PH.D. THESIS

From Sea to Society

Climate Change, Microbial Community Interactions and Assessing Climate Risk on Society

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DEPARTMENT OF MARINE SCIENCES

UNIVERSITY OF
GOTHENBURG

Ph.D. thesis

From Sea to Society | Gurpreet Kaur Kahlon 2017

ISBN 978-91-629-0330-5 (PRINT)
ISBN 978-91-629-0331-2 (PDF)
http://hdl.handle.net/2077/53897

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Cover concept: Gurpreet K. Kahlon
Cover illustration: Ann Hillberg

ISBN: 978-91-629-0330-5 (PRINT)
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Printed in Gothenburg, Sweden 2017
By BrandFactory AB

With doubt and dismay you are smitten
You think there's no chance for you?
Why, the best books haven't been written
The best race hasn't been run,
The highest peak hasn't been climbed yet,
The mightiest rivers aren't spanned,
Don't worry and fret, faint hearted,
The chances have just begun,
For the Best jobs haven't been started,
The Best work hasn't been done.
-Berton Braley

Abstract

Unicellular organisms, microalgae and bacteria, less than one millimeter in size make the world go round. Phytoplankton and cyanobacteria, for example, plays an inevitable role contributing 50-85% to the world's oxygen via photosynthesis. Bacteria, one of the first life forms to appear on earth, are vital for nutrient cycling both in the terrestrial, limnetic and marine ecosystems. Both phytoplankton and marine bacteria in addition form an important foundation of marine food webs. The adversities of climate change loom large on the two, as impacts at the level of primary production will most likely have a bottom up effect across the different trophic levels. Warming of the earth system is proceeding at a rate that is unprecedented, yet its effects on the world's largest ecosystem, the marine pelagic realm, are largely unknown. Using experimental approaches including mesocosm settings and gene-expression analysis, this thesis aims to increase our understanding of climate-induced processes in a costal pelagic tropical ecosystem in southwest coastal Arabian Sea. The results show that sea surface warming and freshening is likely to cause an increase in the abundance of phytoplankton in winter season. While in the summer, freshening of sea surface water can be a leading cause for the increase in phytoplankton growth. The findings presented in this thesis also highlight the tight coupling between phytoplankton and bacterial community. Any impact of warming and freshening is subsequently propagated to bacteria as increased phytoplankton biomass supports increased biomass of bacteria. To understand the underlying bases of this phytoplankton-bacterial interrelationship, it is important to recognize the communication over species borders. We looked for signs of existing cross signalling between a predominant bacterial species, *Vibrio harveyi*, and a dominant diatom species, *Skeletonema marinoi.* In *V. harveyi*, quorum sensing (QS), cell-to-cell signalling among bacteria, indirectly regulates bacterial virulence gene expression. Previous studies have shown this communication being interrupted by inhibitory compounds produced by some species of macro- and microalgae. Our results indicate activation of QS in *V. harveyi* strains during the experiment period, but QS did not regulate the expression levels of virulence factors. This suggests that the QS activity of *V. harveyi* was not interrupted by the presence of *S. marinoi* strain, implying that this diatom-bacteria relationship may in this respect be considered a commensalistic relationship. In the face of climate change, our results make important predictions for the future scenarios suggesting faster growth and development of microbial blooms, both phytoplankton and bacteria including pathogenic bacteria and toxin-producing phytoplankton. These can accumulate in commercially important bivalves, subsequently passing to higher trophic levels. This may have considerable implications for seafood safety, putting human health at high risk. We propose an integrated science-social science framework for assessment of human vulnerability to climate change effects in marine ecosystems. This can help to weigh, more explicitly and systematically, human vulnerability influenced by complex and simultaneous environmental and socio-economic changes.

Keywords: Phytoplankton, Bacteria, Climate change, Quorum sensing, Microbial blooms, Vulnerability factors, Arabian Sea, Southwest India

Sammanfattning

Celler mindre än en millimeter i storlek av mikroalger och bakterier är primära drivkrafter i jordens ekosystem. Det bidrar med 50-85% av världens syreproduktion via fotosyntes. Bakterier, de första livsformerna på jorden, är fortfarande avgörande för näringscykeln både i terrestra, limniska och marina ekosystemet. Både växtplankton och marina bakterier utgör en viktig grund för produktionen av biomassa i haven. Klimatförändringar som ger effekter på primärproduktionen får sannolikt också följdverkningar genom "bottom-up" effekter på övriga trofiska nivåer. Jordens uppvärmning sker med en historiskt oöverträffad hastighet, medan dess effekter på världens största ekosystem, det marina pelagiska ekosystemet, är i stort sett okända. Denna avhandling syftar till att öka vår förståelse för klimatpåverkan på växtplankton- och bakteriesystemen i ett pelagiskt tropiskt ekosystem i kustnära områden i sydvästra Arabiska havet. I avhandlingen används experimentella angreppssätt bl.a. mesocosm försök och gen-uttrycks analyser. Resultaten visar att uppvärmning tillsammans med utsötning av de ytnära vattnet sannolikt kommer att leda till en ökning av växtplankton biomassan under vintersäsongen. Under sommarsäsong kommer utsötningen ensamt att vara den avgörande faktorn för ökad växtplanktonbiomassa. Avhandlingens resultat lyfter också fram den täta kopplingen mellan växtplankton- och bakteriesamhällena. Uppvärmning och utsötning ger nämligen indirekta effekter på bakteriebiomassan genom att bakterierna gynnas av att biomassan av växtplankton ökar.

För att förstå de bakomliggande faktorerna för samspelet mellan växtplankton och bakterier är det viktigt att också ta hänsyn till den kommunikation som sker över artgränserna. Vi letade därför efter tecken på kemisk signalering mellan en dominerande bakterieart, *Vibrio harveyi* och en dominerande art av kiselalg, *Skeletonema marinoi*. Hos denna bakterie (liksom hos andra bakterier) finns en cell-till-cell-signalering som normalt reglerar virulens (alltså hur patogen en cell är). Tidigare studier har visat att denna kommunikation mellan bactericeller avbryts av hämmande föreningar som produceras av vissa arter av makro- och mikroalger. Våra resultat indikerar aktivering av denna signalering i stammar av bakterien under experimentperioden, men kiselalgens närvaro påverkade inte uttrycksnivåerna av virulensfaktorer. Slutsatsen är att signaleringen mellan bakteriecellerna inte avbröts av kiselalgen och man kan därför se alg-bakterieförhållandet mer som ett neutralt förhållande.

Mot bakgrund av pågående klimatförändring visar våra resultat att framtidens klimat kan resultera i snabbare tillväxt och utveckling av planktonblomningar och medföljande ökning av bakterier i vattenmassan, där även patogena bakterier och toxinproducerande växtplankton kan bli vanligare. Detta kan leda till att smittor och gifter ackumuleras i kommersiellt viktiga musslor som genom sin filtrering tar upp och koncentrerar dessa organismer. Detta kan ha betydande konsekvenser för konsumtionen av skaldjur i dessa områden, och påverka människors hälsa. Vi föreslår här ett integrerat och mångvetenskapligt ramverk för bedömning av mänsklig sårbarhet som en följd av klimatpåverkan i marina ekosystem. Detta kan bidra till att på ett tydligare och mer systematiskt sätt väga in hur samhället påverkas av komplexa och samtidiga miljö- och socioekonomiska förändringar.

List of Papers

This thesis is based on the following research papers, referred to in the text by their corresponding numerals.

Paper I **Kaur-Kahlon G**, Kumar S, Rehnstam-Holm A-S, Rai A, Bhavya P, Edler L, Singh A, Andersson B, Karunasagar I, Ramesh R, Godhe A (2016) Response of a coastal tropical pelagic microbial community to changing salinity and temperature. *Aquatic Microbial Ecology* 77:37-50

Paper II **Kaur-Kahlon G**, Kumar S, Rehnstam-Holm A-S, Singh A, Rai A, Bhavya P, Edler L, Andersson B, Karunasagar I, Ramesh R, Johannesson K (Manuscript) Seasonal responses to future climate in a tropical pelagic community

Paper III **Kaur-Kahlon G**, Kumar K, Johannesson K, Karunasagar I, Godhe A, Ruwandeepika HA (Manuscript) Quorum sensing regulation of virulence gene expression in *Vibrio harveyi* in the presence of a marine diatom (*Skeletonema marinoi*)

Paper IV Knutsson P, **Kaur-Kahlon G**, Godhe A, Bhatta R, Karunasagar I, Turner L, Narayana R, Kadfak A, Johansson S (Manuscript) Assessing complex climate risk: the case of climate induced harmful microbial blooms along the Indian southwest coast

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Contents

Section 1

Preface

1.1. Climate change

Climate change is a reality that has now been accepted by most of the countries worldwide. Human activities of the past are responsible for the current state of affairs. It is inevitable and the best we can do is to mitigate its impact. Inter-governmental panel on climate change was founded in 1988 with the aim to provide the world with the scientific knowledge on climate change and its subsequent socio-economic impacts. It forms its assessment reports based on peer-reviewed and non-peer-reviewed published sources. According to its fifth assessment report, the average global temperature can rise by 2.6-4.8°C by 2100 if greenhouse gas emissions continue at their current rate (Fig. 1).

Of all, climate change impacts loom large on marine ecosystems. It is believed that the resilience of marine ecosystems to adjust to these changes will likely reduce by both the range of factors and their rate of change (Pörtner et al. 2014). The current rate of change is phenomenal, where species may not have enough time to adapt under progressing climate change scenarios.

1.2. Sea water temperature and salinity changes- *Global and tropical*

Vertically averaged ocean temperatures are increasing globally and will likely continue to increase in the near term (Kirtman et al. 2013). Subtropical and tropical regions of Asia are warming the most at the ocean surface. World’s coastlines are a home to about 60% of the world’s population, living with in 100 Km from coastlines. More than 70% of these coastlines indicate a significant warming of sea surface temperature (SST) during the last 3 decades. The rate of rise in SSTs is higher, on average, along coastlines than in the global oceans. Multi-model projections present that it is highly likely that positive trends in coastal SST will continue (Wong et al. 2014).

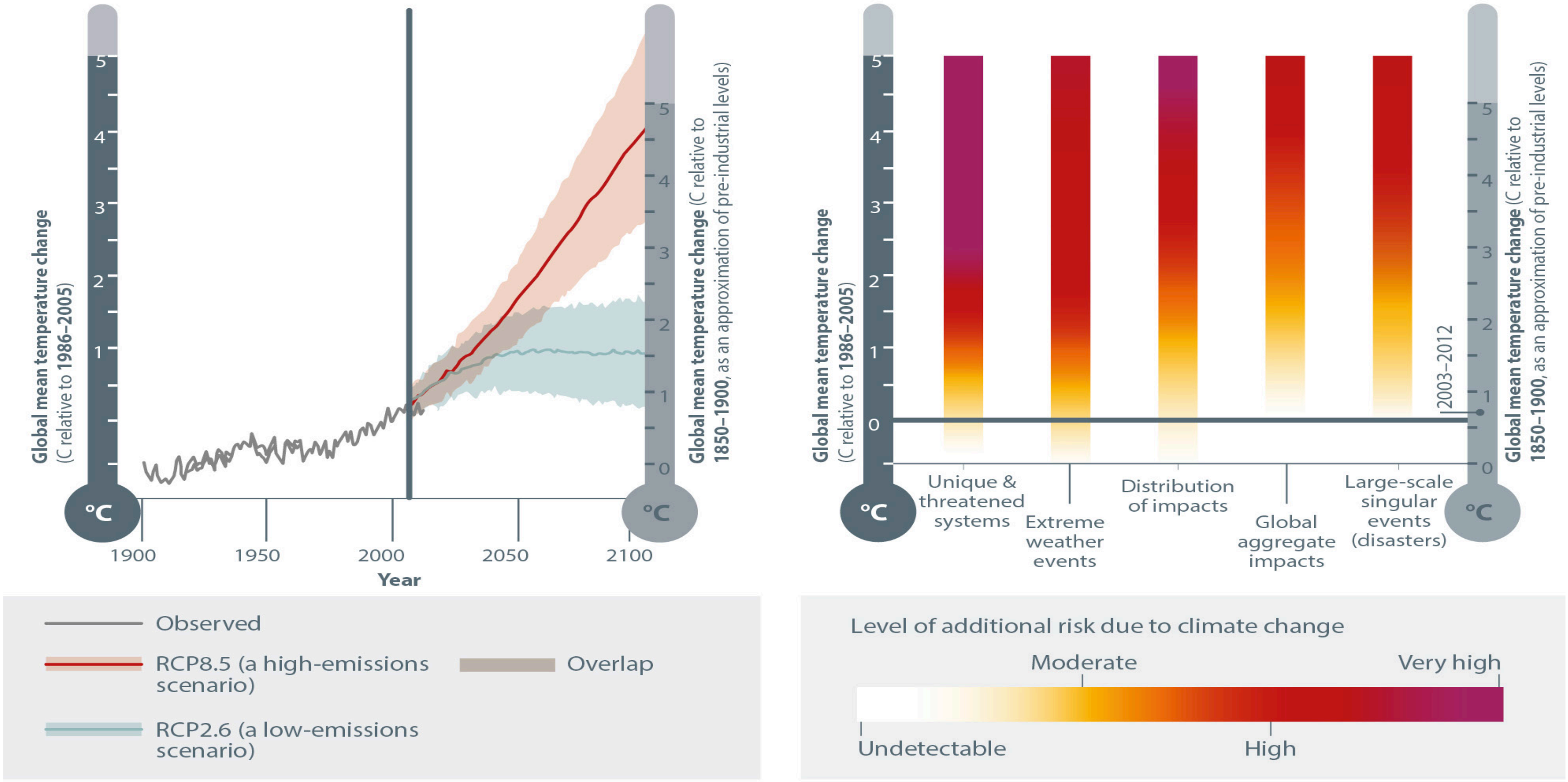
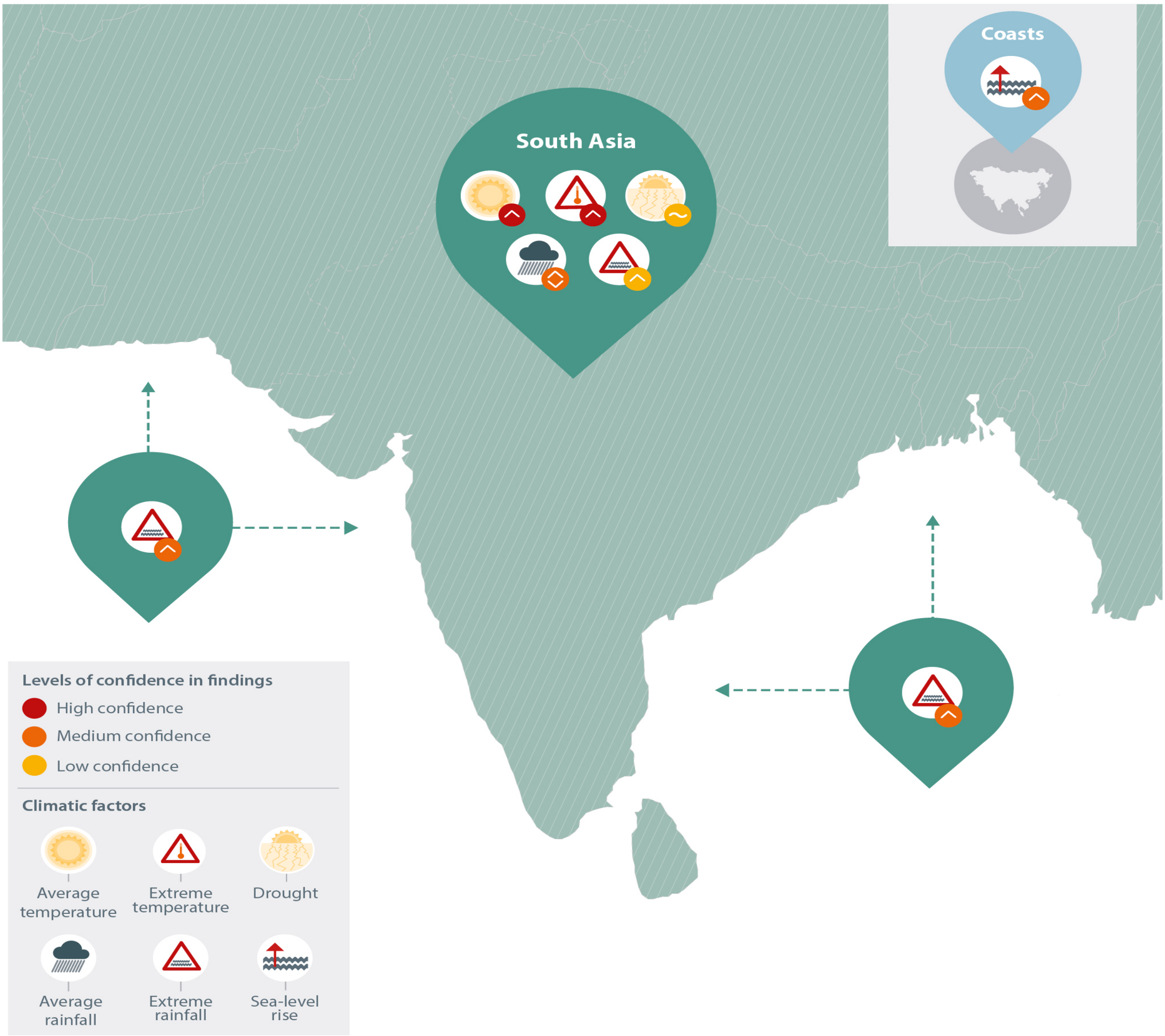


Fig. 1 The graph on the left depicts observed and projected global annual average temperature; on the right global risks under increasing levels of climate change as reported by IPCC 2014. *Source*: Climate and Development Knowledge Network, 2014.

Observations from central Indian region show a building trend of more frequent and heavy rainfall events related to monsoons and fewer weak rainfall events (Goswami et al. 2006). All models and scenarios suggest that this trend will continue and project an increase in both the mean and extreme precipitation in the Indian summer monsoon (Fig. 2) (Christensen et al. 2013).

Fig. 2 Future climate trends of South Asia as reported by IPCC 2014. *Source*: Climate and Development Knowledge Network, 2014.



Symbol	Rainfall	Temperature	Extreme rainfall, extreme temperature, sea-level rise
⌃	up to 30% increasing trend	1–5 °C increasing trend	increasing trend
◇	both increasing and decreasing trends	–	both increasing and decreasing trends
⌄	up to 30% decreasing trend	–	decreasing trend
−	inconsistent trend	inconsistent trend	inconsistent trend
∼	no or only slight change	no or only slight change	no or only slight change

1.3. Phytoplankton and bacteria

1.3.1. Phytoplankton- *the marine primary producers*

Marine plankton is a vital member of marine ecosystem. It fuels not only the marine realm but also the entire globe by contributing 50% of the global primary production and also to the biogeochemical cycling. Marine ecosystem is witnessing a global shift among some groups of plankton where some tropical species are moving towards poles. However, the responses of different plankton communities that depend on the type and extent of linkages between successive trophic levels is not fully known. It is, therefore, important to understand their individual responses and inter-relationships so as to comprehend the climate change impacts on these groups under projected future scenarios.

1.3.2. Bacteria- *its role and communication in the marine realm*

There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in 1ml of fresh water. So, you can imagine how many bacteria there are out in global oceans. Interestingly or not, bacteria have devised their own form of communication through chemical signaling called Quorum sensing (QS). QS occurs within bacterial populations, inter-species and intra-species, in a density-dependent manner (Bassler). It regulates different genes and modulates many biological functions such as bioluminescence, virulence factor expression, biofilm formation, sporulation, and bacterial conjugation (Waters and Bassler 2005, Mangwani et al. 2012). QS is receiving increasing attention from marine ecologists with attempts to understand how different bacterial species interact and may effect the marine algae they co-habitat with.

1.3.3. Phytoplankton-bacterial interaction

Algae and bacteria co-exist in aquatic ecosystems. Studies reveal that several interactions occur between them, which are of particular interest in the field of marine ecology (Waters and Bassler 2005, Hughes and Sperandio 2008, Jayaraman and Wood 2008, Amin et al. 2015). These interactions impact the physiology of both partners, alter the chemistry of their environment, and shape the microbial diversity (Miller and Bassler 2001). One type of interaction is through interfering with QS. Bacteria employ QS during colonization and invasion of their eukaryotic hosts. QS-mediated interactions widely influence algal-bacterial symbiotic relationships, which in turn determine community organization, population structure, and ecosystem functioning. Understanding these QS-mediated ecological processes may shed light on the symbiotic interactions between algae host and associated bacteria.

1.4. Changing climate and society

Climate change poses risks to human and natural systems. Risks related to climate change arise from climate-related hazards (climate trends and extremes) and the vulnerability of exposed societies, communities or systems (in terms of livelihoods, infrastructure, ecosystem services and governance systems). Poor people along the coastlines heavily depend on natural resources that are directly influenced by changes in weather and climate. Compared to other regions, South Asia exhibits the greatest exposure in terms of population and assets (Jongman et al. 2012). Fifth assessment report of IPCC also suggests that such populations and assets exposed to coastal risks will increase significantly in the coming decades due to population growth, economic development and urbanization (Wong et al. 2014). Growing temperatures (ocean warming), rising sea levels and changing rainfall patterns (hyposalinity) closely regulate fisheries sector. Climate-related declines in seafood productivity will have impact on livelihoods and exports, increasing poverty levels and also affecting urbanizing areas (Hijioka et al. 2014). In short, in costal zones of South Asia, rapid population growth, urbanization, economic growth and changes in land use could interact with climate change resulting in an increase in the vulnerability of certain groups to future climate change (Klein et al. 2014).

1.4.1 Microbial blooms- toxic algae and pathogenic bacteria

Harmful algal blooms (HABs) have been increasing in size and frequency worldwide, a fact that many experts attribute to global climate change. It is predicted that under the influence of ongoing and predicted climate change scenarios, the occurrence of harmful algal blooms will continue to increase.

Coastal ecosystems and populations are directly impacted and most vulnerable to such blooms. There are increasing evidences of strong linkages between HABs and climate fluctuations (Dale et al. 2006). Ocean warming, nutrient fluctuations, enhanced surface stratification and eutrophication in coastal areas will likely stimulate HABs, and may also cause shifts in seasonal cycles and blooms (Johns et al. 2003). Additionally, ocean acidification may exacerbate the toxicity of species in coastal oceans under nutrient-limited conditions (Sun et al. 2011).

Bacterial abundance has been seen to be highly controlled by phytoplankton biomasses and community composition. Continued warming of tropical and temperate coastal habitats, excessive nutrient loading leading to phytoplankton and zooplankton blooms, will subsequently exacerbate the occurrence and threat of pathogenic bacterial species. For example, sub-tropical countries like India where cholera is endemic, outbreaks correlate with warming and increasing algal blooms.

1.4.2 Toxic bivalves- consumption and production

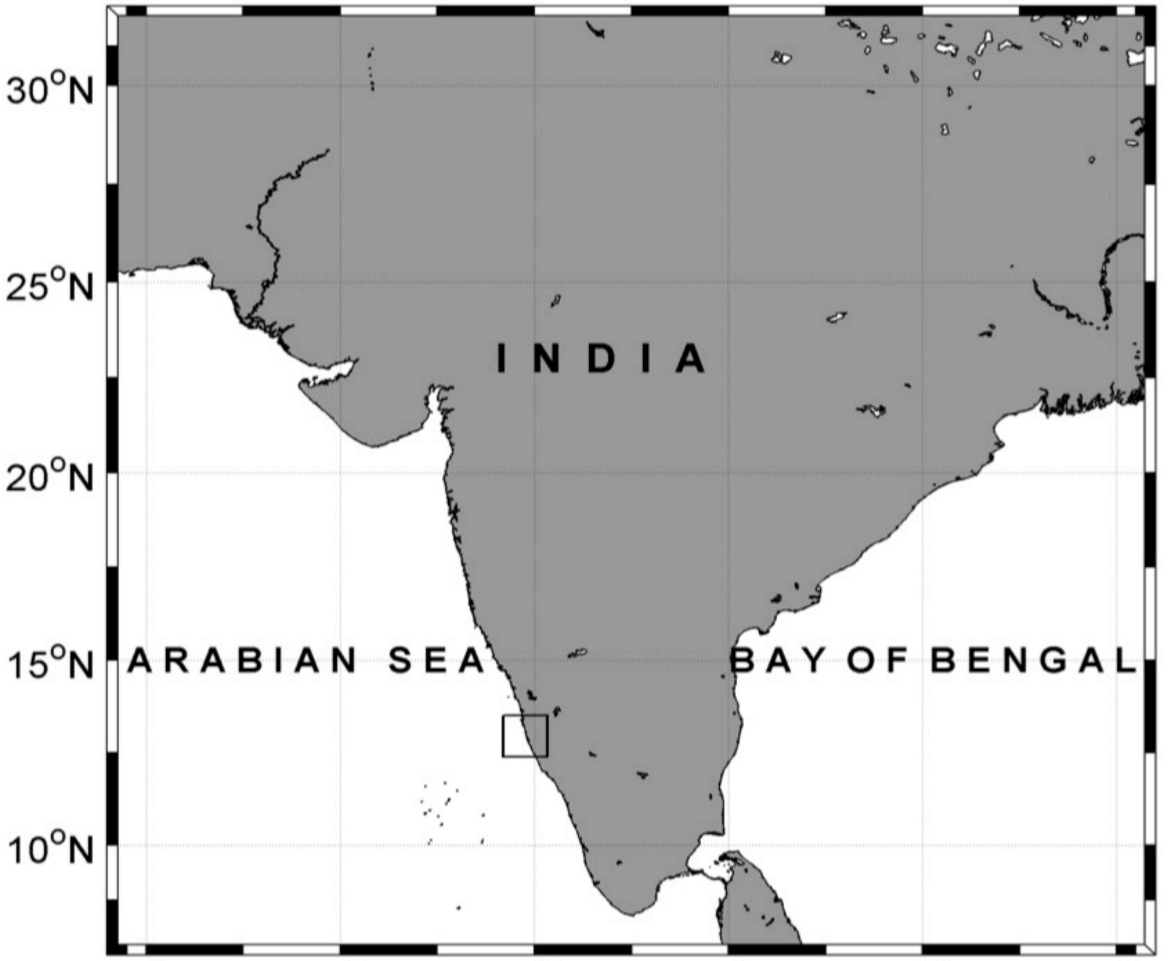
Three billion people receive almost 20% of their average per capita intake of animal protein from seafood, where an annual 63 million tons of molluscs and crustaceans from marine aquaculture, mostly concentrated in coastal areas, form a part of global seafood production (Prakash and Stigler 2012). The growth of aquaculture has decelerated, but is still considered a development opportunity and a need in regions such as South Asia. Bivalve production contributes substantially to the fisheries sector in India. Also, the coastal communities along the southwest and southeast coast of India depend on bivalves as a source of food. Warming may increase the risk of disease outbreaks or bacterial infections, in marine organisms including bivalves, and ultimately, humans (Altizer et al. 2013, Burge et al. 2014). Climate-induced shifts in ecosystems and fisheries production will create significant challenges to sustainability and management, particularly for communities with fewer resources and lower adaptive capacity, including South Asian coastal populations.

Section 2

Study area

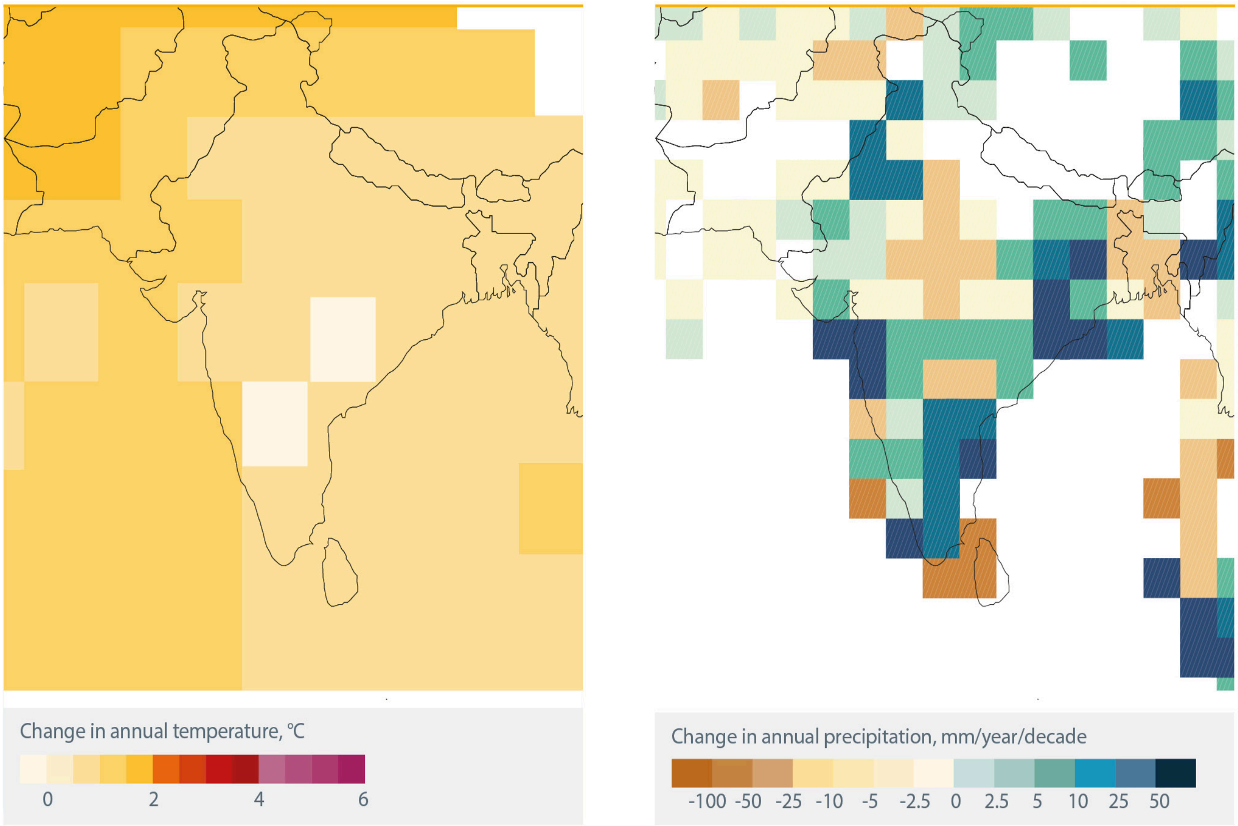
Studies reported in **Paper I, II and IV** were conducted in Mangalore, India. Mangalore is one of the major port cities on the west coast of India. It is surrounded by Arabian Sea on the west and Western Ghats on the east. It has a tropical monsoon climate and is under the influence of the Southwest monsoon. The plankton inocula for the mesocosm experiments (**Paper I and II)** were collected 13-15 km south of Mangalore (Fig. 3).

Fig. 3 A map of study area and sample collection. The small square represents the city of Mangalore, located on the west coast of India. The samples were collected 13-15 km south of the city.



The four different treatments chosen for mesocosm experiments were designed based on long-term trends and multi-model projections of sea surface temperature and salinity. Fig. 4 below gives an overview of annual average temperature and rainfall in Indian region during1901-2012.

Fig. 4 Figure on the left shows the changes in annual average temperature in South Asia between 1901-2012, while figure on the right shows changes in annual average rainfall in South Asia (India) from 1951-2012. *Source*: IPCC 2014, adapted from Climate and Development Knowledge Network, 2014.



Section 3

Aims and objectives

Different studies that form a part of this thesis were carried out with the aim to (1) understand the individual responses and inter-relationships between the two important compartments of microbial loop, phytoplankton and bacteria, (2) to comprehend the climate change impacts on these groups under projected future scenario and (3) assess the complex climate risks that coastal communities are likely to be vulnerable to and identify the potential socio-economic vulnerability factors.

In **Paper I**, mesocosm experiment simulating the future scenarios of warming and hyposalinity was conducted in December 2011 (winter) to study the individual responses of phytoplankton community and associated heterotrophic bacteria to these changes in sea surface temperature and salinity. Additionally, attempts were also made to identify and understand the inter-relationship between phytoplankton and bacterial communities.

Based on the observations from **Paper I**, it was interesting to see how the two communities will behave and respond to warming and hyposalinity conditions during different seasons. Another mesocosm experiment (**Paper II**) was performed during March 2013 (early summer).

In order to understand better the relationship between phytoplankton and bacterial community, it is important to recognize and identify any sings of existing communication between the two. A quorum sensing study **(Paper III)** was, therefore, conducted to see how the presence of a dominant phytoplankton species *Skeletonema marinoi* can influence the quorum sensing regulated virulence gene expression of a predominant *Vibrio harveyi* bacteria.

Last but not the least, attempts were made to assess the complex climate risks that coastal communities are likely to be vulnerable to and identify the potential socio-economic vulnerability factors using the case of climate induced harmful microbial blooms along the Indian southwest coast **(Paper IV).**

Section 4

Main findings of this thesis

This thesis touches on the following main subjects:

4.1 Tropical pelagic community under future climate: *responses and interactions*

The fifth assessment report presents a long list of changes that scientists have observed around the world. Since the mid-19th century, the average increase in the temperature of the Earth's surface has been 0.85°C (IPCC 2013). Globally averaged surface and vertically averaged ocean temperatures have increased and will continue to increase in the near term (Kirtman et al. 2013). More than 70% of the world's coastlines have witnessed a significant warming of sea surface temperature (SST) and it is highly likely that these positive trends in coastal SST will continue (Wong et al. 2014).

Heavy rains in different parts of India cause a significant discharge of freshwater by many large rivers into the Bay of Bengal, causing the reduction in salinity (Qasim et al. 1972). The advection of these low salinity waters from the Bay of Bengal by the East Indian Coastal Current (EICC) during the NE Monsoon is responsible for the seasonal freshening of surface waters in SE Arabian Sea (Gopalakrishna et al. 2005, Nyadjro et al. 2012).

These climate induced hydrographic changes affect the microbial community. Long term phytoplankton and environmental monitoring data demonstrates a shift in phytoplankton abundance and richness with the changing sea surface temperature and salinity (Godhe et al.). There is a need to understand ongoing trends and build up possible future scenarios to contemplate better what future holds for the marine pelagic tropical ecosystem.

With this vision, mesocosm studies were conducted during two different seasons (winter and early summer) using the natural microbial community from southwest coast of India. During the first experiment conducted in December 2011 (**Paper I**) different treatments were set up representing ambient winter conditions (28°C, 35 PSU), hyposaline (28°C, 31 PSU), warm (31°C, 35 PSU), and a combination of hyposaline and warm conditions (31°C, 31 PSU), Maximum phytoplankton and bacterial growth was observed in the warm-hyposaline combined treatment, followed by the warm treatment. Hyposalinity, however, did not favour microbial growth resulting in an overall low phytoplankton and bacterial biomass in all the hyposaline treatments. The hyposaline conditions had the most considerable influence, manifesting a significantly lower primary production, and the most divergent microphytoplankton species community among the different treatments. Hyposalinity combined with warming, however, maintained a higher primary production.

On the contrary, the early summer microbial community sampled in March 2013 (**Paper II**) gave a completely different response. This mesocosm experiment followed a similar set up like the previous representing different treatments as follows: ambient (30°C, 36 PSU), hyposaline (30°C, 32 PSU), warm (33°C, 36 PSU), warm and hyposaline (33°C, 32 PSU). The hyposaline treatment turned out to be the most supportive condition for microbial growth. Thus hyposaline waters promoted increase in phytoplankton biomass, while warming had the opposite effect, and both factors together had insignificant effect. Structural equation modelling (SEM) was employed as a tool to partition the net effects of these treatments into direct and indirect effects and to look for possible interactions between the different compartments of the microbial loop (Fig. 5). The structural equation modelling emphasized the interrelationship between phytoplankton biomass and bacterial abundance for both the seasonal communities **(Paper I and II)**. It elaborated on our observation of climate change impacts being propagated to bacteria from phytoplankton. Increased phytoplankton biomass under hyposaline conditions resulted in a subsequent indirect positive effect on bacterial biomass in hyposaline waters (**Paper II**). Similarly, in the winter mesocosm, positive influence of hyposalinity and warming was reflected as an indirect increase in bacterial abundance in this treatment (**Paper I**). Comparing the two mesocosm studies (**Paper I and II)** undertaken during winter (December 2011) and early summer (March 2013) brings out a distinct difference in response of the two seasonal communities. The summer response was clearly different from the winter response, thus highlighting the climate effects being seasonal even in a tropical sea. Although the underlying processes differ, the combined results of these two mesocosm experiments suggest faster growth and development of microbial blooms, both phytoplankton and bacteria, both under summer and winter conditions. This suggests that under the influence of climate change tropical coastal zones, both in winter and summer, will experience faster growth and development of microbial blooms, both phytoplankton and bacteria, with subsequent significant effects on higher trophic levels.

Fig. 5 Structural equation model (SEM) based on the estimates of phytoplankton biomass (measured as µg C L^{-1}) and bacterial abundance (measured as cells ml^{-1}) for the (a) summer (March) and (b) winter (December, reproduced from(Kaur-Kahlon et al. 2016) microbial communities. Ovals represent treatments and rectangles represent measured variables. Black and grey arrow paths indicate significant ($p < 0.05$) positive and negative influences, respectively. Please note that only significant paths are presented in the figure but see Table 2 for coefficients of all paths for the summer experiment and Kaur-Kahlon et al. 2016 for the winter experiment. At each path the standardized coefficients are represented and interpreted as follows: If, for example, hyposalinity goes up by 1 SD during summer, the phytoplankton biomass of the summer community goes up by 0.243 SD. R^2 value for endogenous variables indicates the degree of variance explained by the model.

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4.2 Quorum sensing regulation of bacterial virulence gene expression in the presence of a marine diatom: *de facto means to understand phytoplankton-bacterial interaction*

Algae are ubiquitous in the marine environment, and the ways in which they interact with bacteria are of particular interest in the field of marine ecology. Phytoplankton-bacteria interactions impact the physiology of both partners, alter the chemistry of their environment, and shape microbial diversity. The relationship between algae and bacteria is not well-known and studied with respect to quorum sensing (QS). This is a system of chemical stimuli and response correlated to population density. QS is pivotal in driving community structure and modulating bacterial gene expression, pathogenicity, antibiotic resistance, and bacterial-host interactions. These interactions widely influence algal-bacterial relationships, which in turn determine community organization, population structure, and ecosystem functioning. Understanding cross-talk over species borders, may shed light on the interactions between algal host and associated bacteria. *Vibrio harveyi*, a predominant bacterial species and *Skeletonema marinoi*, a dominant diatom species were used as models to probe for any signs of existing cross-signalling between the two different compartments of the microbial loop (**Paper III**). Four treatment combinations (Q1-Q4), featuring different combinations of *Vibrio* and *Skeletonema* strains of different concentrations were used (Table 1) to study the regulation of gene expression of a virulence regulator, *luxR* (a bioluminescence gene), and virulence factors *vhp* (metalloprotease) and *chiA* (chitinase) genes.

Table 1 Different concentration combinations of *S. marinoi* and *V. harveyi* used for the experiment

XXXXXXXXX UNPUBLISHED DATA

In *V. harveyi*, QS regulates bioluminescence and *luxR* indirectly regulates the virulence gene expression (*vhp*, *chiA*). QS regulation of these genes was monitored in four selected strains (one non-virulent, one strongly virulent and 2 different wild type strains) during the infection of axenic cultures of a marine diatom (*S. marinoi*). We observed QS activation, represented as a significant *luxR* gene response, in all the strains among all the treatments except for the non-virulent (QS-) strain, as expected. The virulence factors *vhp* and *chiA*, however, were not regulated by QS since their expression levels in the virulent (QS+) and wild type strains were not different from that in the non-virulent strain. For example, Fig 6 illustrates this best (**Paper III**). This implies that the presence of this strain of *S. marinoi* did not interfere with the QS activity of *V. harveyi* and this diatom-bacteria relationship may in this respect be considered a commensalistic relationship.

This study suggests how QS might modulate microbial behaviour, affecting algal-bacterial relationship, and abundance. This corroborates the results of the two mesocosm studies (**Paper I and II**), where we found a positive coupling between phytoplankton and bacterial biomasses, which likely mirrors a relationship of commensalism between *V. harveyi* and *S. marinoi.* In view of ongoing climate change, it is, therefore, likely that any impact of changed hydrographic conditions on the phytoplankton community will have a consequent and positive effect on the bacteria (**Paper I, II and III**).

Fig. 6 Expression values for genes (a) *luxR*, (b) *vhp* and (c) *chiA* over a 48h period in treatment Q3 (*Skeletonema* =2x10^4 cells ml^{-1}, *Vibrio* =10^7 cells ml^{-1}). Y-axis= Relative expression to *rpoA*, X-axis= Time elapsed (h). Error bars represent standard error of mean (**Paper III)**.

XXXXXXXXX UNPUBLISHED DATA

4.3 Assessing complex climate risk: *the case of climate induced harmful microbial blooms along the Indian southwest coast*

In response to ongoing climate change, the geographic range, seasonal activities and migration patterns of many terrestrial, freshwater and marine species have shifted. The abundance of species has changed, as have interactions among species. Climate change will have widespread impacts on South Asian society and South Asians' interaction in any of its many natural environments (Ipcc). As one example, human health and near-shore ecosystems may be directly impacted by climate change promoting increase in harmful algal blooms (Edwards et al. 2006). Similarly, diseases, such as cholera and its pathogen the marine bacterium *Vibrio cholera* has shown outbreaks in coastal populations in South Asia that have been associated with increased temperatures and algal blooms. Coastal ecosystems and populations are directly impacted and most vulnerable to such blooms. There are increasing evidences of strong linkages between HABs and climate fluctuations (Dale et al. 2006). Ocean warming, nutrient fluctuations, enhanced surface stratification and eutrophication in coastal areas will likely stimulate HABs, and may also cause shifts in seasonal cycles and blooms (Johns et al. 2003). Additionally, ocean acidification may aggravate the toxicity of species in coastal oceans under nutrient-limited conditions (Sun et al. 2011).

Based on these global trends and observations from our previous studies (**Paper I, II and III**), it is highly likely that climate change will cause an increase in the occurrence of harmful microbial blooms along the southwest coast of India. We propose an integrated framework for assessment of human vulnerability to climate change effects in marine ecosystems (**Paper IV).** In this study, we used the rapidly urbanizing city of Mangalore, situated on the southwest of India, to examine the effects of climate driven changes in blooms of toxic algae and bacteria, and how these effects may propagate through fisheries to the society. The proposed framework is an analytical hierarchy process guided multi-criteria analysis, where relevant vulnerability factors, both biophysical and socio-economic, are identified and systematically organized into a hierarchical framework. The biophysical factors; climate change induced hydrographic conditions, abundance of toxic bloom-forming phytoplankton and pathogenic bacteria, were chosen based on our results from **Paper I and II**. The future local patterns of urbanization, poverty, economic development, and fishery sector development together with risk response capacity were identified as socio-economic vulnerability factors of this area (**Paper IV**).

To ensure a more comprehensive application of the framework, a workshop was arranged in December 2016 to engage local academic and non-academic representatives in using the framework as a heuristic tool. All participants assigned a weight to each of the five socio-economic vulnerability factors in influencing human consumption and production of bivalves, the two core vulnerability mechanisms, which lie at the center of this hierarchical framework.

Overall, these five vulnerability factors were considered to have an influence on human consumption/production of bivalves. Participant responses confirmed that context-relevant processes of socio-economic change do have an influence on the extent to which people in Mangalore will be vulnerable to the future risk of increased occurrence of toxic bivalves. These socio-economic vulnerability factors, therefore, do carry substantial weight in determining if and how climate change induced microbial blooms is a risk worth the attention of policy makers. In these times of complex and simultaneous environmental and socio-economic changes, the suggested framework will allow more explicit and systematic assessment of human vulnerability. Furthermore, this paper illustrates the need to bring together knowledge from different disciplines, on different environmental and socio-economic processes, into integrated assessments of human vulnerability.

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