actually, *Proteobacteria* in both sites showed high abundances at all salinities, till oversaturation. However, among the BSB communities those less related to the salinity showed a strong dominance of *Proteobacteria*, whereas those more related to this driving factor were characterised by a strong dominance of *Bacteroidetes* (Figs 5.23, 5.25). As discussed above, *Bacteroidetes* is generally very high at near- and over-saturation salinities. This taxon was very high in BSB (mainly at salinities > 225‰) and definitely lower in P37; on the contrary, *Actinobacteria* was very high in P37 (mainly at salinities 40-110‰), but definitely lower in BSB. A possible explanation could be due to the different salinity conditions; BSB showed always medium-high salt concentration (favourable to *Bacteroidetes*, as discussed above), whereas in P37 the salinity had frequently much lower values (for 11 months salinity was in the range 35-84‰).

A rather unforeseen outcome was the almost regular presence of *Cyanobacteria* as major taxon at salinities ≥ 184‰ in BSB, while it was always a minor taxon at lower salinities. In marine salterns ponds, this phylum is generally abundant at medium-low NaCl concentrations, whereas at the highest salinities its presence is controversial (Benlloch et al. 2002; Ghai et al., 2011; Boujelben et al., 2012; Dillon et al., 2013; Fernandez et al., 2014a; Gomariz et al., 2015; Kambourova et al., 2017; Mora-Ruiz et al., 2018; Couto-Rodríguez and Montalvo-Rodríguez, 2019). However, also in P37 we found *Cyanobacteria* with quite high abundance at salinities higher than 180‰.

At the genus level, more differences in the pattern of taxa were observed between BSB and the ponds; in BSB, a greater diversity of major genera occurred.

The most abundant genera retrieved in BSB were *Pontimonas*, *Sediminimonas, Celeribacter, Spiribacter, Psychroflexus, Halomonas* and *Salinibacter*. Among them, *Salinibacter* was the only genus classified as extremely halophilic (Viver et al., 2018). Its high abundance in BSB samples at salinities > 200‰ and its trend shown by GLM analysis (Fig. 5.27) were consistent with this classification. For the other most abundant genera, the current study (Fig. 5.27) confirmed the slight or moderate halophilic behaviour generally attributed to these taxa (Wang et al., 2009; Jin et al., 2016; Gan et al., 2018; Jami et al., 2016; León et al., 2018).

The only "incongruence" concerned *Pontimonas* that, as already mentioned, it is considered a slightly halophilic marine bacterium. Instead, it was always detected in BSB (characterised by salinities ≥104‰) and its highest abundances were recorded at 154-284‰ salinity, confirming our previous observations suggesting it as a possible moderate or even borderline-extreme halophile.

6.3 Metagenetic profiling of the bacterial communities of the Kuril Kamchatka Trench

The current work was aimed to get an in-depth investigation of the bacterial communities of the Kuril Kamchatka Trench, which is mostly unknown under the microbiology point of view. Although this area has been investigated since 1949, the studies were principally focused to characterise its oceanographic, chemical and physical features and to investigate its productivity, plankton and benthic fauna distribution (Bogorov, 1972; Zenkevich, 1963; Fisher and Brandt, 2015). Studies carried out on the KKT microbial diversity are definitely scant and, as far as we know, only two works involved sampling in the KKT region or nearby areas (Jing et al., 2017; Li et al., 2018). They dealt with samples just collected by CTD at a maximum depth of 3000 m (bathyal waters), and in addition, the work of Jing et al. (2017) investigated the archaeal communities only.

As already mentioned, all KKT communities were characterised by low evenness (Tab. 5.5), having Gini Indices > 0.9; therefore, they showed presence of few dominant OTUs. Except for KKT1, all BS showed high diversity, as indicated by the high Shannon indices (≥ 5.0). Differently, among AHS diversity was much variable (Shannon index 2.686-5.818); all the deepest samples (at about 9500m) showed high diversity. The PCA and the RDA analyses showed that BS and AHS clearly clustered in two distinct groups, and they oriented according to the depth, confirming the expected driving effects of this parameter on the community structure.

All the KKT communities were mainly dominated by 3 phyla (*Proteobacteria*, *Bacteroidetes* and *Actinobacteria*), being *Proteobacteria* the predominant taxon. In addition, *Firmicutes* and *Planctomycetes* were quite abundant in all communities. Instead,

Marininicrobia and *Verrucomicrobia* were found as major phyla in BS, and usually as rare taxa in AHS. On the contrary, *Acidobacteria* was detected as major phyla in AHS only. The mentioned work of Li et al. (2018), mainly investigated surface waters with some deeper samples (1000 and 3000 m) in order to get a comparison of the prokaryotic communities subject to different oceanic currents. The results obtained by these authors from the deepest samples could be compared with those of our BS. Excluding the general notably dominance of *Proteobacteria*, the assemblages of the community described in the work, although similar under the composition point of view, were quite different regarding the relative abundance of *Marinimicrobia*, *Bacteroidetes* and *Verrucomicrobia*. In addition, they did not observe rather high abundance of *Firmicutes*.

At the phylum level the comparison could be also done with papers dealing with other oceanic trenches (i.e. Mariana Trench, Japan Trench, Puerto Trench, Yap Trench). Only some studies agreed with the general predominance of *Proteobacteria*, found in our work both the bathyal and in the abysso-hadal zones (i.e. Eloe et al., 2011; Nunoura et al., 2016; Peoples et al., 2018; Zhang et al., 2018; Liu et al., 2019; Wang et al., 2019), whereas others reported dominance of other taxa.

For example, in the work of Nunoura et al. (2015), carried out in the Challenger Deep in the Mariana Trench, *Proteobacteria* dominated in the bathyal zone (1000-2000 m) and in the hadal zone at 10000 m, while *Bacteroidetes* dominated in the abysso-hadal zone from 6000 to 9000 m.

In the same area, Nunoura et al. (2018) observed a very strong abundance of *Protobacteria* in hadal waters at 10257 m, whereas *Chloroflexi* and into a second extent *Bacteroidetes*, *Planctomycetes*, *Marinimicrobia* and *Gemmatimonadetes* were the most abundant taxa in the sediments. In a later work (Gao et al., 2019), characterising the Challenger Deep prokaryotic communities, in most of samples taken from the hadal

waters, *Marinimicrobia* was the most abundant bacterial phylum. Dominance of *Marinimicrobia* was found also in hadal waters and sediments of various deep sites (Nunoura et al., 2015, 2018; Liu et al., 2018; Cui et al., 2019; Huang and Wang, 2019). In our case, except for KKT14 (7136.7) and KKT26 (9540.2), this phylum was always a rare taxon in AHS.

On the whole, it is clearly evident that, already at the phylum level, the bacterial communities of the marine trenches differentiated both within the same areas and among different geographic zones.

At the genus level, a high number of unassigned OTUs were evidenced, mainly in AHS (44.9-68.5%). Although considering possible technical bias (i.e. the rather short amplicon length), this outcome would suggest that the majority of the KKT bacterial diversity remains still unknown, particularly in the deepest waters. However, among the assigned OTUs, *Acinetobacter* was the most represented genus in all KKT samples and the most abundant in BS. Although among the most abundant genera, *Colwellia*, *Pseudomonas*, *C. Pelagibacter* and *Zhongshania* showed generally much lesser abundance than *Acinetobacter*. All these taxa, except *Zhongshania*, were higher in BS than AHS (Fig. 5.35). In addition, *Acinetobacter*, *Pseudomonas* and in particular *Candidatus Pelagibacter* showed a significant decrease at increasing depth (Fig. 5.36).

Acinetobacter is a very cosmopolitan genus including various species with different ecological roles (from pathogeny to xenobiotic biodegradation), in a wide array of terrestrial and aquatic environments, including oceanic trenches (Takami et al., 1997; Maruyama et al., 2000; Horikoshi and Tsujii, 2012; Zhang et al., 2018). A special attention could be given to *Zhongshania*, which was usually a major genus in AHS and the most abundant taxa in KKT12 and KKT13. Psychrophilic and psychrotolerant

members of this genus were isolated from various marine environments (from Antarctic coastal waters to tidal sediments of the Yellow Sea), at maximum depth of 500 m (Jang et al., 2011; Li et al., 2011; Naysim et al., 2014; On et al., 2018). Our findings, as far as we know, represented the first detection of these bacteria in the deepest oceans.

Chapter 10

CONCLUSIONS

The current work was aimed to obtain an in-depth characterisation of the bacterial communities of some extreme marine environments, which were never studied before or that received very scarce scientific attention under the microbiological point of view:

- Kandalaksha Bay (Russia), an intertidal/estuarine Arctic zone

- The “Saline di Tarquinia” marine solar salterns (Italy), a hypersaline environment

- The Kuril Kamchatka Trench (Northwest Pacific Ocean), an oceanic trench.

A metagenetic approach was used in order to increase the knowledge on the bacterial life in these aquatic ecosystems, obtaining detailed information regarding their community structure and composition, and to establish a solid base for further investigations.

Our results showed how KB community assemblages were clearly influenced by water temperature and salinity, which are affected by the unique hydrodynamic regime, characterised by strong tidal currents (in particular in the surface layers), and intense inputs of freshwaters (i.e. runoff of freshwater and precipitations). The rather high similarity among the bacterial communities sampled at the water surface or sub-surface, could be attributed to the peculiar patterns of water mixing recorded in the area. By contrast, the much stable deep layers showed quite different communities, which, in particular, had a definitely lower bacterial diversity and were much more dominated.

As for the ST environment, the variation of the bacterial communities found along the salinity gradient was related to various factors (sampling site, sampling year, salinity, and sampling month). These communities showed an increasing simplification of the bacterial diversity along the gradient, particularly if compared to those of the nearby sea.

BSB, which once was filled with the waters taken from the crystallisers (i.e. P37), represents now a very different ecosystem, since it has been isolated for a long time from the saltpan system. In fact, the composition of its bacterial communities appeared quite different from those of the nearby P37 pond. Actually, it was clear that its communities underwent to a different evolutionary development. Differently form the saltern ponds, BSB community structure was influenced only by salinity. BSB, is a unique closed ecosystems, while the nearby ponds represent a very dynamic open environment with continue relations with the adjacent sea, which supplies new inputs of biodiversity and nutrients.

As for the KKT area, evident differences were recorded between the community of the bathyal samples and those of the abysso-hadal zone. The variations among the assemblages were obviously explained by the depth differences. The metagenetic survey allowed obtaining a rather well defined picture of the KKT bacterial diversity, but a very high number of Unassigned OTUs were recorded (mainly at the genus level), suggesting that the majority of the its bacterial diversity is still unknown. Thus, this deep oceanic trench would deserve to be further investigated, even more than the two other ecosystems studied in this work.

Although they were definitely different each other, some general remarks could be done observing common features among the environments investigated in this work.

On the whole, our results revealed that even under highly extreme conditions the bacterial diversity of the marine environment is quite abundant. This could be somehow surprising considering the very extreme chemical-physical parameters recorded in these ecosystems (in particular those related to some ST and KKT samples), which could be considered going beyond the boundaries of life. Although the great diversity recorded, all the bacterial communities showed poor evenness and, consequently, presence of few dominant taxa/OTUs. Moreover, the presence of the most abundant genera, characterising the bacterial communities of each environment, was recorded nearly in the entire range of the parameters that were found to be the community drivers determining the extremophilic features of the studied sites.

To increase the knowledge regarding these very interesting environments, as future perspectives, some further investigations could be planned. First of all, the bacterial assemblages of the communities could be analysed at the OTU level, in order to understand their specific contribute to the broad adaptation evidenced for the various abundant genera. In addition, to complete the information, regarding the microbial diversity of the studied ecosystems, the investigation could be extended to other microbial groups, such as Archaea and Fungi. Furthermore, it would be useful to perform metagenomic analyses for the most interesting samples.

In this context, some investigation are already in course.

ACKNOWLEDGEMENTS

My first thanks are given to the staff of the laboratories of Microbiology and Microbial Ecology of Fungi, Bacteria and Algae (Department of Ecology and Biology, University of Tuscia, Viterbo, Italy). A particular mention to Dr. Paolo Barghini and Dr. Marcella Pasqualetti.

I wish to thank Prof. Maria Belen Rodelas (Laboratory of Microbiology, Faculty of Pharmacy, University of Granada, Spain) and Her staff, for their precious help during my stay in Granada.

I wish to thank Prof. Andrea Franzetti (Laboratory of Microbiology, Department of Earth and Environmental Sciences, University of Milano – Bicocca, Italy) and His staff, for their great contribution to my knowledge in NGS library preparation and data sequence analyses.

I wish to thank Prof. Roberto Ambrosini (Department of Environmental Science and Policy, University of Milano, Italy) for his help in data analysis and in the comprehension of various bioinformatic and statistic tools.

All my appreciation to the Informatic System Service of the University of Tuscia for their help with hardware and software setup. Special thanks to Dr. Stefano Mastrostefano for His professional support and precious help.

My very special thanks to Prof. Massimiliano Fenice, for its professional and continuous help during the PhD course, allowing my personal and professional development within an exciting learning path. During my PhD course, He was always source of inspiration. He introduced me in the various aspects of microbiology and microbial ecology, which went well beyond the results reported in this work.

REFERENCES

Agogué, H., Joux, F., Obernosterer, I., & Lebaron, P. (2005). Resistance of marine bacterioneuston to solar radiation. Applied and environmental microbiology, 71(9), 5282-5289

Alfinito, S., Iberite, M., & Fumanti, B. (1990). The algal microflora of the salt works of Tarquinia (Italy). Hydrobiologia, 203(3), 137-146.

Alldredge, A. L., & Silver, M. W. (1988). Characteristics, dynamics and significance of marine snow. Progress in oceanography, 20(1), 41-82.

Aller, R. C., & Rude, P. D. (1988). Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. Geochimica et Cosmochimica Acta, 52(3), 751-765.

Andrade, L., González-López, J., Fenice, M., Martínez-Toledo, M.V., Pesciaroli, C., Maza- Márquez, P., Juárez-Jiménez, B., 2014. Application of Response Surface Methodology (RSM) for Culture Conditions and Biomass Production of Psychrophilic Microalgae Isolated from High Mountains Lake During the ice-free Season. International Journal of Environmental Research, 8 (3), 799-812.

Angeletti, D., Cimmaruta, R., & Nascetti, G. (2010). Genetic diversity of the killifish *Aphanius fasciatus* paralleling the environmental changes of Tarquinia salterns habitat. Genetica, 138(9-10), 1011-1021.

Angeletti, D., Cimmaruta, R., Sebbio, C., Bellisario, B., Carere, C., & Nascetti, G. (2017). Environmental heterogeneity promotes microgeographic genetic divergence in the Mediterranean killifish *Aphanius fasciatus* (Cyprinodontidae). Ethology Ecology & Evolution, 29(4), 367-386.

Antón, J., Rosselló-Mora, R., Rodríguez-Valera, F., & Amann, R. (2000). Extremely halophilic bacteria in crystallizer ponds from solar salterns. Applied and environmental microbiology, 66(7), 3052-3057.

Aquilanti, A. (2015). Studio della diversità batterica delle Saline di Tarquinia: approcci colturali e coltura-indipendenti. PhD Thesis, Tuscia University, Viterbo, Italy

Arrigo, K. R., & Thomas, D. N. (2004). Large scale importance of sea ice biology in the Southern Ocean. Antarctic Science, 16(04), 471-486.

Austin, B. (1988). Marine microbiology.: Cambridge University Pres, Cambridge

Baati, H., Guermazi, S., Amdouni, R., Gharsallah, N., Sghir, A., & Ammar, E. (2008). Prokaryotic diversity of a Tunisian multipond solar saltern. Extremophiles, 12(4), 505-518.

Badamchi, A., & Papizadeh, M. (2017). Comparative phylogeny of the genus *Bordetella* using sequence analysis of 16S rRNA and ompA genes. Journal of Medical Bacteriology, 6(3-4), 1-13.

Badamchi, A., & Papizadeh, M. (2017). Comparative phylogeny of the genus *Bordetella* using sequence analysis of 16S rRNA and ompA genes. Journal of Medical Bacteriology, 6(3-4), 1-13.

Bardou, P., Mariette, J., Escudié, F., Djemiel, C., & Klopp, C. (2014). jvenn: an interactive Venn diagram viewer. BMC bioinformatics, 15(1), 293.

Barghini, P., Giovannini, V., Fenice, M., Gorrasi, S., & Pasqualetti, M. (2018c). High Lutein production by a halo-tolerant strain of *Dunaliella* sp. (Chlorophyceae) isolated from solar salterns in central Italy. Journal of Environmental Protection and Ecology, 19(2), 704-712.

Barghini, P., Pasqualetti, M., Gorrasi, & Fenice, M. (2018a). Study of bacterial diversity of a saltern crystallisation pond (‘Saline di Tarquinia’, Italy) and its correlation with salinity variations. Journal of Environmental Protection and Ecology, 19(1), 139-145.

Barghini, P., Pasqualetti, M., Gorrasi, & Fenice, M. (2018b). Bacteria from the "Saline di Tarquinia" marine salterns reveal very atypical growth profiles with regards to salinity and temperature. Mediterranean Marine Science, 19(3), 513-525.

Barghini, P., Silvi, S., Aquilanti, A., Marcelli, M., & Fenice, M. (2014). Bacteria from marine salterns as a model of microorganisms adapted to high environmental variations. Journal of Environmental Protection and Ecology, 15(3), 897.

Belkin, I. M., & Cornillon, P. (2003). SST fronts of the Pacific coastal and marginal seas. Pacific Oceanography, 1(2), 90-113.

Bellisario, B., Carere, C., Cerfolli, F., Angeletti, D., Nascetti, G., & Cimmaruta, R. (2013). Infaunal macrobenthic community dynamics in a manipulated hyperhaline ecosystem: a long-term study. Aquatic biosystems, 9(1), 20.

Bellisario, B., Cerfolli, F., & Nascetti, G. (2010). Spatial network structure and robustness of detritus-based communities in a patchy environment. Ecological research, 25(4), 813-821.

Bellisario, B., Cerfolli, F., & Nascetti, G. (2012). The interplay between network structure and functioning of detritus-based communities in patchy aquatic environment. Aquatic Ecology, 46(4), 431-441.

Benjamini, Y., & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. The annals of statistics, 29(4), 1165-1188.

Benlloch, S., López-López, A., Casamayor, E. O., Øvreås, L., Goddard, V., Daae, F. L., Smerdon, G., Massana, R., Joint, I., Thingstad, F., Pedrós-Alió, C., & Rodríguez-Valera, F. (2002). Prokaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. Environmental Microbiology, 4(6), 349-360.

Berger, V. Y., & Gorbushin, A. M. (2001). Tolerance and Resistance in Gastropod Mollusks Hydrobia ulvaeand H. ventrosafrom the White Sea to Abiotic Environmental Factors. Russian Journal of Marine Biology, 27(5), 314-319.

Berger, V. Y., & Naumov, A. D. (2000). General features of the White Sea. Berichte Polarf, 359, 3-9.

Bergquist, D. C., Ward, T., Cordes, E. E., McNelis, T., Howlett, S., Kosoff, R., Hourdez, S., Carney, R., & Fisher, C. R. (2003). Community structure of vestimentiferan-generated habitat islands from Gulf of Mexico cold seeps. Journal of Experimental Marine Biology and Ecology, 289(2), 197-222.

Biondi M. (2014). Check-list degli uccelli della RNPA Saline di Tarquinia (VT) aggiornata al maggio 2013. In La Riserva Naturale Statale "Saline di Tarquinia". Corpo Forestale dello Stato, Ufficio territoriale per la Biodiversità di Roma. Colletti L. (Ed.).

Blanchet, F. G., Legendre, P., & Borcard, D. (2008). Forward selection of explanatory variables. Ecology, 89(9), 2623-2632.

Bobrov, Y. A., Maksimov, M. P., & Savinov, V. M. (1995). Primary production of phytoplankton. White Sea. Explorations of the faunas of the seas, 42, 50.

Boeuf, D., Humily, F., & Jeanthon, C. (2014). Diversity of Arctic pelagic Bacteria with an emphasis on photoheterotrophs: a review.

Bogorov, V.R., ed. (1972). Fauna of the Kurile–Kamchatka Trench and its Environment, Based on Data of the 38th Cruise of theR/V "Vityaz". IPST, Jerusalem.

Boujelben, I., Gomariz, M., Martínez-García, M., Santos, F., Peña, A., López, C., Antón, J., & Maalej, S. (2012). Spatial and seasonal prokaryotic community dynamics in ponds of increasing salinity of Sfax solar saltern in Tunisia. Antonie Van Leeuwenhoek, 101(4), 845-857.

Bowers, K. J., Mesbah, N. M., & Wiegel, J. (2009). Biodiversity of poly-extremophilic Bacteria: Does combining the extremes of high salt, alkaline pH and elevated temperature approach a physico-chemical boundary for life?. Saline systems, *5*(1), 9.

Bowman, J. S., Rasmussen, S., Blom, N., Deming, J. W., Rysgaard, S., & Sicheritz-Ponten, T. (2012). Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. The ISME journal, 6(1), 11.

Buck, K. R., Chavez, F. P., & Campbell, L. (1996). Basin-wide distributions of living carbon components and the inverted trophic pyramid of the central gyre of the North Atlantic Ocean, summer 1993. Aquatic Microbial Ecology, 10(3), 283-298.

Cadier, M., Gorgues, T., Sourisseau, M., Edwards, C. A., Aumont, O., Marié, L., & Mémery, L. (2017). Assessing spatial and temporal variability of phytoplankton communities' composition in the Iroise Sea ecosystem (Brittany, France): A 3D modeling approach. Part 1: Biophysical control over plankton functional types succession and distribution. Journal of Marine Systems, 165, 47-68.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Gonzalez Peña, A., Goodrich, J. K, Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E, Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., & Huttley, G. A. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature methods, 7(5), 335.

Cardoso, S. S., & Cartwright, J. H. (2017). On the differing growth mechanisms of black-smoker and Lost City-type hydrothermal vents. Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences, 473(2205), 20170387.

Celepli, N., Sundh, J., Ekman, M., Dupont, C. L., Yooseph, S., Bergman, B., & Ininbergs, K. (2017). Meta-omic analyses of Baltic Sea cyanobacteria: diversity, community structure and salt acclimation. Environmental microbiology, 19(2), 673-686.

Cerfolli, F., Bellisario, B., & Battisti, C. (2013). Detritus-based assemblage responses under salinity stress conditions in a disused aquatic artificial ecosystem. Aquatic biosystems, 9(1), 22

Chao, A., & Bunge, J. (2002). Estimating the number of species in a stochastic abundance model. Biometrics, 58(3), 531-539.

Chénard, C., & Lauro, F. M. (Eds.). (2017). Microbial Ecology of Extreme Environments. Springer.

Cho, B. C., Hardies, S. C., Jang, G. I., & Hwang, C. Y. (2018). Complete genome of streamlined marine actinobacterium *Pontimonas salivibrio* strain CL-TW6 T adapted to coastal planktonic lifestyle. BMC genomics, 19(1), 625.

Cho, B. C., Jang, G. I., & Hwang, C. Y. (2015). *Pontimonas*. Bergey's Manual of Systematics of Archaea and Bacteria, 1-5.

Cimmaruta, R., Blasi, S., Angeletti, D., & Nascetti, G. (2010). The recent history of the Tarquinia Salterns offers the opportunity to investigate parallel changes at the habitat and biodiversity levels. Transitional Waters Bulletin, 4(2), 53-59.

Cita, M. B. (2006). Exhumation of Messinian evaporites in the deep-sea and creation of deep anoxic brine-filled collapsed basins. Sedimentary Geology, 188, 357-378.

Claesson, M. J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J. R., Smidt, H., de Vos, W. M., Ross, P, & O'Toole, P. W. (2009). Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. PloS one, 4(8), e6669.

Clarke, K. R., & Gorley, R. N. (2005). PRIMER: Getting started with v6. PRIMER-E Ltd: Plymouth, UK.

Comeau, A. M., Li, W. K., Tremblay, J. É., Carmack, E. C., & Lovejoy, C. (2011). Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. PloS one, 6(11), e27492.

Connelly, T. L., Baer, S. E., Cooper, J. T., Bronk, D. A., & Wawrik, B. (2014). Urea uptake and carbon fixation by marine pelagic bacteria and archaea during the Arctic summer and winter seasons. Applied and environmental microbiology, 80(19), 6013-6022.

Cottrell, M. T., & Kirchman, D. L. (2000). Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Applied and environmental microbiology, 66(12), 5116-5122.

Couto-Rodríguez, R. L., & Montalvo-Rodríguez, R. (2019). Temporal Analysis of the Microbial Community from the Crystallizer Ponds in Cabo Rojo, Puerto Rico, Using Metagenomics. Genes, 10(6), 422.

Cui, G., Li, J., Gao, Z., & Wang, Y. (2019). Spatial variations of microbial communities in abyssal and hadal sediments across the Challenger Deep. PeerJ, 7, e6961.

Danovaro, R., Corinaldesi, C., Dell'Anno, A., & Rastelli, E. (2017). Potential impact of global climate change on benthic deep-sea microbes. FEMS microbiology letters, 364(23), fnx214.

De Filippis, F., La Storia, A., Villani, F., & Ercolini, D. (2013). Exploring the sources of bacterial spoilers in beefsteaks by culture-independent high-throughput sequencing. PLoS One, 8(7), e70222.

Dexter, C., Murray, G. L., Paulsen, I. T., & Peleg, A. Y. (2015). Community-acquired *Acinetobacter baumannii*: clinical characteristics, epidemiology and pathogenesis. Expert review of anti-infective therapy, 13(5), 567-573.

Dillon, J. G., Carlin, M., Gutierrez, A., Nguyen, V., & McLain, N. (2013). Patterns of microbial diversity along a salinity gradient in the Guerrero Negro solar saltern, Baja CA Sur, Mexico. Frontiers in microbiology, 4, 399.

Dolotov, Y. S., Filatov, N. N., Shevchenko, V. P., Nemova, N. N., Rimskii-Korsakov, N. A., Denisenko, N. V., Kutcheva, I. P., Boyarinov, P. M., Petrov, M. P., V. Lifshitz, Kh., Platonov, A. V., Demina, L. L., Kukharev, V. I., Kovalenko, V. N., Zdorovennov, R. E., Rat'kova, T. N., Sergeeva, O. M., Novigatskii, A. N., Pautova, L. A., Filipieva, K. V., Nothig, E.-M., & Loronzen, C. (2005). Monitoring tidal conditions in estuaries of the Karelian Coast of the White Sea. Water resources, 32(6), 611-628.

Donlan, R. M. (2002). Biofilms: microbial life on surfaces. Emerging infectious diseases, 8(9), 881.

Dubilier, N., Mülders, C., Ferdelman, T., de Beer, D., Pernthaler, A., Klein, M., Wagner, M., Erséus, C., Thiermann, F., Krieger, J., Giere, O. & Amann, R. (2001). Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. Nature, 411(6835), 298-302.

Dul'tseva, N. M., Dubinina, G. A., & Lysenko, A. M. (1996). Isolation of marine filamentous sulfur bacteria and description of the new species *Leucothrix thiophila* sp. nov. Microbiology (Mikrobiologiya), 65(1), 79-87

.

Duperron, S., Sibuet, M., MacGregor, B. J., Kuypers, M. M., Fisher, C. R., & Dubilier, N. (2007). Diversity, relative abundance and metabolic potential of bacterial endosymbionts in three Bathymodiolus mussel species from cold seeps in the Gulf of Mexico. Environmental Microbiology, 9(6), 1423-1438.

Dupont, C. L., Larsson, J., Yooseph, S., Ininbergs, K., Goll, J., Asplund-Samuelsson, J., McCrow, J. P., Celepli, N., Zeigler Allen, L., Ekman, M., Lucas, A. J., Hagström, Å., Thiagarajan, M., & Bergman, B. (2014). Functional tradeoffs underpin salinity-driven divergence in microbial community composition

Durden, J. M., Bett, B. J., Jones, D. O., Huvenne, V. A., & Ruhl, H. A. (2015). Abyssal hills–hidden source of increased habitat heterogeneity, benthic megafaunal biomass and diversity in the deep sea. Progress in Oceanography, 137, 209-218.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26(19), 2460-2461.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature methods, 10(10), 996.

Eloe, E. A., Fadrosh, D. W., Novotny, M., Allen, L. Z., Kim, M., Lombardo, M. J., Yee-Greenbaum, J., Yooseph, S., Allen, E. E., Lasken, R., Williamson, S. J., & Bartlett, D. H. (2011). Going deeper: metagenome of a hadopelagic microbial community. PLoS One, 6(5), e20388.

Ercolini, D., Ferrocino, I., Nasi, A., Ndagijimana, M., Vernocchi, P., La Storia, A., Laghi, L., Mauriello, G., Guerzoni, M. E., & Villani, F. (2011). Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. Applied and environmental microbiology, 77(20), 7372-7381.

Fernández González, A. B., León León, M. J., Vera Gargallo, B., Sánchez-Porro Álvarez, C., & Ventosa Ucero, A. (2014a). Metagenomic sequence of prokaryotic microbiota from an intermediate-salinity pond of a saltern in Isla Cristina, Spain. Genome Announcements, 2 (1), 1-2.

Fernandez, A. B., Ghai, R., Martin-Cuadrado, A. B., Sanchez-Porro, C., Rodriguez-Valera, F., & Ventosa, A. (2013). Metagenome sequencing of prokaryotic microbiota from two hypersaline ponds of a marine saltern in Santa Pola, Spain. Genome Announcements, 1(6), e00933-13.

Fernández, A. B., Ghai, R., Martin-Cuadrado, A. B., Sanchez-Porro, C., Rodriguez-Valera, F., & Ventosa, A. (2014b). Prokaryotic taxonomic and metabolic diversity of an intermediate salinity hypersaline habitat assessed by metagenomics. FEMS microbiology ecology, 88(3), 623-635

Fernández, A. B., Vera-Gargallo, B., Sánchez-Porro, C., Ghai, R., Papke, R. T., Rodriguez-Valera, F., & Ventosa, A. (2014c). Comparison of prokaryotic community structure from Mediterranean and Atlantic saltern concentrator ponds by a metagenomic approach. Frontiers in microbiology, 5, 196.

Fischer, V., & Brandt, A. (2015). Composition of the abyssal infauna of the Kuril–Kamchatka area (NW Pacific) collected with a box corer. Deep Sea Research Part II: Topical Studies in Oceanography, 111, 26-33.

Flombaum, P., Gallegos, J. L., Gordillo, R. A., Rincón, J., Zabala, L. L., Jiao, N., Karl, D. M., Li, W. K. W., Lomas, M. W., Veneziano, D., Vera, C. S., Vrugt, J. A., & Martiny, A. C. (2013). Present and future global distributions of the marine *Cyanobacteria Prochlorococcus* and *Synechococcus*. Proceedings of the National Academy of Sciences, 110(24), 9824-9829.

Frondoni, R., & Iberite, M. (2002). The halophile vegetation of the sedimentary coast of Lazio (central Tyrrhenian district, Italy). Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 136(1), 49-67.

Fuhrman, J. A., Cram, J. A., & Needham, D. M. (2015). Marine microbial community dynamics and their ecological interpretation. Nature Reviews Microbiology, 13(3), 133.

Gage, J. D., & Tyler, P. A. (1991). Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press.

Gan, L., Long, X., Zhang, H., Hou, Y., Tian, J., Zhang, Y., & Tian, Y. (2018). *Halomonas saliphila* sp. nov., a moderately halophilic bacterium isolated from a saline soil. International journal of systematic and evolutionary microbiology, 68(4), 1153-1159.

Gandolfi, I., Canedoli, C., Imperato, V., Tagliaferri, I., Gkorezis, P., Vangronsveld, J., Padoa Schioppa, E., Papacchini, M., Betsetti, G., & Franzetti, A. (2017). Diversity and hydrocarbon-degrading potential of epiphytic microbial communities on Platanus x acerifolia leaves in an urban area. Environmental pollution, 220, 650-658.

Gao, Z. M., Huang, J. M., Cui, G. J., Li, W. L., Li, J., Wei, Z. F., Chen, J., Xin, Y.-Z., Cai, D.-S., Zhang, A.-Q., & Wang, Y. (2019). In situ meta-omic insights into the community compositions and ecological roles of hadal microbes in the Mariana Trench. Environmental microbiology, 21(11), 4092-4108.

Gasol, J. M., Zweifel, U. L., Peters, F., Fuhrman, J. A., & Hagström, Å. (1999). Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Applied and Environmental Microbiology, 65(10), 4475-4483.

Ghai, R., Pašić, L., Fernández, A. B., Martin-Cuadrado, A. B., Mizuno, C. M., McMahon, K. D., Papke, R. T., Stepanauskas, R., Rodriguez-Brito, B., Rohwer, F., Sánchez-Porro, C., Ventosa, A., & Rodríguez-Valera, F. (2011). New abundant microbial groups in aquatic hypersaline environments. Scientific reports, 1, 135.

Gini, C. (1912) Variabilità e mutabilità. Contributo allo studio delle distribuzioni e delle relazioni statistiche. C. Cuppini, Bologna.

Giovannoni, S. J., Britschgi, T. B., Moyer, C. L., & Field, K. G. (1990). Genetic diversity in Sargasso Sea bacterioplankton. Nature 345: 60-63

Goffredi, S. K., Jones, W. J., Erhlich, H., Springer, A., & Vrijenhoek, R. C. (2008). Epibiotic bacteria associated with the recently discovered Yeti crab, *Kiwa hirsuta*. Environmental microbiology, 10(10), 2623-2634.

Gomariz, M., Martínez-García, M., Santos, F., Rodriguez, F., Capella-Gutiérrez, S., Gabaldon, T., Rosselló-Móra, R., Meseguer, I., & Antón, J. (2015). From community approaches to single-cell genomics: the discovery of ubiquitous hyperhalophilic *Bacteroidetes* generalists. The ISME journal, 9(1), 16.

Gómez-Villegas, P., Vigara, J., & León, R. (2018). Characterization of the Microbial Population Inhabiting a Solar Saltern Pond of the Odiel Marshlands (SW Spain). Marine drugs, 16(9), 332.

Good, I. J. (1953). The population frequencies of species and the estimation of population parameters. Biometrika, 40(3-4), 237-264.

Gorelova, O. A., Kosevich, I. A., Baulina, O. I., Fedorenko, T. A., Torshkhoeva, A. Z., & Lobakova, E. S. (2009). Associations between the White Sea invertebrates and oxygen-evolving phototrophic microorganisms. Moscow University biological sciences bulletin, 64(1), 16-22.

Gorlenko, V., Puchkova, N. N., & Demchev, V. V. (1985). Photosynthetic microorganisms of supralittoral bath of White Sea. In Dokl Biol Sci (Vol. 5, pp. 66-72).

Grossmann, S., & Dieckmann, G. S. (1994). Bacterial standing stock, activity, and carbon production during formation and growth of sea ice in the Weddell Sea, Antarctica. Applied and environmental microbiology, 60(8), 2746-2753.

Guan, Y., Ngugi, D. K., Vinu, M., Blom, J., Alam, I., Guillot, S., Ferry, J.G., & Stingl, U. (2019). Comparative genomics of the genus *Methanohalophilus*, including a newly isolated strain from Kebrit Deep in the Red Sea. Frontiers in microbiology, 10, 839.

Halse, S.A., Cale, D., Jasinska, E.J., Shiel, R.J., 2002. Monitoring change in aquatic invertebrate biodiversity: sample size, faunal elements and analytical methods. Aquatic Ecology, 36, 395–410.

Hamidou Soumana, I., Linz, B., & Harvill, E. T. (2017). Environmental origin of the genus *Bordetella*. Frontiers in microbiology, 8, 28.

Han, D., Ha, H. K., Hwang, C. Y., Lee, B. Y., Hur, H. G., & Lee, Y. K. (2015). Bacterial communities along stratified water columns at the Chukchi Borderland in the western Arctic Ocean. Deep Sea Research Part II: Topical Studies in Oceanography, 120, 52-60.

Heinen, W. (1974). Proceedings of the first European workshop on microbial adaptation to extreme environments. Biosystems, 6, 57-80.

Helmke, E., & Weyland, H. (2004). Psychrophilic versus psychrotolerant bacteria-occurrence and significance in polar and temperate marine habitats. Cellular and molecular biology, 50(5), 553-561.

Helmuth, B. S., & Hofmann, G. E. (2001). Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. The Biological Bulletin, 201(3), 374-384.

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. The ISME journal, 5(10), 1571.

Herring, P. (2002). The biology of the deep ocean. Oxford University Press.

Hess, W. R., Garczarek, L., Pfreundt, U., & Partensky, F. (2016). Phototrophic Microorganisms: The Basis of the Marine Food Web. In The Marine Microbiome (pp. 57-97). Springer, Cham.

Hiraoka, S., Hirai, M., Matsui, Y., Makabe, A., Minegishi, H., Tsuda, M., Juliarni, Rastelli, E., Danovaro, R., Corinaldesi, C., Kitahashi, T., Tasumi, E., Nishizawa, M., Takai, K., Nomaki, H., & Nunoura, T. (2019). Microbial community and geochemical analyses of trans-trench sediments for understanding the roles of hadal environments. The ISME Journal, 1-17.

Horikoshi, K., & Tsujii, K. (Eds.). (2012). Extremophiles in deep-sea environments. Springer Science & Business Media.

Howland, R. J. M., Pantiulin, A. N., Millward, G. E., & Prego, R. (1999). The hydrography of the Chupa Estuary, White Sea, Russia. Estuarine, coastal and shelf science, 48(1), 1-12.

Hu, Y. O., Karlson, B., Charvet, S., & Andersson, A. F. (2016). Diversity of pico-to mesoplankton along the 2000 km salinity gradient of the Baltic Sea. Frontiers in microbiology, 7, 679.

Huang, J. M., & Wang, Y. (2019). Genomic differences within the phylum *Marinimicrobia*: From waters to sediments in the Mariana Trench. Marine genomics, 100699.

Huang, S., Wilhelm, S. W., Harvey, H. R., Taylor, K., Jiao, N., & Chen, F. (2012). Novel lineages of *Prochlorococcus* and *Synechococcus* in the global oceans. The ISME journal, 6(2), 285.

Huber, J. A., Welch, D. B. M., Morrison, H. G., Huse, S. M., Neal, P. R., Butterfield, D. A., & Sogin, M. L. (2007). Microbial population structures in the deep marine biosphere. science, 318(5847), 97-100.

Iversen, N., & Jorgensen, B. B. (1985). Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark) 1. Limnology and Oceanography, 30(5), 944-955.

Jami, M., Lai, Q., Ghanbari, M., Moghadam, M. S., Kneifel, W., & Domig, K. J. (2016). *Celeribacter persicus* sp. nov., a polycyclic-aromatic-hydrocarbon-degrading bacterium isolated from mangrove soil. International journal of systematic and evolutionary microbiology, 66(4), 1875-1880.

Jamieson, A. (2015). The hadal zone: life in the deepest oceans. Cambridge University Press.

Jamieson, A. J., Fujii, T., Mayor, D. J., Solan, M., & Priede, I. G. (2010). Hadal trenches: the ecology of the deepest places on Earth. Trends in Ecology & Evolution, 25(3), 190-197.

Jang, G. I., Cho, Y., & Cho, B. C. (2013). *Pontimonas salivibrio* gen. nov., sp. nov., a new member of the family *Microbacteriaceae* isolated from a seawater reservoir of a solar saltern. International journal of systematic and evolutionary microbiology, 63(6), 2124-2131.

Jang, G. I., Hwang, C. Y., Choi, H. G., Kang, S. H., & Cho, B. C. (2011). Description of *Spongiibacter borealis* sp. nov., isolated from Arctic seawater, and reclassification of *Melitea salexigens* Urios et al. 2008 as a later heterotypic synonym of *Spongiibacter marinus* Graeber et al. 2008 with emended descriptions of the genus *Spongiibacter* and *Spongiibacter marinus*. International journal of systematic and evolutionary microbiology, 61(12), 2895-2900.

Jensen, P. R., & Fenical, W. (1994). Strategies for the discovery of secondary metabolites from marine bacteria: ecological perspectives. Annual Reviews in Microbiology, 48(1), 559-584.

Jiasheng, W., Wang, Y., & Li, Q. (2007). Potential contributions of extremophiles to hydrocarbon resources in marine extreme environments: A review. Frontiers of Earth Science in China, 1(4), 444-451.

Jin, M., Gai, Y., Guo, X., Hou, Y., & Zeng, R. (2019). Properties and applications of extremozymes from deep-sea extremophilic microorganisms: A mini review. Marine drugs, 17(12), 656.

Jin, S., Xia, J., Dunlap, C. A., Rooney, A. P., & Du, Z. J. (2016). *Psychroflexus saliphilus* sp. nov., isolated from a marine solar saltern. International journal of systematic and evolutionary microbiology, 66(12), 5124-5128.

Jing, H., Cheung, S., Xia, X., Suzuki, K., Nishioka, J., & Liu, H. (2017). Geographic Distribution of Ammonia-Oxidizing Archaea along the Kuril Islands in the Western Subarctic Pacific. Frontiers in microbiology, 8, 1247.

Kambourova, M., Tomova, I., Boyadzhieva, I., Radchenkova, N., & Vasileva-Tonkova, E. (2017). Phylogenetic analysis of the bacterial community in a crystallizer pond, Pomorie salterns, Bulgaria. Biotechnology & Biotechnological Equipment, 31(2), 325-332.

Karner, M., & Herndl, G. J. (1992). Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria. Marine Biology, 113(2), 341-347.

Kennish, M. J. (2019). Practical handbook of marine science. 4th edn. CRC press.

Kersters, K., Hinz, K. H., Hertle, A., Segers, P., Lievens, A., Siegmann, O., & De Ley, J. (1984). *Bordetella avium* sp. nov., isolated from the respiratory tracts of turkeys and other birds. International Journal of Systematic and Evolutionary Microbiology, 34(1), 56-70.

Kimbrel, J. A., Ballor, N., Wu, Y. W., David, M. M., Hazen, T. C., Simmons, B. A., Singer, S. W., & Jansson, J. K. (2018). Microbial community structure and functional potential along a hypersaline gradient. Frontiers in Microbiology, 9, 1492.

Kirchman, D. L. (2018). Processes in microbial ecology. Oxford University Press.

Kirchman, D. L., Cottrell, M. T., & Lovejoy, C. (2010). The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. Environmental Microbiology, 12(5), 1132-1143.

Kirschner, A. K. T., & Velimirov, B. (1999). Benthic bacterial secondary production measured via simultaneous 3H-thymidine and 14C-leucine incorporation, and its implication for the carbon cycle of a shallow macrophyte-dominated backwater system. Limnology and Oceanography, 44(8), 1871-1881.

Koskinen, K., Hultman, J., Paulin, L., Auvinen, P., & Kankaanpää, H. (2011). Spatially differing bacterial communities in water columns of the northern Baltic Sea. FEMS microbiology ecology, 75(1), 99-110.

Kravchishina, M. D., Burenkov, V. I., Kopelevich, O. V., Sheberstov, S. V., Vazyulya, S. V., & Lisitzin, A. P. (2013). New data on the spatial and temporal variability of the chlorophyll a concentration in the White Sea. In Doklady Earth Sciences (Vol. 448, No. 1, p. 120). Springer Science & Business Media.

Kravchishina, M. D., Mitzkevich, I. N., Veslopolova, E. F., Shevchenko, V. P., & Lisitzin, A. P. (2008). Relationship between the suspended particulate matter and microorganisms in the White Sea waters. Oceanology, 48(6), 837-854.

Lanzuisi E. (2009). Waterbirds of a Mediterranean wetland, Tarquinia Salinas. Regional and local environmental variables and management prescriptions. PhD Thesis, Tuscia University, Viterbo, Italy

Lee, S. H., Dahms, H. U., Kim, Y., Choy, E. J., Kang, S. H., & Kang, C. K. (2014). Spatial distribution of small phytoplankton composition in the Chukchi Sea. Polar biology, 37(1), 99-109.

Legendre, P., & Legendre, L. (2012). Numerical Ecology 3rd English edn Elsevier: Amsterdam. The Netherlands.

León, M. J., Aldeguer-Riquelme, B., Antón, J., Sánchez-Porro, C., & Ventosa, A. (2017). *Spiribacter aquaticus* sp. nov., a novel member of the genus *Spiribacter* isolated from a saltern. International journal of systematic and evolutionary microbiology, 67(8), 2947-2952.

León, M. J., Fernández, A. B., Ghai, R., Sánchez-Porro, C., Rodriguez-Valera, F., & Ventosa, A. (2014). From metagenomics to pure culture: isolation and characterization of the moderately halophilic bacterium *Spiribacter salinus* gen. nov., sp. nov. Applied and Environmental Microbiology, 80(13), 3850-3857.

León, M. J., Hoffmann, T., Sánchez-Porro, C., Heider, J., Ventosa, A., & Bremer, E. (2018). Compatible solute synthesis and import by the moderate halophile *Spiribacter salinus*: Physiology and genomics. Frontiers in Microbiology, 9, 108.

Leyva-Díaz, J. C., Poyatos, J. M., Barghini, P., Gorrasi, S., & Fenice, M. (2017). Kinetic modeling of *Shewanella baltica* KB30 growth on different substrates through respirometry. Microbial cell factories, 16(1), 189.

Li, H. J., Zhang, X. Y., Chen, C. X., Zhang, Y. J., Gao, Z. M., Yu, Y., Chen, X. L., Chen, B., & Zhang, Y. Z. (2011). *Zhongshania antarctica* gen. nov., sp. nov. and *Zhongshania guokunii* sp. nov., gammaproteobacteria respectively isolated from coastal attached (fast) ice and surface seawater of the Antarctic. International journal of systematic and evolutionary microbiology, 61(9), 2052-2057.

Li, Y., Jing, H., Xia, X., Cheung, S., Suzuki, K., & Liu, H. (2018). Metagenomic insights into the microbial community and nutrient cycling in the western subarctic Pacific Ocean. Frontiers in Microbiology, 9, 623.

Li, Y., Wang, Z., & Lin, X. (2016). Microbial community structure of Arctic seawater as revealed by pyrosequencing. Acta Oceanologica Sinica, 35(6), 78-84.

Linz, B., Ma, L., Rivera, I., & Harvill, E. T. (2019). Genotypic and phenotypic adaptation of pathogens: lesson from the genus: *Bordetella*. Current opinion in infectious diseases, 32(3), 223-230.

Liu, J., Zheng, Y., Lin, H., Wang, X., Li, M., Liu, Y., Yu, M., Zhao, M., Pedentchouk, N., Lea-Smith, D. J., Todd, J. D., Magill, C. R., Zhang, W.-J., Zhou, S., Song, D., Zhong, H., Xin, Y., Yu, M., Tian, J. & Zhang, X.-H. (2019). Proliferation of hydrocarbon-degrading microbes at the bottom of the Mariana Trench. Microbiome, 7(1), 47.

Liu, R., Wang, L., Liu, Q., Wang, Z., Li, Z., Fang, J., Zhang, L., & Luo, M. (2018). Depth-resolved distribution of particle-attached and free-living bacterial communities in the water column of the New Britain Trench. Frontiers in Microbiology, 9, 625.

López-Pérez, M., Ghai, R., Leon, M. J., Rodríguez-Olmos, Á., Copa-Patiño, J. L., Soliveri, J., Sanchez-Porro, C., Ventosa, A., & Rodriguez-Valera, F. (2013). Genomes of "*Spiribacter*", a streamlined, successful halophilic bacterium. BMC genomics, 14(1), 787.

MacElroy, R. D. (1974). Some comments on the evolution of extremophiles. Biosystems, 6(1), 74-75.

Maki, J. S. (2002). Neuston microbiology: life at the air–water interface. Encyclopedia of Environmental Microbiology.

Malyutina, M. V., Chernyshev, A. V., & Brandt, A. (2018). Introduction to the SokhoBio (Sea of Okhotsk Biodiversity Studies) expedition 2015. Deep Sea Research Part II: Topical Studies in Oceanography, 154, 1-9.

Mann, K. H., & Lazier, J. R. (2013). Dynamics of marine ecosystems: biological-physical interactions in the oceans. John Wiley & Sons.

Mapelli, F., Barozzi, A., Michoud, G., Merlino, G., Crotti, E., Borin, S., & Daffonchio, D. (2017). An updated view of the microbial diversity in deep hypersaline anoxic basins. In Adaption of Microbial Life to Environmental Extremes (pp. 23-40). Springer, Cham.

Martin, W., Baross, J., Kelley, D., & Russell, M. J. (2008). Hydrothermal vents and the origin of life. Nature Reviews Microbiology, 6(11), 805.

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(2), 835-846.

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R., & Hugenholtz, P. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME journal, 6(3), 610.

McIntyre, A. (2010) Life in the World's Oceans: Diversity, Distribution and Abundance. John Wiley & Sons Ltd, Chicester

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. PLoS computational biology, 10(4), e1003531.

Meier, D. V., Pjevac, P., Bach, W., Hourdez, S., Girguis, P. R., Vidoudez, C., Amann, R., & Meyerdierks, A. (2017). Niche partitioning of diverse sulfur-oxidizing bacteria at hydrothermal vents. The ISME journal, 11(7), 1545.

Melnikov, I. A., Korneeva, G. A., Zhitina, L. S., & Shanin, S. S. (2003). Dynamics of ecological–biochemical characteristics of sea ice in coastal waters of the White Sea. Biology Bulletin of the Russian Academy of Sciences, 30(2), 164-171.

Merino, N., Aronson, H. S., Bojanova, D. P., Feyhl-Buska, J., Wong, M. L., Zhang, S., & Giovannelli, D. (2019). Living at the Extremes: Extremophiles and the Limits of Life in a Planetary Context. Frontiers in microbiology, 10, 780.

Merlino, G., Barozzi, A., Michoud, G., Ngugi, D. K., & Daffonchio, D. (2018). Microbial ecology of deep-sea hypersaline anoxic basins. FEMS microbiology ecology, 94(7), fiy085.

Michelou, V. K., Cottrell, M. T., & Kirchman, D. L. (2007). Light-stimulated bacterial production and amino acid assimilation by *Cyanobacteria* and other microbes in the North Atlantic Ocean. Applied and Environmental Microbiology, 73(17), 5539-5546.

Mitarai, S., Watanabe, H., Nakajima, Y., Shchepetkin, A. F., & McWilliams, J. C. (2016). Quantifying dispersal from hydrothermal vent fields in the western Pacific Ocean. Proceedings of the National Academy of Sciences, 113(11), 2976-2981.

Mitra, A., & Zaman, S. (2016). Basics of marine and estuarine ecology. Springer.

Mitsuzawa, K., Holloway, G., (1998).Characteristics of deep currents along trenches in the northwest Pacific. J. Geophys. Res. Oceans 1978–2012(103), 13085–13092.

Moore, J. K., Doney, S. C., Glover, D. M., & Fung, I. Y. (2001). Iron cycling and nutrient-limitation patterns in surface waters of the World Ocean. Deep Sea Research Part II: Topical Studies in Oceanography, 49(1), 463-507.

Mora-Ruiz, M. D. R., Cifuentes, A., Font-Verdera, F., Pérez-Fernández, C., Farias, M. E., González, B., Orfila, A., & Rosselló-Móra, R. (2018). Biogeographical patterns of bacterial and archaeal communities from distant hypersaline environments. Systematic and applied microbiology, 41(2), 139-150.

Munn, C. B, (2011) Marine Microbiology: Ecology and Applications. 2nd edn. Garland Science, New York

Nagahama, T. (2006). Yeast biodiversity in freshwater, marine and deep-sea environments. In Biodiversity and ecophysiology of yeasts (pp. 241-262). Springer, Berlin, Heidelberg.

Nakanishi, M., & Hashimoto, J. (2011). A precise bathymetric map of the world's deepest seafloor, Challenger Deep in the Mariana Trench. Marine Geophysical Research, 32(4), 455-463.

Naysim, L. O., Kang, H. J., & Jeon, C. O. (2014). *Zhongshania aliphaticivorans* sp. nov., an aliphatic hydrocarbon-degrading bacterium isolated from marine sediment, and transfer of *Spongiibacter borealis* Jang et al. 2011 to the genus *Zhongshania* as *Zhongshania borealis* comb. nov. International journal of systematic and evolutionary microbiology, 64(11), 3768-3774.

Nunoura, T., Hirai, M., Yoshida-Takashima, Y., Nishizawa, M., Kawagucci, S., Yokokawa, T., Miyazaki, J., Koide, O., Makita, H., Takaki, Y., Sunamura M., &Takai, K. (2016). Distribution and niche separation of planktonic microbial communities in the water columns from the surface to the hadal waters of the Japan Trench under the eutrophic ocean. Frontiers in microbiology, 7, 1261.

Nunoura, T., Nishizawa, M., Hirai, M., Shimamura, S., Harnvoravongchai, P., Koide, O., Morono, Y., Fukui, T., Inagaki, F., Miyazaki, J., Takaki, Y., & Takai, K. & Takaki, Y. (2018). Microbial diversity in sediments from the bottom of the Challenger Deep, the Mariana Trench. Microbes and environments, ME17194.

Nunoura, T., Takaki, Y., Hirai, M., Shimamura, S., Makabe, A., Koide, O., Kikuchi, T., Miyazaki, J., Koba, K., Yoshida, N., Sunamura, M., & Takai, K. (2015). Hadal biosphere: insight into the microbial ecosystem in the deepest ocean on Earth. Proceedings of the National Academy of Sciences, 112(11), E1230-E1236.

On, Y. G., Oh, J. S., & Roh, D. H. (2018). *Zhongshania marina* sp. nov., isolated from deep-sea water. International journal of systematic and evolutionary microbiology, 69(2), 542-546.

Ortega-Retuerta, E., Joux, F., Jeffrey, W. H., & Ghiglione, J. F. (2013). Spatial variability of particle-attached and free-living bacterial diversity in surface waters from the Mackenzie River to the Beaufort Sea (Canadian Arctic). Biogeosciences, 10(4), 2747-2759.

Pantyulin, A. N. (2003). Hydrological system of the White Sea. Oceanology, 43(SUPP).

Park, S. J., Kang, C. H., & Rhee, S. K. (2006). Characterization of the microbial diversity in a Korean solar saltern by 16S rRNA gene analysis. Journal of microbiology and biotechnology, 16(10), 1640-1645.

Pašić, L., Rodriguez-Mueller, B., Martin-Cuadrado, A. B., Mira, A., Rohwer, F., & Rodriguez-Valera, F. (2009). Metagenomic islands of hyperhalophiles: the case of *Salinibacter ruber*. Bmc Genomics, 10(1), 570.

Pasqualetti, M., Bernini, R., Carletti, L., Crisante, F., & Tempesta, S. (2010). Salinity and nitrate concentration on the growth and carotenoids accumulation in a strain of *Dunaliella salina* (Chlorophyta) cultivated under laboratory conditions. Transitional Waters Bulletin, 4(2), 94-104.

Paulsen, M. L., Doré, H., Garczarek, L., Seuthe, L., Müller, O., Sandaa, R. A., Bratbak, G., & Larsen, A. (2016). *Synechococcus* in the Atlantic gateway to the Arctic Ocean. Frontiers in Marine Science, 3, 191.

Pedrós-Alió, C., Potvin, M., & Lovejoy, C. (2015). Diversity of planktonic microorganisms in the Arctic Ocean. Progress in oceanography, 139, 233-243.

Peoples, L. M., Donaldson, S., Osuntokun, O., Xia, Q., Nelson, A., Blanton, J., Allen, E. E., Church, M. J., & Bartlett, D. H. (2018). Vertically distinct microbial communities in the Mariana and Kermadec trenches. PloS one, 13(4), e0195102.

Pernice, M. C., Giner, C. R., Logares, R., Perera-Bel, J., Acinas, S. G., Duarte, C. M., Gasol., J. M., & Massana, R. (2016). Large variability of bathypelagic microbial eukaryotic communities across the world's oceans. The ISME journal, 10(4), 945.

Pesciaroli, C., Barghini, P., Cerfolli, F., Bellisario, B., & Fenice, M. (2015b). Relationship between phylogenetic and nutritional diversity in Arctic (Kandalaksha Bay) seawater planktonic bacteria. Annals of microbiology, 65(4), 2405-2414.

Pesciaroli, C., Cupini, F., Selbmann, L., Barghini, P., & Fenice, M. (2012). Temperature preferences of bacteria isolated from seawater collected in Kandalaksha Bay, White Sea, Russia. Polar biology, 35(3), 435-445.

Pesciaroli, C., Rodelas, B., Juarez-Jiménez, B., Barghini, P., & Fenice, M. (2015a). Bacterial community structure of a coastal area in Kandalaksha Bay, White Sea, Russia: possible relation to tidal hydrodynamics. Annals of microbiology, 65(1), 443-453.

Pittino, F., Maglio, M., Gandolfi, I., Azzoni, R. S., Diolaiuti, G., Ambrosini, R., & Franzetti, A. (2018). Bacterial communities of cryoconite holes of a temperate alpine glacier show both seasonal trends and year-to-year variability. Annals of Glaciology, 59(77), 1-9.

Plominsky, A. M., Delherbe, N., Ugalde, J. A., Allen, E. E., Blanchet, M., Ikeda, P., Santibañez, F., Hanselmann, K., Ulloa, O., De la Iglesia, R., von Dassow, P., Astorga, M., Gálvez, M. J., González, M. L., Henríquez-Castillo, C., Vaulot, D., Lopes do Santos, A., van den Engh, G., Gimpel, C., Bertoglio, F., Delgado, Y., Docmac, F., Elizondo-Patrone, C., Narváez, S., Sorroche, F., Rojas-Herrera, M. & Trefault N. (2014). Metagenome sequencing of the microbial community of a

solar saltern crystallizer pond at Cáhuil Lagoon, Chile. Genome Announc., 2(6), e01172-14.

Plominsky, M. A., Henríquez, C. A., Delherbe, N., Podell, S., Ramirez, S., Ugalde, J. A., Santibañez, J. F., van den Engh, G., Hanselmann, K., Ulloa, O., De la Iglesia, R., Allen, E. E., & Trefault, N. (2018). Distinctive archaeal composition of an artisanal crystallizer pond and functional insights into salt-saturated hypersaline environment adaptation. Frontiers in microbiology, 9, 1800.

Poli, A., Finore, I., Romano, I., Gioiello, A., Lama, L., & Nicolaus, B. (2017). Microbial diversity in extreme marine habitats and their biomolecules. Microorganisms, 5(2), 25.

Qiu, B. (2001). Kuroshio and Oyashio currents. Ocean Currents: A Derivative of the Encyclopedia of Ocean Sciences, 61-72.

Rabold, S., Gorlenko, V. M., & Imhoff, J. F. (2006). *Thiorhodococcus mannitoliphagus* sp. nov., a new purple sulfur bacterium from the White Sea. International journal of systematic and evolutionary microbiology, 56, 1945-1951.

Raffel, T. R., Register, K. B., Marks, S. A., & Temple, L. (2002). Prevalence of *Bordetella avium* infection in selected wild and domesticated birds in the eastern USA. Journal of wildlife diseases, 38(1), 40-46.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C., Levin, L., Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B.E., Smith, C.R., Tittensor, D.P., Tyler, P.A., Vanreusel A., & Vecchione, M. (2010). Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. Biogeosciences, 7(9), 2851-2899.

Ramos-Barbero, M. D., Martin-Cuadrado, A. B., Viver, T., Santos, F., Martinez-Garcia, M., & Antón, J. (2019). Recovering microbial genomes from metagenomes in hypersaline environments: The Good, the Bad and the Ugly. Systematic and applied microbiology, 42(1), 30-40.

Reboleiro-Rivas, P., Juarez-Jimenez, B., Martinez-Toledo, M.V., Rodelas, M., Andrade, L., Gonzalez-Lopez, J., Fenice, M., 2013. Bacterial communities structure in a high mountain lake during the ice-free season: cultural and PCR-TGGE investigations. International Journal of Environmental Research, 7, 685–696.Reeder, J., & Knight, R. (2010). Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. Nature methods, 7(9), 668.

Reysenbach, A. L., & Cady, S. L. (2001). Microbiology of ancient and modern hydrothermal systems. TRENDS in Microbiology, 9(2), 79-86.

Rieck, A., Herlemann, D. P., Jürgens, K., & Grossart, H. P. (2015). Particle-associated differ from free-living bacteria in surface waters of the Baltic Sea. Frontiers in microbiology, 6, 1297.

Rodriguez-Brito, B., Li, L., Wegley, L., Furlan, M., Angly, F., Breitbart, M., Buchanan, J., Desnues, C., Dinsdale, E., Edwards, R., Felts, B., Haynes, M., Liu, H., Lipson, D., Mahaffy, J., Martin-Cuadrado, A. B., Mira, A., Nulton, J., Pašić, L., Rayhawk, S., Rodriguez-Mueller, J., Rodriguez-Valera, F., Salamon, P., Srinagesh, S., Thingstad, T. F., Tran, T., Vega Thurber, R., Willner, D., Youle M., & Rohwer, F. (2010). Viral and microbial community dynamics in four aquatic environments. The ISME journal, 4(6), 739.

Rogers, A. D. (2015). Environmental change in the deep ocean. Annual Review of Environment and Resources, 40, 1-38.

Ross, D. A. (1995) An Introduction to Oceanography, Longman

Salgaonkar, B. B., Mani, K., & Bragança, J. M. (2013). Accumulation of polyhydroxyalkanoates by halophilic archaea isolated from traditional solar salterns of India. Extremophiles, 17(5), 787-795.

Sanden, G. N., & Weyant, R. S. (2015). *Bordetella*. Bergey's Manual of Systematics of Archaea and Bacteria, 1-20

Saralov, A. I. (2019). Adaptivity of archaeal and bacterial extremophiles. Microbiology, 88(4), 379-401.

Savvichev, A. S., Rusanov, I. I., Yusupov, S. K., Bairamov, I. T., Pimenov, N. V., Lein, A. Y., & Ivanov, M. V. (2003). The process of microbial sulfate reduction in sediments of the coastal zone and littoral of the Kandalaksha Bay of the White Sea. Microbiology, 72(4), 478-489.

Savvichev, A. S., Rusanov, I. I., Yusupov, S. K., Pimenov, N. V., Lein, A. Y., & Ivanov, M. V. (2004). The biogeochemical cycle of methane in the coastal zone and littoral of the Kandalaksha Bay of the White Sea. Microbiology, 73(4), 457-468.

Savvichev, A. S., Rusanov, I. I., Zakharova, E. E., Veslopolova, E. F., Mitskevich, I. N., Kravchishina, M. D., Lein, A. Y. & Ivanov, M. V. (2008). Microbial processes of the carbon and sulfur cycles in the White Sea. Microbiology, 77(6), 734-750.

Selivanova, E. A., Poshvina, D. V., Khlopko, Y. A., Gogoleva, N. E., & Plotnikov, A. O. (2018). Diversity of prokaryotes in planktonic communities of saline Sol-Iletsk lakes (Orenburg oblast, Russia). Microbiology, 87(4), 569-582.

Shannon, C. E. (1948). A mathematical theory of communication. Bell system technical journal, 27(3), 379-423.

Shaporenko, S. I., Koreneva, G. A., Pantyulin, A. N., & Pertsova, N. M. (2005). Characteristics of the ecosystems of water bodies separating from Kandalaksha Bay of the White Sea. Water Resources, 32(5), 469-483.

Shurigin, V., Hakobyan, A., Panosyan, H., Egamberdieva, D., Davranov, K., & Birkeland, N. K. (2019). A glimpse of the prokaryotic diversity of the Large Aral Sea reveals novel extremophilic bacterial and archaeal groups. MicrobiologyOpen, e850.

Sieburth, J. M. N. (1979). Sea microbes. Oxford University Press, New York

Singh, P., Jain, K., Desai, C., Tiwari, O., & Madamwar, D. (2019). Microbial Community Dynamics of Extremophiles/Extreme Environment. In Microbial Diversity in the Genomic Era (pp. 323-332). Academic Press.

Speight, M. R., & Henderson, P. A. (2013). Marine ecology: concepts and applications. John Wiley & Sons.

Stan-Lotter, H., & Fendrihan, S. (2012). Adaption of microbial life to environmental extremes. Vienna: Springer Vienna.

Steele, J. H., Turekian, K. K., & Thorpe, S. A. (2010). Encyclopedia of ocean sciences.

Stenzel, T., Pestka, D., Tykałowski, B., Śmiałek, M., Koncicki, A., & Bancerz-Kisiel, A. (2017). Detection of *Bordetella avium* by TaqMan real-time PCR in tracheal swabs from wildlife birds. Polish journal of veterinary sciences, 20(1), 31-36.

Sumich, J. L., & Morrissey, J. F. (2004) Introduction to the biology of marine life 8th edn. Jones and Bartlett Publishers, Inc, Sudbury

Takami, H., Inoue, A., Fuji, F., & Horikoshi, K. (1997). Microbial flora in the deepest sea mud of the Mariana Trench. FEMS Microbiology Letters, 152(2), 279-285.

Takami, H., Inoue, A., Fuji, F., & Horikoshi, K. (1997). Microbial flora in the deepest sea mud of the Mariana Trench. FEMS Microbiology Letters, 152(2), 279-285.

Talukder, A. R. (2012). Review of submarine cold seep plumbing systems: leakage to seepage and venting. Terra Nova, 24(4), 255-272.

Taylor-Mulneix, D. L., Hamidou Soumana, I., Linz, B., & Harvill, E. T. (2017). Evolution of *Bordetellae* from environmental microbes to human respiratory pathogens: Amoebae as a missing link. Frontiers in cellular and infection microbiology, 7, 510.

Team, R. C. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Tempesta, S., Paoletti, M., & Pasqualetti, M. (2010). Morphological and molecular identification of a strain of the unicellular green alga *Dunaliella* sp. isolated from Tarquinia Salterns. Transitional waters bulletin, 4(2), 60-70.

Ter Braak, C. J. F., & Smilauer, P. (2012). Canoco 5, Windows release (5.00). Software for mutivariate data exploration, testing, and summarization. Biometris, Plant Research International, Wageningen.

Thurber, A. R., Sweetman, A. K., Narayanaswamy, B. E., Jones, D. O., Ingels, J., & Hansman, R. L. (2014). Ecosystem function and services provided by the deep sea. Biogeosciences, 11(14), 3941-3963.

Tiirik, K., Nõlvak, H., Oopkaup, K., Truu, M., Preem, J. K., Heinaru, A., & Truu, J. (2014). Characterization of the bacterioplankton community and its antibiotic resistance genes in the Baltic Sea. Biotechnology and applied biochemistry, 61(1), 23-32.

Timperio, A. M., Gorrasi, S., Zolla, L., & Fenice, M. (2017). Evaluation of MALDI-TOF mass spectrometry and MALDI BioTyper in comparison to 16S rDNA sequencing for the identification of bacteria isolated from Arctic sea water. PloS one, 12(7), e0181860.

Tremblay, G., Belzile, C., Gosselin, M., Poulin, M., Roy, S., & Tremblay, J. É. (2009). Late summer phytoplankton distribution along a 3500 km transect in Canadian Arctic waters: strong numerical dominance by picoeukaryotes. Aquatic Microbial Ecology, 54(1), 55-70.

Trigui, H., Masmoudi, S., Brochier-Armanet, C., Barani, A., Grégori, G., Denis, M., Dukan, S., & Maalej, S. (2011). Characterization of heterotrophic prokaryote subgroups in the Sfax coastal solar salterns by combining flow cytometry cell sorting and phylogenetic analysis. Extremophiles, 15(3), 347-358.

Tsiamis, G., Katsaveli, K., Ntougias, S., Kyrpides, N., Andersen, G., Piceno, Y., & Bourtzis, K. (2008). Prokaryotic community profiles at different operational stages of a Greek solar saltern. Research in Microbiology, 159(9-10), 609-627.

Tsili, M., & Zacharopoulos, D. (2017). Does Temperature Effects Propagation and Grow of Cracks in a Submarine Volcano That is under a Hot-spot?. American Journal of Mechanical Engineering, 5(2), 58-63.

Tyler, P. A. (Ed.). (2003). Ecosystems of the deep oceans (Vol. 28). Elsevier

Tzetlin, A. B., Mokievsky, V. O., Melnikov, A. N., Saphonov, M. V., Simdyanov, T. G., & Ivanov, I. E. (1997). Fauna associated with detached kelp in different types of subtidal habitats of the White Sea. Hydrobiologia, 355(1-3), 91-100.

Valentine, D. L. (2002). Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. Antonie van Leeuwenhoek, 81(1-4), 271-282.

Van Audenhaege, L., Fariñas-Bermejo, A., Schultz, T., & Van Dover, C. L. (2019). An environmental baseline for food webs at deep-sea hydrothermal vents in Manus Basin (Papua New Guinea). Deep Sea Research Part I: Oceanographic Research Papers.

Van den Hove, S., & Moreau, V. (2007). Deep-sea Biodiversity and Ecosystems: A Scoping Report on Their Socio-economy, Management and Governanace (No. 184). UNEP/Earthprint.

van der Wielen PWJJ, Bolhuis H, Borin S, et al. (2005) The enigma of prokaryotic life in deep hypersaline anoxic basins. Science 307: 121–123.

Ventosa, A., Fernández, A. B., León, M. J., Sánchez-Porro, C., & Rodriguez-Valera, F. (2014). The Santa Pola saltern as a model for studying the microbiota of hypersaline environments. Extremophiles, 18(5), 811-824.

Vershinin, A., Moruchkov, A., Morton, S. L., Leighfield, T. A., Quilliam, M. A., & Ramsdell, J. S. (2006). Phytoplankton composition of the Kandalaksha Gulf, Russian White Sea: Dinophysis and lipophilic toxins in the blue mussel (Mytilus edulis). Harmful Algae, 5(5), 558-564.

Viver, T., Orellana, L., González-Torres, P., Díaz, S., Urdiain, M., Farías, M. E., Benes, V., Kaempfer, P., Shahinpei, A., Amoozegar, M. A., Amann, R., Anton, J., ´ Konstantinidis, K. T., & Rossello-Mora, R. (2018). Genomic comparison between members of the *Salinibacteraceae* family, and description of a new species of *Salinibacter* (*Salinibacter altiplanensis* sp. nov.) isolated from high altitude hypersaline environments of the Argentinian Altiplano. Systematic and applied microbiology, 41(3), 198-212.

Waleron, M., Waleron, K., Vincent, W. F., & Wilmotte, A. (2007). Allochthonous inputs of riverine picocyanobacteria to coastal waters in the Arctic Ocean. FEMS microbiology ecology, 59(2), 356-365.

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol., 73(16), 5261-5267.

Wang, Y. X., Wang, Z. G., Liu, J. H., Chen, Y. G., Zhang, X. X., Wen, M. L., Xu, L. H., Peng, Q., & Cui, X. L. (2009). *Sediminimonas qiaohouensis* gen. nov., sp. nov., a member of the *Roseobacter* clade in the order *Rhodobacterales*. International journal of systematic and evolutionary microbiology, 59(7), 1561-1567.

Wang, Y., & Qian, P. Y. (2009). Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PloS one, 4(10), e7401.

Wang, Y., Gao, Z. M., Li, J., He, L. S., Cui, G. J., Li, W. L., Chen, J., Xin, Y. Z., Cai, D. S., & Zhang, A. Q. (2019). Hadal water sampling by in situ microbial filtration and fixation (ISMIFF) apparatus. Deep Sea Research Part I: Oceanographic Research Papers, 144, 132-137.

Watling, L., Guinotte, J., Clark, M. R., & Smith, C. R. (2013). A proposed biogeography of the deep ocean floor. Progress in Oceanography, 111, 91-112.

Wilson, B., Müller, O., Nordmann, E. L., Seuthe, L., Bratbak, G., & Øvreås, L. (2017). Changes in marine prokaryote composition with season and depth over an arctic polar year. Frontiers in Marine Science, 4, 95.

Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., De Vos, P., Verstraete, W., & Boon, N. (2009). Initial community evenness favours functionality under selective stress. Nature, 458(7238), 623.

Xu, W., Pang, K. L., & Luo, Z. H. (2014). High fungal diversity and abundance recovered in the deep-sea sediments of the Pacific Ocean. Microbial ecology, 68(4), 688-698.

Yergeau, E., Michel, C., Tremblay, J., Niemi, A., King, T. L., Wyglinski, J., Kenneth, L., & Greer, C. W. (2017). Metagenomic survey of the taxonomic and functional microbial communities of seawater and sea ice from the Canadian Arctic. Scientific reports, 7, 42242.

Zenkevich, L.A. (1963). Biology of the Seas of the USSR George Allen & Unwin LTD.,

Zhang, J., Ma, G., Deng, Y., Dong, J., Van Stappen, G., & Sui, L. (2016). Bacterial diversity in Bohai Bay solar saltworks, China. Current microbiology, 72(1), 55-63.

Zhang, X., Xu, W., Liu, Y., Cai, M., Luo, Z., & Li, M. (2018). Metagenomics reveals microbial diversity and metabolic potentials of seawater and surface sediment from a hadal biosphere at the Yap Trench. Frontiers in microbiology, 9, 2402.

Zhaxybayeva, O., Stepanauskas, R., Mohan, N. R., & Papke, R. T. (2013). Cell sorting analysis of geographically separated hypersaline environments. Extremophiles, 17(2), 265-275.

Zucconi, L., Onofri, S., Cecchini, C., Isola, D., Ripa, C., Fenice, M., Madonna, S., Reboleiro-Rivas, P., & Selbmann, L. (2016). Mapping the lithic colonization at the boundaries of life in Northern Victoria Land, Antarctica. Polar Biology, 39(1), 91-102.

Zwirglmaier, K., Jardillier, L., Ostrowski, M., Mazard, S., Garczarek, L., Vaulot, D., Not, F., Massana, R., Ulloa, O., & Scanlan, D. J. (2008). Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environmental microbiology, 10(1), 147-161.