Ecological impact of land-derived anthropogenic nutrients and organic matter on tropical estuarine and coastal systems of Hainan, China

Dissertation submitted by

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In partial fulfillment of the requirements for the degree of doctor of natural sciences (Dr. rer. nat.)

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<tr>
<td>AVS</td>
<td>acid volatile sulphide</td>
</tr>
<tr>
<td>Chl a</td>
<td>chlorophyll a</td>
</tr>
<tr>
<td>C$_\text{org}$</td>
<td>particulate organic carbon</td>
</tr>
<tr>
<td>C/N</td>
<td>molar ratio of C$_\text{org}$ and TN</td>
</tr>
<tr>
<td>CRS</td>
<td>chromium reducible sulphide</td>
</tr>
<tr>
<td>DIN</td>
<td>dissolved inorganic nitrogen</td>
</tr>
<tr>
<td>DIP</td>
<td>dissolved inorganic phosphorus</td>
</tr>
<tr>
<td>DO</td>
<td>dissolved oxygen</td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
</tr>
<tr>
<td>DOM</td>
<td>dissolved organic matter</td>
</tr>
<tr>
<td>DON</td>
<td>dissolved organic nitrogen</td>
</tr>
<tr>
<td>DW</td>
<td>dry weight</td>
</tr>
<tr>
<td>$\delta^{13}$C$_\text{org}$</td>
<td>stable organic carbon isotope ratio</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>stable nitrogen isotope ratio</td>
</tr>
<tr>
<td>$\delta^{34}$S</td>
<td>stable sulphur isotope ratio</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>H$_2$S</td>
<td>hydrogen sulphide</td>
</tr>
<tr>
<td>LANCET</td>
<td>Land-Sea Interactions along Coastal Ecosystems of Tropical China: Hainan</td>
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<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>NE</td>
<td>north-east</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>ammonia</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>ammonium</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>nitrite</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>nitrate</td>
</tr>
<tr>
<td>NO$_x^-$</td>
<td>nitrite + nitrate</td>
</tr>
<tr>
<td>OM</td>
<td>organic matter</td>
</tr>
<tr>
<td>PE</td>
<td>poly ethylene</td>
</tr>
<tr>
<td>POM</td>
<td>particulate organic matter</td>
</tr>
<tr>
<td>TN</td>
<td>total nitrogen</td>
</tr>
<tr>
<td>PO$_4^{3-}$</td>
<td>phosphate</td>
</tr>
<tr>
<td>Si(OH)$_4$</td>
<td>silicate</td>
</tr>
<tr>
<td>SE</td>
<td>south-east</td>
</tr>
<tr>
<td>TSM</td>
<td>total suspended matter</td>
</tr>
<tr>
<td>WWE</td>
<td>Wenchang/Wenjiao Estuary</td>
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<tr>
<td>ZnAct</td>
<td>zinc acetat</td>
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</tbody>
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Abstract

Human development of the coastal zone causes cumulative effects on the nutrient and organic matter (OM) transport to coastal waters, especially by increased inputs, modified transport and altered biological processing. These effects are among the primary reasons for increasing degradation and losses of valuable coastal habitats, such as seagrasses and coral reefs. Generally, fertilizers from agriculture are thought to be the main source for nutrient enrichment in aquatic ecosystems. This may be different in the Southeast Asian region, where pond aquaculture for the production of shrimp and fish has expanded rapidly during the last decades at the expense of natural wetlands.

The major objective of this thesis was to study on the example of NE Hainan, tropical China, how land-based activities alter nutrient and OM fluxes to tropical coastal waters, and how these, in turn, affect the health and ecological functions of coastal habitats.

Biogeochemical, ecological and stable isotope methods, as well as experiments with bioindicators were used to determine the sources, pathways and fate of nutrients and OM in the Wenchang/Wenjiao Estuary (WWE) and nearshore coastal waters. Furthermore, the state of the seagrass meadows in three back-reef areas was determined. Samples and measurements were obtained in riverine, estuarine and coastal waters and sediments, as well as from potential land-based sources, including agriculture fields and aquaculture ponds and drainage channels, during four field campaigns between 2006 and 2009, one of that in the wake of a typhoon. The samples were analyzed for inorganic nutrients, dissolved and particulate organic carbon and nitrogen, for their stable isotope composition ($\delta^{13}$C$_{\text{org}}$ and $\delta^{15}$N), and chlorophyll a (chl a). At the three coastal sites, Ye Lin, Qingge and Chang qi gang, which varied in pond area in their hinterland (0.04, 2.4 and 8.7 km$^2$, respectively), pond-effluent related nutrient and OM fluxes to their respective back-reef areas were determined. Moreover, species abundance, shoot density and above- and below-ground biomass of the seagrasses at stations along a coastal transect perpendicular to the shore were assessed. Leaves of Thalassia hemprichii collected from the same stations were analyzed for epiphyte loads, $\delta^{15}$N and sulphur isotopic composition ($\delta^{34}$S). In addition, a bioassay experiment was conducted incubating offshore water in dialysis bags at the stations along the coastal transects.

Results indicate that effluents from aquaculture ponds lead to high nutrient and OM inputs to the WWE and coastal waters, in particular dissolved ammonium and dissolved organic nitrogen. Those stimulate phytoplankton growth, causing eutrophication of estuarine and nearshore coastal waters. While effluents released into
the WWE are removed from the water column to large extents due to efficient phytoplankton uptake in the estuarine lagoon during low to moderate precipitation conditions, aquaculture ponds releasing their effluents directly into coastal waters cause continuous nutrient enrichment in usually oligotrophic back-reef areas, especially close to shore. Additionally, precipitation-induced freshwater pulses, which were strongest after typhoon events, are responsible for export of nutrients and OM from the agriculture-dominated hinterland of the WWE into coastal waters. These export pulses add to water quality deterioration in the region with the highest frequency of tropical storms worldwide and, are likely aggravated due to the loss of nutrient-reducing and sediment-retaining mangroves in the course of land use change.

δ^{15}N values ranging from 5-12‰ in suspended matter, seagrass leaves and epiphytes, were among the highest values reported worldwide. This, as well as increases in δ^{15}N from <7‰ up to 14‰ in the phytoplankton bioassays incubated close to shore, reflect the immense impact of ^15N-rich pond effluents on coastal waters of the effluent-exposed back-reef areas of Qingge and Chang qi gang, exceeding 2.5 km from the shore. Higher epiphyte loads on *T. hemprichii* leaves at these back-reef areas than at those of Ye Lin indicate seagrasses at effluent-exposed sites to be at risk of shading and reduced photosynthesis by enhanced algae growth. δ^{34}S values from 15-18‰ in *T. hemprichii* leaves that were lower than 21‰, the typical δ^{34}S value for seawater, reveal temporal intrusion of toxic sulphides into leaf tissue at effluent exposed stations as a result of decomposition of high OM loads leading to sub-/anoxic OM-processing in sediments. Seagrass meadows close to the shore at the pond-affected sites had a much lower species abundance (≤2 species), shoot density (≤100 shoots m⁻²) and total biomass (<350 g m⁻²) compared to that at Ye Lin (≤6 species, 2000-3000 shoots m⁻² and 350-670 g m⁻², respectively), indicating adverse growth conditions for seagrasses exposed to high nutrient loads from pond effluents.

This implies that large-scale pond aquaculture, especially when placed directly adjacent to coastal back-reef areas, causes severe environmental problems to coastal habitats, likely lowering their resilience permanently and making them susceptible to other pressures, including the consequences of typhoon events. Thus, coastal waters in regions with significant brackish water pond aquaculture production are likely at high risk, because persistent effects of nutrient enrichment by pond effluents add to the rain-derived pulsed effects of fertilizer-rich runoff from agriculture fields. As in global estimations of nutrient fluxes to coastal seas only those nutrients are considered that are directly exported via precipitation runoff, it is conceivable that still increasing pond aquaculture is an underestimated threat for coastal ecosystems in many (sub)tropical regions.
ZUSAMMENFASSUNG


Das Hauptziel der vorliegenden Arbeit war es, im nordöstlichen Hainan, China, exemplarisch zu untersuchen, wie landbasierte Aktivitäten den Eintrag von Nährstoffen und OM in tropische küstennahe Gewässer verändern, und wie dies wiederum die Gesundheit und ökologische Funktionen von Küstenhabitaten beeinflusst.

Unter Anwendung biogeochemischer, isotopengeochemischer und ökologischer Methoden sowie durch Experimente mit Bioindikatoren wurden die Quellen, die Transformationsprozesse und der Verbleib von Nährstoffen und OM im Wenchang/Wenjiao-Ästuar (WWE) und angrenzenden küstennahen Gewässern bestimmt. Des Weiteren wurde der Gesundheitszustand der Seegräser in drei küstennahen Rückriffbereichen untersucht. Die Probennahmen und Messungen erfolgten sowohl in der Wassersäule und im Sediment der Flüsse, des Ästuars und der Rückriffbereiche, als auch in Gebieten, die potentielle Quellen vermehrten Nährstoffeintrags sind, darunter insbesondere landwirtschaftliche Produktionsflächen, Aquakulturteiche und Drainagekanäle. Die Proben wurden während vier Feldaufenthalten in den Jahren 2006 bis 2009 entnommen, einmal während eines Taifuns. Sie wurden auf anorganische Nährstoffe, den Gehalt von gelöstem und partikulärem organischem Kohlenstoff und Stickstoff, auf die Zusammensetzung der stabilen Isotopen des organischen Kohlenstoffs ($\delta^{13}C_{\text{org}}$) und Stickstoffs ($\delta^{15}N$) sowie auf Chlorophyll a hin analysiert. An drei Untersuchungsbereichen entlang der Küsten von Ye Lin, Qingge und Chang qi gang mit jeweils unterschiedlich großer Aquakulturfläche im Hinterland (0,04, 2,4 und 8,7 km²) wurden Nährstoff- und OM-Flüsse von den Abflüssen der Teichanlagen in die jeweiligen Rückriffbereiche bestimmt. Gleichzeitig wurden an mehreren Stationen entlang eines Transsekts senkrecht zur Küstenlinie die Artenvielfalt, die Triebdichte, sowie die ober und


Zusätzlich konnten speziell nach einem Taifun niederschlagsbedingte Süßwasserimpulse festgestellt werden, die zusätzliche hohe Konzentrationen von Nährstoffen und OM aus den landwirtschaftlichen Nutzflächen im Hinterland des Ästuars in die Küstengewässer transportieren. Da die Region zu den am häufigsten von tropischen Stürmen betroffenen Gebieten der Erde zählt, ist die regelmäßige zusätzliche Verschlechterung der Wasserqualität durch die nährstoffreichen Süßwasserimpulse ein weiterer ernst zu nehmender Stressfaktor der Küstenhabitate. Verschärft wird die Situation durch die weitgehend verloren gegangene Filterfunktion des stark reduzierten Mangrovenbestandes im WWE.

δ^{15}N-Werte von 5-12‰ in suspendiertem Material, Seegrasblättern und Epiphyten gehörten zu den höchsten, die weltweit je gemessen wurden. Das küstenferne Phytoplankton, welches in den Bioassays an den küstenannen Stationen inkubiert wurde, zeichnete einen enormen Anstieg in δ^{15}N von <7‰ auf bis zu 14‰. Diese Werte zeigen den enormen Einfluss der δ^{15}N-reichen Teichabwässer auf die Wasserqualität der Rückriffbereiche von Qingge and Chang qi gang, der sogar über eine Distanz von 2,5 km hinaus festgestellt werden konnte. Der stärkere Epiphytenbefall von *T. hemprichii* in diesen Gebieten im Vergleich zu Ye Lin zeigt, dass Seegräser in Bereichen, die den Teichabwässern ausgesetzt sind, durch das verstärkte Algenwachstum von Beschattung und reduzierter Photosynthese-Aktivität betroffen sind. δ^{34}S-Werte in *T. hemprichii*-Blättern lagen zwischen 15‰ und 18‰ und damit deutlich unter dem Normalwert von 21‰ in Meerwasser. Dies ist durch ein
vorübergehendes Eindringen von Sulfiden in das Blattgewebe zu erklären, wo Seegräser den Teichabflüssen besonders ausgesetzt sind und im Sediment hohe Mengen von OM teilweise unter sub- oder anaeroben Bedingungen abgebaut werden. Die nahe an den Aquakulturen gelegenen Seegraswiesen weisen zudem eine wesentlich geringere Artenvielfalt (≤2 Spezies), Triebsdichte (≤100 Triebe m\(^{-2}\)) und absolute Biomasse (<350 g m\(^{-2}\)) auf, als diejenigen an der Station Ye Lin (≤6 Spezies, 2000-3000 Triebe m\(^{-2}\) und 350-670 g m\(^{-2}\)). Dies deutet auf schlechte Wachstumsbedingungen unter dem Einfluss der Teichabwässer hin.

CHAPTER I

Herbeck, L.S., Unger, D., Wu, Y., Jennerjahn, T.C.

Effluent, nutrient and organic matter export from shrimp and fish ponds causing eutrophication in coastal and back-reef waters of NE Hainan, tropical China.

Journal: Continental Shelf Research
Current status: Submitted
Contributions: L. Herbeck developed the specific ideas of this study, conducted the field sampling and the sample and data analyses, and wrote the manuscript with scientific and editorial advice by the co-authors.

CHAPTER II


The impact of anthropogenic activities on nutrient dynamics in the tropical Wenchanghe and Wenjiaobeo Estuary and Lagoon system in East Hainan, China.

Journal: Marine Chemistry 125: 49-68.
Current status: Published, 2011
Contributions: L. Herbeck took part in the sampling campaigns, contributed data for and wrote part 3.8 “nutrients in the aquaculture waters”, and commented on the whole manuscript.

CHAPTER III

Krumme, U., Herbeck, L.S., Wang, T.

Tide- and rainfall-induced variations of physical and chemical parameters in a mangrove-depleted estuary of East Hainan (South China Sea).

Journal: Marine Environmental Research
Current status: Submitted
Contributions: L. Herbeck was involved in the sample processing in the field lab, was responsible for the nutrient analysis, compiled the nutrient data, wrote the parts of the manuscript related to nutrient dynamics and commented on the whole text of the manuscript.
CHAPTER IV

Unger, D., Herbeck, L.S., Li, M., Bao, H., Wu, Y, Zhang, J.

Sources, transformation and fate of particulate amino acids and hexosamines under varying hydrological regimes in the tropical Wenchang/Wenjiao Rivers and estuary, Hainan, China.

Journal: Continental Shelf Research.
Current status: Submitted
Contributions: L. Herbeck took part in the fieldwork, was responsible for parts of the sample analyses (TSM, POC, δ13Corg), developed the maps and contributed to the manuscript text.

CHAPTER V


Typhoon-induced precipitation impact on nutrient and suspended matter dynamics of a tropical estuary affected by human activities in Hainan, China.

Journal: Estuarine Coastal and Shelf Science 93: 375-388
Current status: Published, 2011
Contributions: L. Herbeck shared the field work with D. Unger, U. Krumme and S.M. Liu, conducted the sample and data analyses, developed the manuscript idea, and wrote the manuscript with scientific and editorial advice by the co-authors.

CHAPTER VI

Herbeck, L.S., Unger, D., Scharfbillig, A., Jennerjahn, T.C.

Tracing nutrient dispersal from aquaculture pond effluents using nitrogen stable isotope ratios (δ15N) in seagrass, epiphytes and phytoplankton bioassays.

Journal: Marine Ecology Progress Series
Current status: In preparation
Contributions: L. Herbeck developed the study design and experimental set-up, was responsible for the field work and sample and data analyses and wrote the manuscript with scientific and editorial advice by the co-authors.
1. SCIENTIFIC BACKGROUND AND OBJECTIVES

1.1 Tropical estuaries and coasts under anthropogenic pressure

Estuaries and coasts, the interfaces between land and the ocean are among the most productive of the earth’s aquatic systems because their physical, chemical and biological characteristics provide a favourable setting for a diverse flora and fauna (Ryther, 1969; Odum, 1971). In tropical regions, they host valuable habitats, such as mangrove forests, seagrass beds and coral reefs. Those comprise important ecological services including trapping and cycling of nutrients, the processing of pollutants, sediment stabilization and wave attenuation, and therewith play a key role in shoreline stabilization (Costanza et al., 1997; Hemminga and Duarte, 2000; Duarte, 2002; Orth et al., 2006; UNEP, 2006). Furthermore, they support animal biomasses and biodiversity of fish and invertebrate species by providing food and shelter (Nagelkerken et al., 2000; Beck et al., 2001). Coasts are considered to be highly dynamic systems that are continuously exposed to changing geomorphological and hydrographical conditions (Nicholls et al., 2007). However, during recent years, anthropogenic pressures increasingly outreach these physical factors in many regions of the world. Human activities in the coastal zone and drainage basins have caused an ongoing degradation and wide-reaching losses of estuarine and coastal habitats (Alongi, 2002; Pandolfi et al., 2003; Waycott et al., 2009).

About half of the world’s population now lives within 200 km of the coast and the number of people inhabiting coastal areas is likely to have doubled by 2025 (Creel, 2003), as further population growth is predicted to also occur predominantly in coastal areas (Hinrichsen, 1994). This development has been especially strong in tropical regions, as in the past decades, population growth has been and will be especially strong in the less developed countries of Asia and Oceania, as well as in Sub-Sahara Africa (U.S. Census Bureau, 2004). In those countries, demographic population growth is accompanied by large-scale migration into coastal urban areas (Montgomery, 2008). Thus, tropical coastal zones have been and will be centres of land use change linked to increasing human land usage for settlement, transportation, food production and recreation.
1.2 Land-based human activities affecting nutrient and organic matter inputs to coastal waters

The main forms of anthropogenic land use change are the development of urban settlements, agriculture and aquaculture at the expense of natural occurring vegetation. Land use changes, in particular the removal of the original vegetation, have a range of direct consequences such as soil erosion, habitat fragmentation and biodiversity declines (Canadell et al., 2007). Effects are, however, not restricted to land as they also bear implications on the nutrient and organic matter (OM) transport to estuarine and coastal waters.

Population growth has increased human waste water discharge from households and industries to natural water bodies (Van Drecht et al., 2009). While there are increasing efforts to treat the waste water effluents, overall nitrogen and phosphorus removal fractions are often still low, especially in developing countries where installations for advanced biological treatment are not widespread (Van Drecht et al., 2009). As a combined effect of rising numbers of people, urbanization and enhanced sewage connectivity, increases in sewage nitrogen and phosphorus discharge up to a factor of 4-5 are predicted for the next 40 years in South Asia (Van Drecht et al., 2009).

It is estimated that agriculture cultivation has transformed about 12% of the earth’s land surface into cropland, while another 22% have been converted to pastures and rangeland (Raddatz, 2007). Worldwide agricultural growth has been facilitated by a rapid rise in industrial fertilizer use, especially associated with the “green revolution” in global agriculture in the 1970s. Since then, fertilizer application has increased by 700% (Foley et al., 2005). The production of reactive nitrogen increased from ~15 Tg yr\(^{-1}\) in 1860 to 156 Tg yr\(^{-1}\) in 1995 and further to 187 Tg yr\(^{-1}\) in 2005 (Galloway et al., 2008), especially by the Haber-Bosch process. Increasing fertilizer usage was necessary to compensate the larger natural nutrient losses of the soils e.g. by denitrification, leaching of NO\(_3^-\) and DON, ammonia volatilization and soil erosion (Brady, 1990) derived by the increased production yields. Though, only about half of these anthropogenic nitrogen inputs are taken up by the crops (Smil, 1999), while the remainder is released to the environment at a much higher rate than in natural ecosystems (Van Drecht et al., 2003). This contributes to a constantly growing supply of “new” nutrients to aquatic ecosystems. For example, in many watersheds in the US and Canada, agriculture is responsible for more than half of the nitrogen and phosphorus inputs to estuaries and coasts (Castro et al., 2003; Driscoll et al., 2003). While future scenarios predict agriculture-related nitrogen and phosphorus fluxes to the ocean to remain unchanged or even decline in North America and Europe as a result of
continuing counter-measures, massive increases are expected in developing countries, especially in South Asia (Bouwman et al., 2009).

Compared to urbanization and agriculture, relatively little attention is paid to aquaculture. While the term “aquaculture” comprises a large range of techniques, pond aquaculture is its primary land-based form of aquaculture. Due to a rising demand of farmed marine resources, pond aquaculture has become an increasingly important form of land use in many subtropical and tropical regions (FAO, 2010). In estuarine and coastal areas, especially in Southern Asia, brackish-water pond aquaculture of shrimp and fish has grown enormously within the past decades. Conventional pond aquaculture relies on fertilization and/or input of food supplies rich in nitrogen content, and often releases significant fractions of the added nitrogen to surrounding waters (Boyd and Tucker, 1998). Globally, pond aquaculture is one of the primary reasons for the immense destruction of mangroves (Alongi, 2002).

Besides the sheer increase in the quantities of nutrients supplied to natural water bodies, the discharge of those substances to the surrounding environments is additionally reinforced through changes of the coastal morphology. Urbanization, agriculture and aquaculture are associated with massive transformations of the coastal landscape, such as the canalization or damming of rivers, removal of original vegetation, including wetlands and road or housing constructions in the watershed. Thereby, important ecosystem functions provided by mangroves and march lands, such as the retention of sediments and water filtration, are lost (Valiela and Cole, 2002). Constructions of roads and houses may additionally increase the imperviousness of the soil. These modifications alter the transport of water from land to sea, thereby changing the biological processing of nutrients and OM (Paerl and Piehler, 2008). This leads to an acceleration of transport mechanisms of nutrients and OM to natural water bodies, especially during storm-water runoff. Additionally, nutrient ratios in coastal waters are modified by decreasing river exports of silica to the sea. This is related to damming that causes burial of an increased diatom biomass in reservoirs (Conley et al., 1993; Humborg et al., 1997).

Increased inputs, as well as modified transport and biological processing related to land-based human activities have led to an overall enhanced release of nutrients and OM to coastal waters. In the 1990s, the estimated global load of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) delivered to the oceans was approximately three times higher than in the 1970’s (Meybeck, 1982; Smith et al., 2003). Since then, the input rates have continuously increased. Depending on the modeling scenario applied, global DIN and DIP loads are predicted to further increase by 2030, or to remain on a comparable level to that in 2000 (Seitzinger et al., 2010). On
a regional level, South Asia alone accounted for over half of the global increase in DIN and DIP river export between 1970 and 2000, and this trend is predicted to continue in the subsequent 30 years (Seitzinger et al., 2010). These estimates indicate that coastal waters globally are increasingly exposed to nutrient loads, and are therefore threatened by eutrophication. The situation in South Asia appears especially alarming.

1.3 Eutrophication of coastal waters and its consequences

The excessive release of nitrogen and phosphate to the environment has emerged as one of the most important direct drivers of biodiversity and ecosystem changes, impacting both terrestrial and aquatic ecosystems, currently even outmatching the impacts of global climate change (Millenium Ecosystem Assessment, 2005). Although increasing nutrient levels can have initial positive effects (for example a rising productivity and higher yields of fisheries resources), they are eventually exceeded by adverse effects, such as eutrophication of inland and coastal waters. Eutrophication is rated amongst the greatest threats to aquatic ecosystem health and integrity worldwide (Balls et al., 1995; Braga et al., 2000; Davis and Koop, 2006). In coastal waters, it is among the primary reasons for biodiversity declines (Lotze et al., 2006), loss and degradation of seagrass beds (Short and Wyllie-Echeverria, 1996; Hemminga and Duarte 2000; McGlathery, 2001; Waycott et al., 2009) and coral reefs (Brodie et al., 2011; Bell, 1992).

The process of eutrophication is usually understood as the biogeochemical response to heavy nutrient loading, leading to an increase in the rate of supply of OM to an ecosystem (Nixon, 1995; Cloern, 2001). More specifically, it has been defined as “the process of enrichment of water with nitrogen and phosphorus that stimulates production leading to enhanced algal growth and sometimes to phytoplankton blooms” (Likens, 1972). Since nitrogen is often the limiting factor for autotrophic production of OM in brackish or marine aquatic environments (Howarth, 1988), nitrogen enrichment is usually the primary causative agent of coastal eutrophication (Paerl and Piehler, 2008; Vitousek et al., 2002).

Typical signs of eutrophication include losses of water clarity and high levels of chlorophyll a (Boynton et al., 1982; Nixon and Pilson, 1983), indicating rapidly accelerated algal growth. Amplified primary production may even lead to blooms of phytoplankton (Paerl, 1988; Richardson, 1997), macroalgae (McGlathery, 2001) and epiphytes (Tomasko and Lapointe, 1991). While an enhanced phytoplankton biomass may be beneficial to consumers up to a certain threshold, negative effects can occur if the increase in nutrients is above the capacity of the system to absorb the increased
phytoplankton production (e.g. Rabalais et al., 2009). An elevated biomass of primary producers is generally responsible for deteriorated light conditions causing shading effects on benthic primary producers, such as seagrasses and corals. Eutrophication can also include changes in community composition of primary producers, as excess nitrogen and phosphorus delivery with respect to silica may lead to shifts from diatoms to non-siliceous algae (Conley et al., 1993). Modification of the silica/nitrogen and silica/phosphorus molar ratios in coastal waters can also promote shifts to plankton communities dominated by harmful and/or toxic algae species (Rabalais et al., 1996; Heisler et al., 2008 and references therein), causing enormous economic loss and serious impacts on human health (Anderson, 1997).

Furthermore, the high loads of primary produced OM triggers an increased microbial activity and related consumption of dissolved oxygen, which may lead to anoxia and hypoxia (Gerlach, 1990; Hagy et al., 2004; Zhang et al., 2010). Since oxygen depletion is stressful or even deadly to fauna and flora, the organisms in an ecosystem escape if possible or may suffer high mortalities (Diaz and Rosenberg, 1995; Glasgow and Burkholder, 2000; Rabalais and Turner, 2001, Diaz and Rosenberg, 2008). Moreover, anaerobic microbial pathways in sediments, such as sulphate reduction, are favoured, leading to accumulation of toxic hydrogen sulphide. Respective poisoning effects have been identified as the key factor in sudden die-off events in seagrass beds observed in several parts of the world (Robblee et al., 1991; Carlson et al., 1994; Seddon, et al., 2000; Plus et al., 2003; Borum et al., 2005).

1.4 Research needs regarding the drivers and effects of eutrophication

Although the effects of eutrophication are well-known, the mechanisms governing its effects are still poorly understood. Coastal waters exhibit varying sensitivities to nitrogen and other nutrient loads that are controlled e.g. by their size, hydrologic properties, morphology, vertical and horizontal mixing characteristics and climatic regimes (Paerl and Piehler, 2008). Therefore, the extent to which nutrient loading promotes eutrophication varies greatly among ecosystems and regions. Also, there is a variety of factors that influence the rate of nutrient and OM export from watersheds, including natural basin characteristics, hydrological and meteorological conditions and management practices (Howarth et al., 1996, 2006; Biggs et al., 2004; Schaefer and Alber, 2007; Sobota et al., 2009; Chen and Hong, 2011; Hughes et al., 2011). The highly differing responses to land use changes worldwide emphasize that
Regional studies are more important than global-scale analyses (Boyer et al., 2006; Alvarez-Cobelas et al., 2008).

The majority of regional studies on nutrient enrichment and potential eutrophication has been carried out in temperate regions, especially in the US and Europe, while much less is known from tropical regions. However, as nutrient inputs to the coastal zone are currently highest in tropical regions, especially South Asia, and are still projected to rise in the future (Seitzinger et al., 2010), human activities there will have the most vigorous effects on the ecology and the elemental cycles of the coastal ecosystems.

In tropical regions, high temperatures as well as episodic climatic events such as tropical storms strongly modify the degree to which eutrophication is manifested in coastal waters (Paerl and Piehler, 2008). The high temperatures generally accelerate conversion processes of OM (Grisi et al., 1998), while higher precipitation rates, which are dominated by short lived flood events, result in an overall higher and more episodic delivery of material to estuarine and coastal systems (Eyre and Balls, 1999). Besides that, more constant input of insolation together with high temperatures enables year round biological activity (Eyre and Balls, 1999).

As most studies on increasing nutrient runoff and associated eutrophication have been carried out in the temperate regions, where agriculture is by far the most important form of land use change, agricultural effects are often the only ones considered. Consequently, they are normally the only basis for global extrapolations (Meybeck, 1982; Smith et al., 2003; Bouwman et al., 2009; Seitzinger et al., 2010). This obstructs the view on other forms of land use changes, which in other regions of the world might have similar or even higher relevance for changes in the nutrient discharge to coastal ecosystems. For example, there is still not much known about the potential effects of nutrient release from land-based aquaculture. While aquaculture represents a much smaller nutrient input source than agriculture globally, it may be highly relevant locally (Paerl and Piehler, 2008). This is especially true for tropical regions, such as in SE Asia, where land-based aquaculture development has become a very important form of land use within the past decades. Nitrogen and phosphate budgets have been investigated for intensive shrimp farms in Thailand, Australia and the US (e.g. Hopkins et al., 1993; Briggs and Funge-Smith, 1994; Jackson et al., 2003). These studies reported on considerable nitrogen yields between 35 and 140 t km\(^{-2}\) yr\(^{-1}\) that are released from ponds. However, the effects of these on water quality in the adjacent environment have rarely been studied. Apart from that, the majority of the few existing assessments of pond aquaculture on adjacent creeks and coastal waters focused on small-scale pond culture, where only few ponds are maintained within a
relatively large area (e.g. Jones et al., 2001; Costanzo et al., 2004; Lin and Fong, 2008). Recent developments in many Asian countries, however, concentrated on large scale pond culture with agglomerations of pond complexes covering several hundred ha of coastal or estuarine areas, as e.g. found in Thailand, Vietnam and China. The Island Hainan in the south of China is a typical example for recent coastal development in SE Asia. Mangroves, coral reefs and seagrasses occur along the coastal shoreline of the tropical island, especially in the north-eastern and southern parts. These ecosystems are increasingly threatened, as the island has developed into an important centre for the production of aquaculture goods (Fig. 1). Besides that, it has experienced enormous population increase simultaneously with agriculture development over the past 60 years (Fig. 1). The agriculture area has been decreasing during the past 12 years due to booming aquaculture growth as well as the increasing infrastructure development for tourism. However, the GDP of agriculture yields from Hainan increased approximately 6 times between 1990 and 2006 (Statistical Bureau of Hainan Province, 2007), indicating an intensification of agriculture, most likely due to fertilization and pesticide usage. Furthermore, crop production changed from a grain-dominated agriculture to tropical fruits, tea, coffee and other plants of high economic value. All these developments make Hainan an interesting area for studying the effects of land use change, especially that of large-scale pond aquaculture, on adjacent tropical estuarine and coastal ecosystems.

Fig. 1: Development of the population (red circles), agriculture area (green triangles) and aquaculture area (blue squares) in Hainan province from 1952–2006. Data were obtained from the Hainan Statistical Yearbook (Statistical Bureau of Hainan Province, 2007). Aquaculture area includes coastal brackish water ponds as well as fresh water ponds.
1.5 Objectives and research questions

The overall aim of this thesis was to investigate how land-based anthropogenic activities change the biogeochemistry and ecological functions of adjacent, tropical, estuarine and coastal ecosystems, taking NE Hainan as a case study.

The following objectives and specific research questions were addressed:

Objective I
To determine the impact of land-based human activities on the fluxes and fate of nutrients and organic matter in estuarine and coastal waters of NE Hainan.

Specific research questions:

- Is there nutrient and OM enrichment in estuarine and coastal waters of Hainan?
- Which are the main sources, pathways and sinks of these nutrients and OM?
- Which are the predominant factors causing spatial and temporal variability in the concentration and composition of nutrients and OM?

Objective II
To evaluate the ecological consequences of anthropogenically increased biogeochemical fluxes on the estuarine and coastal system with special reference to seagrass meadows.

Specific research questions:

- Are there indicators of eutrophication in estuarine and coastal systems?
- Which associated processes affect coastal habitats, and which factors control (aggravate/mitigate) their impact?
- What is the current state of seagrasses in NE Hainan and how may it change in the future?

To approach these objectives, a suite of biogeochemical and ecological methods and experiments was conducted during four sampling campaigns to the NE coast of Hainan. This work was carried out within the German-Chinese LANCET project (Land-Sea Interaction along Coastal Ecosystems of Tropical China: Hainan) from December 2006 to December 2011.
2. MATERIAL AND METHODS

In the following, the study area and methods used during field and lab work of the thesis project are briefly presented. Study sites, sampling design and analytical methods are described in detail in the individual thesis chapters.

2.1 Field work

2.1.1 Study area

The study area is located at the NE coast of Hainan (Fig. 2a) and comprises a coastline of ~45 km. The northern part of the study area encompasses the Wenchang/Wenjiao Estuary (WWE; Fig 2b), a mid-size estuary that is fed by two lowland rivers (Wenchang and Wenjiao) debouching into a shallow (mean depth: 3 m), kidney-shaped lagoon (Bamen Bay). The estuarine lagoon comprises ~40 km² and is connected to the sea via a narrow channel. It has a catchment area of ~900 km² (Zeng and Zeng, 1989). The coastline is fringed by coral reefs in varying distance from the shore ranging from ~0.5 km in Ye Lin up to 3 km in Chang qi gang (Fig. 2b). Seagrass meadows, mainly dominated by the species *Thalassia hemprichii* and *Enhalus acoroides*, occur in their back-reef areas.

The region is characterized by a tropical monsoon climate with a dry season from November to April and a rainy season from May to October (Fig. 3). The NE coast is the major landfall corridor of typhoons in Hainan (Zeng and Zeng, 1989) and is annually affected by ~8 typhoons and directly hit by ~2.6 typhoons (Liu, 1984 cited in Huang, 2003). Typhoons usually occur between July and September and associated rainfall accounts for 35-60% of the total annual precipitation of 1500-2000 mm (Huang, 2003; Wang et al. 2008a; Wang et al., 2010). Hainan’s NE coast is subject to mixed semidiurnal microtides with a tidal range of about 0.5 m at neap and 1.5 m at spring tide.

The watershed of the study area has experienced far-reaching land use changes, especially in the past five decades. While mangrove forests still covered vast areas in the 1960’s, 73 % of it has been lost and their area was reduced to currently ~750 ha in the WWE (CHAPTER III). Instead, aquaculture ponds for the production of shrimp and fish now cover about 40 km² over the study area, half of that situated within the estuarine bay of the WWE. The other 20 km² of pond area are located directly along the coastline with 0.04 km² in Ye Lin, 2.4 km² in Qingge and 8.7 km² in Chang qi.
gang (CHAPTER I). Effluents from those ponds are directly released into the back-reef areas via drainage channels.

The major form of land use in the hinterland of the estuary and coastal shore is agriculture, mainly dominated by cultivation of mixed fruits and vegetables as well as coconut and rice. The area around Wenchang City is urbanized (116,000 inhabitants in 2006) and waste water is drained into the rivers without any treatment. During recent years, an increasing number of hotel complexes and other tourist facilities have been built in the area, contributing to residential effluents. Further background information on the WWE is presented in the study area sections of CHAPTER I, II, III, IV, and V. Information about the coastal back-reef areas in Ye Lin, Chang qi gang and Qingge and their hinterland are given in detail in CHAPTER I and VI.

![Map of study area](image_url)

**Legend**
- Gray: Towns
- Pink: Aquaculture ponds
- Green: Mangrove
- Light green: Seagrass
- Dark blue: Coral reef

**Fig. 2:** a) Location of the study area at the NE coast of Hainan and position of the weather stations Haikou and Qionghai (black dots) and b) overview on the study area including location of the specific study sites WWE, Ye Lin, Chang qi gang and Qingge (red frames), as well as coastal habitats, towns and aquaculture ponds. The map is based on satellite images (Geo EyeTM, 2009).
2.1.2 Sampling campaigns

Four sampling campaigns were carried out in the study area between 2006 and 2009. They took place during the dry season in December 2006, the rainy seasons in August 2007 and July/August 2008 and the transition period between dry and rainy season in March/April 2009 (Fig. 4). In 2008, samples were collected before and after a typhoon, which brought heavy rainfall to the study area. The WWE was sampled during all campaigns, whereas the work in the three coastal back-reef areas of Ye Lin, Chang qi gang and Qingge was carried out only during the expeditions in July/August 2008 and March/April 2009. All sampling campaigns were undertaken jointly with our Chinese project partners of the LANCET project.

Fig. 4: Record of daily precipitation for Haikou and Qionghai (Fig. 2a), the two weather stations closest to the study area, during the sampling campaigns in December 2006, July/August 2007 and 2008, and March/April 2009. Periods, during which samples were collected, are marked by a gray-shaded rectangle for each field campaign. Strong rainfall in August 2008 was associated with typhoon Kammuri. Source: Climate Centre, Utah State University, http://climate.usurf.usu.edu/products/.
2.1.3 Biogeochemical sampling

Sampling of water, suspended matter (TSM) and sediments along estuarine and near-shore coastal waters was conducted from small boats or from the shore. In the coastal back-reef areas, sample collection was accomplished by snorkelling or from small fishing boats. Terrestrial plants, soils and water samples from aquaculture ponds and drainage channels were collected at different stations during land excursions.

Salinity, temperature, pH and dissolved oxygen (DO) were measured in situ using a WTW multi-parameter probe and a Hach Lange LDO™ HQ40d portable dissolved oxygen meter. Water samples were collected with a bucket and a Niskin bottle for surface and bottom water, respectively. Water samples for the analysis of dissolved matter were immediately filtered through single use Sartorius Minisart® membrane filters, fixed and stored cool and dark. Water samples for suspended matter were stored in PE tanks until filtration onto pre-combusted and pre-weighed Whatman GF/F filters in the field lab the same day. The filtrate was acidified to pH 1.5 with concentrated hydrochloric acid and stored frozen in 1L PE bottles. Surface sediments and soils were collected directly with a spoon or with a grab sampler and sediment cores were taken using hand-corers. Seagrasses and terrestrial plants were collected, sorted and rinsed, and combined to pooled samples usually consisting of 2-10 leaves or shoots. Samples of sediments, soils and plants were stored cool in plastic bags or glass vials until they were dried at 40 °C in the field lab within the same day after collection or fixed with zinc acetate. Filters were also dried at 40 °C and stored in plastic boxes. All samples were shipped to Germany for analytical evaluation.

2.1.4 Experimental work

A phytoplankton bioassay was carried out in the three investigated back-reef areas (CHAPTER VI) based on a method modified from Dalsgaard and Krause-Jensen (2006). Offshore water was incubated over 4 days in dialysis bags at 4-6 stations along perpendicular transects reaching from the shore line to the reef crest (Fig. 5).
MATERIAL & METHODS

Fig. 5: Position of the sampling transects for the experimental work and the seagrass assessment at the three coastal back-reef areas Ye Lin, Chang qi gang and Qingge. Stations were situated in a distance of 50, 100, 250, 500, 1000 and 2500 m from the shoreline, depending on the size of the respective back-reef areas.

2.1.5 Seagrass assessment

A seagrass assessment was carried out along the coastal transects in the three investigated back-reef areas (Fig. 5):

Shoot density of the seagrass species occurring at a station was determined by counting the number of shoots of each seagrass species present inside a quadrate (n=30). The quadrate size varied between 200 cm² and 2500 cm² depending on shoot numbers. Quadrates were placed approximately 5 m apart from each other along a grid (20 quadrates parallel and 10 quadrates perpendicular to the shore).

Above- and belowground biomass was obtained by collecting all seagrass tissue within a randomly placed 20 cm x 20 cm quadrate attached to the ground by iron pegs (n=5). The number of shoots in the quadrate was counted before plant collection. In the laboratory, plants were rinsed carefully to remove excess salts and were sorted according to species and above- and belowground parts. Epiphytes of *T. hemprichii* and *E. acoroides* were removed from the leaves by gentle scraping with a knife and were kept in glass vials. Leaf epiphytes of all other species were removed by rinsing in 10% HCl and additional scraping, if necessary. Seagrass fractions and epiphytes were
dried on aluminium foil tares at 40 °C and weighed for dry weight (DW) determination. Above- and belowground biomass (per leaf and per shoot) as well as its ratio were calculated for all species. Epiphyte load was calculated as the biomass of epiphytes on a certain number of leaf shoots divided by the biomass of the shoots (mg epiphyte DW g⁻¹ leaf DW).

2.1.6 Background information

Interviews with randomly selected pond owners were carried out in order to collect information about operating characteristics of fish and shrimp farms in the area (CHAPTER I). Interviews were based on a semi-structured questionnaire with minutes translated and transcribed from memory.

Information about the spatial extent of coastal habitats (mangrove, seagrass, coral reef) as well as urban settlements and aquaculture ponds was received by analysis of satellite images (Geo EyeTM, 2009). Images were geo-referenced, groundtruthed and digitized using ESRI ArcGIS 9.

2.2 Analytical work

A range of analytical methods was applied for the various samples of water, suspended matter, sediments, soils and plants. Tab. 1 gives an overview on the various parameters determined, and the according measurement devices and methods used. The respective methods are described in detail in the thesis chapters except for the determination of acid volatile sulphides (AVS) and chromium reducible sulphides (CRS) in coastal sediments as well as of stable sulphur isotopes (δ³⁴S) in seagrass tissue.

AVS and CRS were analyzed in samples fixed with 1 M ZnAct using the two-step distillation technique of Fossing and Jørgensen (1989). Homogenized sediment subsamples (~4 g) were transferred to a distillation flask, and 10 ml of 50% ethanol was added. After degassing with N₂ for 15 min, 8 ml of 6 M HCl was added to the slurry and distillation was conducted at room temperature for 30 min to release AVS. Subsequently, the slurry was distilled for 30 min while boiling after addition of 16 ml of reduced Cr²⁺ to release CRS. AVS and CRS released during distillations were trapped in 10 ml of 250 mM ZnAct. The pools of AVS and CRS were determined by analyzing H₂S in the distillates by the photometric method of Cline (1969).
Powdered leaf, root and rhizome tissues of the seagrass *T. hemprichii* were rolled in tin cups together with ~8 mg vanadium pentoxide, and were analyzed for the δ^{34}S by ISO-ANALYTICAL (Cheshire, UK) using Elemental Analysis Isotope Ratio Mass Spectrometry (EA-IRMS).

### Tab. 1: Overview of parameters measured and methods applied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample material</th>
<th>Measurement device and methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrients</td>
<td>water</td>
<td>Skalar SAN(^\text{TM}) System (Grasshoff et al., 1999)</td>
</tr>
<tr>
<td>DOC</td>
<td>water</td>
<td>Apollo 9000</td>
</tr>
<tr>
<td>DON</td>
<td>water</td>
<td>Shimadzu</td>
</tr>
<tr>
<td>Chl a</td>
<td>suspended matter</td>
<td>Lovibond Spectro 1.0 photometer (Lorenzen, 1967)</td>
</tr>
<tr>
<td>TN</td>
<td>suspended matter</td>
<td>Turner 10-Au fluorometer (Arar and Collins, 1997)</td>
</tr>
<tr>
<td>Corg</td>
<td>suspended matter</td>
<td>Carbo-Ehba NA 2.00 (Verardo et al., 1990)</td>
</tr>
<tr>
<td>AVS/CRS</td>
<td>suspended matter</td>
<td>Flash 1112 EA &amp; Thermo Finnigan Delta Plus MS</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>suspended matter</td>
<td>Biochrom 30 amino acid analyzer</td>
</tr>
<tr>
<td>δ(^{15})N</td>
<td>suspended matter</td>
<td>Flash 1112 EA &amp; Thermo Finnigan Delta Plus MS</td>
</tr>
<tr>
<td>δ(^{13})Corg</td>
<td>suspended matter</td>
<td>Flash 1112 EA &amp; Thermo Finnigan Delta Plus MS</td>
</tr>
<tr>
<td>δ^{34}S</td>
<td>seagrass</td>
<td>EA-IRMS at ISO-ANALYTICAL (Cheshire, UK)</td>
</tr>
</tbody>
</table>
3. RESULTS AND SYNOPTIC DISCUSSION

3.1 Impact of land-based human activities on nutrient and organic matter fluxes and fate in estuarine and coastal waters of NE Hainan

Three different kinds of land-based human activities were identified to potentially have an impact on the nutrient and organic matter (OM) fluxes, transformation processes and fate in the Wenchang/Wenjiao Estuary (WWE) as well as in the back-reef areas of the NE coast of Hainan:

- **Agriculture**, dominating the catchment area
- **Residential areas**, including the cities Wenchang and Wenjiao
- **Brackish-water pond aquaculture**, with ponds covering approximately 40 km² of the study area and significant related effluent, nutrient and OM fluxes (CHAPTER I).

It is conceivable that variable exposure to these sources as well as different transport and transformation processes cause spatial and temporal variability in nutrient and OM distribution in the estuarine and coastal waters. Additionally, the amplitude of associated impacts on the ecosystems underlies a number of factors, of which precipitation was identified as the most important.

After describing the overall distribution of nutrients and OM in the WWE and coastal back-reef areas in the following, their sources, pathways and fate under low to moderate and under heavy precipitation conditions are discussed, before the spatial and temporal effects of other factors are elucidated.

3.1.1 Distribution of nutrients and organic matter in estuarine and coastal waters

Comparison of the results from the different sampling events during the four campaigns revealed a strong spatial and temporal variability in concentration and composition of inorganic nutrients (CHAPTER I, II, III, V) and dissolved and particulate OM (CHAPTERs I, II and I, IV, V, VI, respectively) over the estuarine gradient of the WWE (CHAPTER I, II, III, IV, V) as well as over an offshore gradient in three coastal back-reef areas (CHAPTER I, IV).

In the upper estuary of the WWE, nutrient concentrations from usually 35-110 μM DIN and 0.4-1.5 μM PO₄³⁻ (CHAPTER II, III) significantly exceed nutrient concentrations <10 μM DIN and <0.3 μM PO₄³⁻ reported from pristine river-estuarine systems of comparable sizes in NE Australia and the Baltic (Eyre and Balls, 1999;
RESULTS & SYNOPTIC DISCUSSION

Humborg et al., 2003), and were mostly above average global conditions (Smith et al., 2003). In contrast, concentrations of mostly <10 μM DIN and <0.5 μM PO₄³⁻ near the estuarine outlet point to nutrient decreases along the estuarine gradient to rather pristine conditions. Such gradients were similarly observed for dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) along the estuary (CHAPTER II). Similarly, particulate organic matter (POM) content tended to be higher in the upper estuary (~13% POC and ~1.7% TN) than close to the outlet (~3% POC and ~0.4% TN), even though TSM concentrations showed an opposite trend, increasing along an downstream gradient from ~15 mg L⁻¹ to ~30 mg L⁻¹ (CHAPTER IV, V). Decreasing concentrations of nutrients and OM generally suggest the land-derived input of external nutrients (and OM) into the upper WWE causing enrichment in the upper and middle estuary. However, associated with heavy rain, nutrient and dissolved organic matter (DOM) concentration were temporarily elevated up to 50 μM DIN, 1 μM PO₄³⁻ and 25 μM DON over the entire estuarine gradient. The same trend also accounted for OM composition of the TSM. This points to temporal nutrient and OM enrichment of the entire WWE resulting from different nutrient and OM inputs, transport and processing.

In coastal back-reef areas, average DIN and PO₄³⁻ concentrations close to shore were much higher in coastal water that had a large pond area in the hinterland (Qingge and Chang qi gang; 9-14 μM DIN and 1.2-1.5 μM PO₄³⁻) than those with few aquaculture ponds on shore (Ye Lin; 5 μM DIN and 0.6 μM PO₄³⁻; CHAPTER I, VI). These nearshore DIN concentrations were also ~3 times higher than 1-2 km further offshore (3-4 μM DIN) indicating substantial land-derived nutrient inputs into coastal waters (CHAPTER I, VI). Despite a considerable variability in nutrient concentrations between the different sampling events, concentrations at these back-reef areas were mostly several times higher than those reported from pristine coastal tropical waters of <2 μM DIN and <0.5 μM PO₄³⁻ (e.g. Szmant, 2002). This points to nutrient enrichment of varying temporal intensity derived directly from the coastal shore. Concentrations of DON, DOC (CHAPTER I) and TN (CHAPTER VI) showed a similar trend, revealing OM distribution to be also affected by land-based sources.

The spatial and temporal variability in the nutrient and OM distribution in estuarine and nearshore coastal waters is explained by the following processes.
3.1.2 Sources, pathways and fate of nutrients and organic matter under low to moderate precipitation conditions

In the following, the main sources, pathways and sinks affecting nutrients and OM in the WWE and nearshore coastal back-reef areas under low to moderate precipitation conditions are discussed and summarized in Tab. 3a. Additionally, the main biogeochemical processes involved are illustrated in a simplified sketch (Fig. 6).

![Summary sketch of biogeochemical processes in WWE](image)

**Fig. 6: Simplified sketch summarizing the major biogeochemical processes in the WWE under low-moderate precipitation as presented in CHAPTER V (Herbeck et al., 2011).**

**IN THE WENCHANG/WENJIAO ESTUARY (WWE)**

The composition of nitrogen and $\delta^{15}$N of NH$_4^+$, NO$_3^-$ and TSM suggests two main sources affecting the nutrient and OM of the WWE under low to moderate rain conditions.

A relatively high NO$_3^-$ contribution (~55%) of DIN and low $\delta^{15}$N values of the TSM (~1.5‰) in the upper estuary reveal an input of nutrients from agriculture into the rivers Wenchang and Wenjiao (CHAPTER II, V), since effluents from agriculture fields fertilized by artificial fertilizers are usually high in NO$_3^-$ (Mian et al., 2009) and have a low $\delta^{15}$N of -2 to 2‰ (Lee et al., 2008). Additionally, benthic nutrient fluxes from the sediment as a result of strong benthic recycling contribute as internal source to the
observed high nutrient concentrations in the upper catchment area (CHAPTER V). This is conceivable because fractionation during remineralization of the high OM inputs into the sediment would likely also lead to fluxes of DIN with a low $\delta^{15}N$.

In contrast, a predominance of $\text{NH}_4^+$ (~80%) in the DIN pool of large parts of the downstream estuarine area points to $\text{NH}_4^+$ and DON from shrimp and fish ponds as main sources, as these are the dominant nitrogen components in their effluents (CHAPTER I, II, V). Nitrogen in pond effluents was usually heavily enriched in $^{15}N$ due to volatilization of ammonia ($\text{NH}_3$) and other discrimination processes during recycling of OM from feed and faeces that accumulate in shrimp and fish ponds over crop production (CHAPTER VI). Values of ~17‰ $\delta^{15}N-\text{NH}_4^+$ and ~7‰ $\delta^{15}N-\text{NO}_3^-$ are the first existing measurements from aquaculture ponds, and confirmed the assumptions of previous studies that pond effluents are enriched in $^{15}N$ (CHAPTER VI). Preliminary data of the $\delta^{15}N-\text{NH}_4^+$ and $\delta^{15}N-\text{NO}_3^-$ in waters of the WWE (Fig. 7), which were on similarly high levels than in aquaculture effluents, suggest aquaculture effluents to be the dominant nitrogen source in the estuarine lagoon.

![Distribution of $\delta^{15}N-\text{NH}_4^+$ and $\delta^{15}N-\text{NO}_3^-$](image)

Fig. 7: Distribution of $\delta^{15}N-\text{NH}_4^+$ and $\delta^{15}N-\text{NO}_3^-$, in the Wenchang/Wenjiao Estuary, NE Hainan, during July/August 2008 and March/April 2009. Samples were taken under low to moderate rain conditions.

Theoretically, it is also conceivable that untreated municipal effluents as well as runoff from agriculture fields that were fertilized with manure added to the high nutrient and OM loads from aquaculture effluents, because both potential sources are also known to mainly consist of $\text{NH}_4^+$ and to be enriched in $^{15}N$ (Jones et al., 2001).
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However, if municipal effluents were of importance in the area, they would rather have affected the upper estuary near the cities Wenchang and Wenjiao, where nutrient composition and δ^{15}N reflected agriculture as the main source. Effluents from fields fertilized with manure are unlikely to be the source of the high nutrients and OM, as artificial fertilizers based on N-fixation are rather applied on the fields in the catchment area than manure (pers. comm. local farmers). With simultaneous consideration of the large area of the estuarine lagoon that is covered by shrimp and fish ponds (∼20 km²), from which an estimated volume of 210 *10^6 m³ effluents is annually released (CHAPTER I), it is concluded that aquaculture effluents were the predominant source accounting for the nutrient concentrations. It is conceivable, though, that wastes from fish net cages, which cover ∼5 ha in the estuarine lagoon contribute to the pond-based aquaculture effluents.

Uptake of the nutrients by phytoplankton was the main transformation process in the upper estuary and the estuarine lagoon. In-situ production thus represents a major sink for the nutrients, while at the same time it is the main source of the OM in the WWE. This was indicated by the primarily autochthonous origin of the TSM, as indicated by a C/N ratio close to the Redfield ratio of 6.7 (Redfield et al., 1963), δ^{13}C_{org} of the TSM ranging from -22 to -32‰, typical for a mixture of marine (-18 to -22‰; Fischer, 1991) and fresh water phytoplankton (-29 to -32‰; Martinelli et al., 1999), as well as the composition of particulate amino acids and hexosamines (IV, V). In contrast, the contribution of soils and sediments was generally low, only slightly increasing towards the outlet of the estuarine lagoon due to tidal-driven resuspension of sediments (CHAPTER IV). Phytoplankton biomass is sustained by agriculture derived nutrients, especially NO_3^-, in the upper estuary as indicated by the low δ^{15}N (∼1.5‰), while uptake of aquaculture-derived nutrients, especially ^15N-enriched NH_4^+ as well as inputs of TSM high in δ^{15}N with effluents from aquaculture ponds resulted in the high δ^{15}N values of TSM of usually >7‰ in the estuarine lagoon (CHAPTER V). The distinct spatial difference between sources of nutrients and OM between the upper catchment area and the estuarine lagoon was most likely derived from immediate consumption of large parts of the in-situ production (Maier, 2010) from agriculture derived sources in the upper estuary (CHAPTER V). Therefore, the impact of effluents from agriculture fertilizers is mainly restricted to the upper catchment area, while impacts of nutrients from aquaculture ponds predominate in the estuarine lagoon of the WWE.

Besides phytoplankton, other primary producers such as remaining mangroves and water hyacinths also take up the nutrients from aquaculture effluents, as reflected by the elevated δ^{15}N values in the plant tissues (Tab. 2a; CHAPTER V). Uptake by
primary producers resulted in decreasing nutrient concentrations along the estuarine downstream gradient. Additional processes reducing nutrient concentrations are tidal driven dilution with nutrient-poor marine waters, as indicated by high salinities in the estuarine lagoon (CHAPTER I, II, III, IV, V), as well as PO₄³⁻ removal due to its adsorption behaviour onto suspended particles (CHAPTER II). However, much lower nutrient concentrations than expected from linear mixing of marine and fresh water end-members points to uptake by phytoplankton and other primary producers as the primary process for nutrient removal along the estuarine gradient (CHAPTER V). In the WWE, nutrient uptake by primary producers is likely facilitated by an enhanced residence time due to the enclosed shape of the estuarine lagoon. The efficient consumption of nutrients within the estuarine lagoon leads to a restricted nutrient export from the estuary into coastal waters, despite the high nutrient inputs from aquaculture.

While parts of the phytoplankton biomass are likely exported into coastal waters with tidal movement, most of the phytoplankton biomass remains within the estuarine lagoon, where it is consumed, recycled or buried in the sediment (CHAPTER IV, V). Decomposition of planktonic and aquaculture-derived OM likely displays an additional internal source of nutrients to estuarine waters. Burial of phytoplanktonic OM in estuarine sediments was evident from high δ¹⁵N in sediments of the past 30 years (6-7‰) compared to older sediments originating from before 1990 with a δ¹⁵N of 5-6‰ (Fig. 8). The high δ¹⁵N values of recent sediments thus reflect burial of aquaculture derived nitrogen in estuarine sediments. Consumption of estuarine phytoplankton by primary consumers and further transfer up the food chain was revealed by high δ¹⁵N of 10-14‰ in the estuarine consumers of the study area (Tab. 2a). δ¹⁵N values usually increase by 3-4‰ with each trophic level (Minawage and Wada, 1984). For each trophic level, δ¹⁵N values were higher than those reported from little affected estuaries (McClelland et al., 1997) and comparable to those reported from other estuaries affected by sewage effluents (e.g. McClelland et al., 1997; Schlacher et al., 2005; Hadwen and Arthington, 2007), indicating that the aquaculture-derived nitrogen is transferred through all trophic levels of the food web. Considerable parts of the fish catches in the estuarine lagoon are not used for human consumption, but instead used as so called “trash fish” for feeding shrimps and fish in ponds (Krumme et al., subm.). Their biomass is in parts recycled in the ponds and exported in form of nutrients and OM into estuarine waters. Thus, higher trophic level organisms, such as estuarine fishes may be sinks and sources of nutrients and OM passing the estuarine recycling loop again and again.
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#### Tab. 2: $\delta^{15}$N ranges in various organisms in the WWE (a) and back-reef areas (b) in NE Hainan

<table>
<thead>
<tr>
<th>Organism</th>
<th>$\delta^{15}$N [‰]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) In the estuarine lagoon of the WWE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>phytoplankton</td>
<td>7 - 10</td>
<td>CHAPTER V</td>
</tr>
<tr>
<td>water hyacinths</td>
<td>7 - 12</td>
<td>CHAPTER V</td>
</tr>
<tr>
<td>mangroves</td>
<td>6 - 8</td>
<td>Herbeck, et al. (unpubl. data)</td>
</tr>
<tr>
<td>crustaceans</td>
<td>7 - 8</td>
<td>Herbeck, et al. (unpubl. data)</td>
</tr>
<tr>
<td>fishes (detritivores)</td>
<td>11 - 14</td>
<td>Krumme et al. (unpubl. data)</td>
</tr>
<tr>
<td>fishes (herbivores)</td>
<td>11 - 14</td>
<td>Krumme et al. (unpubl. data)</td>
</tr>
<tr>
<td>fishes (zooplanktivores)</td>
<td>11 - 15</td>
<td>Krumme et al. (unpubl. data)</td>
</tr>
<tr>
<td>b) In the coastal back-reef areas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>seagrass</td>
<td>5 - 9</td>
<td>CHAPTER VI</td>
</tr>
<tr>
<td>epiphytes</td>
<td>7 - 10</td>
<td>CHAPTER VI</td>
</tr>
<tr>
<td>macroalgae</td>
<td>6 - 8</td>
<td>Scharfbillig (2009)</td>
</tr>
<tr>
<td>fishes</td>
<td>10 - 17</td>
<td>Scharfbillig (2009)</td>
</tr>
<tr>
<td>bivalves</td>
<td>8 - 9</td>
<td>Scharfbillig (2009)</td>
</tr>
<tr>
<td>gastropodes</td>
<td>7 - 9</td>
<td>Scharfbillig (2009)</td>
</tr>
<tr>
<td>crustaceans</td>
<td>8 - 12</td>
<td>Scharfbillig (2009)</td>
</tr>
<tr>
<td>polychaetes</td>
<td>11 - 14</td>
<td>Scharfbillig (2009)</td>
</tr>
</tbody>
</table>

![Fig. 8: $\delta^{15}$N along depth in a) a dated mangrove sediment core from the WWE (Bao et al., subm.), and b) a sediment core from the middle of Bamen Bay (Herbeck and Unger, unpubl. data)](image-url)
RESULTS & SYNOPTIC DISCUSSION

IN THE COASTAL BACK-REEF AREAS

High $\delta^{15}$N values of usually $>$8‰ of various primary producers in coastal waters close to the shore at the three back-reef areas of Ye Lin, Qingge and Chang qi gang, indicate the uptake of $^{15}$N enriched DIN (CHAPTER VI). Strong increases in $\delta^{15}$N from $\sim$6‰ to up to 13‰ over time of offshore phytoplankton incubated close to the shore in a bioassay experiment also proved that mainly the $^{15}$N enriched ammonium released from aquaculture ponds was taken up (CHAPTER VI). This implies that effluents from coastal aquaculture ponds that are directly released to coastal waters via drainage channels are a significant nutrient source for coastal back-reef areas and are responsible for the observed nutrient enrichment in coastal waters.

Decreasing $\delta^{15}$N values in phytoplankton, seagrass and epiphytes in combination with water column DIN in offshore direction indicate a removal of pond derived nitrogen and increasing nitrogen contributions from unaffected marine sources. Similar to estuarine waters, uptake by primary producers, including phytoplankton, benthic and epiphytic algae and seagrasses, as well as tidal-driven mixing with nutrient-poor ocean water were likely the main removal processes. Nevertheless, $\delta^{15}$N in epiphyte and seagrass tissues higher than 5-7‰, the typical $\delta^{15}$N value of seawater (Miyake and Wada, 1967; Wada et al., 1975), and significant elevation of $\delta^{15}$N after incubation in a bioassay reflect the persisting impact of aquaculture-derived nitrogen at the stations furthest offshore. This, together with significant increases in $\delta^{15}$N of incubated offshore water at these stations, indicates that aquaculture effluents affect the entire back-reef areas exceeding 1000 m from the shore in Qingge and 2500 m from the shore in Chang qi gang (CHAPTER VI). This is corroborated by findings of Roder et al. (subm.), who reported high $\delta^{15}$N values in the tissue of the coral *Porites lutea* in five reefs in NE Hainan varying from 6.8-9.0‰ in coral host and 6.9-9.3‰ in zooxanthellae.

The average $\delta^{15}$N measured in the seagrass *T. hemprichii* from the back-reef areas of Hainan is the highest ever measured in this species worldwide (Fig. 9). Similarly, $\delta^{15}$N values in epiphytes were the highest ever measured (CHAPTER VI). This indicates that the seagrass meadows of NE Hainan are extremely exposed to aquaculture wastes.
Comparable to inner-estuarine findings, the main OM sinks are sediment recycling and burial, as well as consumption by higher trophic levels. Indicative for that were high $\delta^{15}$N values of sedimentary OM ($7.5\pm0.4\%_\text{o}; n=12$), as well as those of various consumers in the back-reef areas (Tab. 2b). Parts of the OM might have reached open ocean waters and were buried or processed there. Overall, similar to the estuarine lagoon, the back-reef areas of NE Hainan are substantially enriched with aquaculture derived nutrients.
3.1.3 Sources, pathways and fate of nutrients and organic matter under heavy precipitation

In the following, the main sources, pathways and sinks affecting nutrients and OM in the WWE and nearshore coastal back-reef areas under heavy precipitation are discussed and summarized in Tab. 3b. Additionally, the main biogeochemical processes involved during and shortly after heavy precipitation are illustrated in simplified sketches (Fig. 10).

Fig. 10: Simplified sketches summarizing the major biogeochemical processes in the WWE under heavy precipitation (a) and several days after the occurrence of heavy precipitation (b) as presented in CHAPTER V, Herbeck et al., 2011.

IN THE WENCHANG/WENJIAO ESTUARY (WWE)

During heavy rain events, nutrient and TSM concentrations in the estuarine lagoon were up to 3 times higher compared to “normal” conditions (V). NO$_3^-$ dominated the DIN pool (II, V), and the TSM mainly consisted of terrestrial OM (CHAPTER IV, V). Those alterations were caused by rain-derived erosion and leaching from agriculture
fields, resulting in additional inputs of sediments, soils and nutrients, predominantly NO$_3^-$, into the estuary that even outnumbered the continuing supply of aquaculture effluents. Furthermore, the rainwater itself represents an additional source of nutrients, containing up to 20 μM NO$_3^-$ and NH$_4^+$, respectively, as measured during the typhoon Kammuri (CHAPTER V). Atmospheric nitrogen deposition originating mainly from fossil fuel and other combustion products, as well as agriculture emissions are considered to be a significant “new” N source to estuarine and coastal waters globally (Paerl, 1995, 1997), and especially along the Chinese coast (Duce et al., 2008).

The shift in sources of nutrients and OM was also accompanied by changes in salinity due to an increased freshwater runoff that reduced the residence time in the estuary and flushed the estuarine lagoon (V). The magnitude of estuarine flushing as well as the duration until system recovery depends on rain intensity, and is likely severest during and after typhoons, such as observed during typhoon Kammuri (CHAPTER II, III, IV, V). During estuarine flushing, nutrient uptake by primary producers was intermitted, because phytoplankton was exported from the estuarine lagoon and primary production was light limited due to elevated concentration of allochthonous TSM that was washed into estuarine waters (CHAPTER III, IV, V). Thus, agriculture-derived nutrients and OM that were transported from the upper catchment area into the estuarine lagoon are directly exported, and their sinks are predominantly found in coastal waters (see below).

An immediate flush-out of nutrients and OM from the estuarine lagoon into coastal waters was observed even as a response to comparatively little precipitation associated with a local thunderstorm (CHAPTER III). This indicates that river export is likely aggravated due to losses of mangroves in the estuary as well as natural vegetation in the watershed that remove and/or retain nutrients and OM (CHAPTER III, V). Additionally, the construction of roads, aquaculture ponds and other urban structures raise the imperviousness of the soil, and thereby accelerate the storm water runoff into coastal waters. In summary, urbanization, agriculture and aquaculture development in the WWE have led to an increased exposure of estuarine and coastal waters to nutrients and TSM that are introduced into these systems during and after rain events.

**IN THE COASTAL BACK-REEF AREAS**

Under heavy precipitation, the back-reef areas of NE Hainan receive agriculture-based NO$_3^-$-and terrestrial OM-rich storm water from estuaries and streams in addition to the effluents dominated by NH$_4^+$, as well as phytoplankton and POM that are released via drainage channels along the entire shore line (CHAPTER I). Nutrients,
exported from the WWE after rainfall, are mainly taken up by phytoplankton after settling of the exported terrestrial particles, thereby improving light conditions. In combination with the high amounts of nutrients, this may even cause phytoplankton blooms several days after the rain event, as observed after typhoon Kammuri (CHAPTER V). Seagrasses and other benthic macrophytes at Ye Lin that are exposed to the river plume of the WWE during rain-related freshwater export also contribute to nutrient uptake, as indicated by increasing δ¹⁵N values in seagrasses and epiphytes towards the reef crest (CHAPTER VI). Parts of the deposited planktonic biomass and the exported allochthonous particles are likely recycled an/or buried in the nearshore sediments close to the WWE, as indicated by high sediment loads deposited in coral reefs of Ye Lin (Roder pers. comm.). Other parts may be transported to ocean waters, though the importance of the respective sinks for OM has to be further investigated.

Tab. 3: Overview of the predominant sources, pathways/transformation processes, and sinks of nutrients and OM in the different compartments of the estuary and nearshore coastal zone.

<table>
<thead>
<tr>
<th></th>
<th>Low to moderate precipitation</th>
<th>Heavy precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper WWE</td>
<td>Estuarine lagoon of WWE</td>
</tr>
<tr>
<td><strong>Main Sources</strong></td>
<td>nuts</td>
<td>ag</td>
</tr>
<tr>
<td>nutrients</td>
<td>agriculture effluents</td>
<td>OM-recycling*</td>
</tr>
<tr>
<td>OM</td>
<td>in-situ production</td>
<td>in-situ production</td>
</tr>
<tr>
<td><strong>Main Pathways</strong></td>
<td>uptake</td>
<td>uptake</td>
</tr>
<tr>
<td>nutrients</td>
<td>consumption</td>
<td>consumption</td>
</tr>
<tr>
<td>OM</td>
<td>deposition</td>
<td>deposition</td>
</tr>
<tr>
<td></td>
<td>bacterial remineralization</td>
<td>bacterial remineralization</td>
</tr>
<tr>
<td></td>
<td>tidal transport</td>
<td>tidal transport</td>
</tr>
<tr>
<td><strong>Main Sinks</strong></td>
<td>nutrients</td>
<td>phytoplankton</td>
</tr>
<tr>
<td></td>
<td>hyacinths, algae, mangroves</td>
<td>hyacinths, algae, mangroves</td>
</tr>
<tr>
<td>OM</td>
<td>higher trophic levels</td>
<td>higher trophic levels</td>
</tr>
<tr>
<td></td>
<td>sediment burial</td>
<td>sediment burial</td>
</tr>
</tbody>
</table>

*internal sources
3.1.4 Other factors causing spatial and temporal variability in nutrient and organic matter fluxes and transport

Nutrient and OM regimes underlie various seasonal impacts that are of relevance in the estuarine and nearshore coastal systems. Rain-derived nutrients and OM from agriculture can generally be expected to influence the system more during the rainy season from May to October (Fig. 3), although rain rates in this period are highly variable. Dry periods alternate with extreme rainfall of up to 300 mm d\(^{-1}\), often related to typhoons. This causes a high variability of the nutrient and OM sources and processing during that time and may also account for the differences observed during the two sampling campaigns in August 2007 and 2008 (CHAPTER II, III, IV).

Moreover, inputs of nutrients and OM are likely among the highest at the beginning of the rainy season, because fertilizers and OM that may have accumulated over the dry season are leached all at once and flushed into estuarine and coastal waters with the first rainfall (e.g. Eyre and Balls, 1999; Boonphakdee and Fujiwara, 2008). This so called “first-flush effect” was most likely observed in March/April 2009, at the transition between the dry and the rainy season, when e.g. much higher concentrations of PO\(_4^{3-}\), DOC and DON were found in coastal waters compared to July/August 2008 (CHAPTER I).

In addition, the time of fertilization or harvesting of crops is an important factor influencing seasonality in nutrient and OM input (Beman et al., 2005; Chen and Hong, 2011). However, due to the high variety of crops that are cultured in the catchment area, including various vegetables, fruits and grains, that have different cultivation cycles and according fertilisation demands, no specific trends are expected with this respect. Thus, it is conceivable that the amount of fertilizers introduced into natural water bodies is rather determined by the presence of rain than by the fertilization time.

However, there is a source-specific seasonal variability in nutrient inputs from aquaculture ponds, because shrimp production is intermitted in some ponds during the colder months from December until February, when the growth rate of shrimps is decelerated (CHAPTER I). This can reduce effluent-derived nutrient and OM inputs in some areas during that time.

In addition to seasonal effects, receiving estuarine and coastal waters are exposed to a high local short-term variability of nutrient and OM inputs, as ponds release their effluents pulsed into drainage channels, where they mix with the effluents of other ponds. The effluents may also vary strongly in concentration related to the crop age with effluents from ponds with older animals being more enriched in nutrients and OM (CHAPTER I). This variation in effluent composition contributes to the large variability in coastal nutrient concentrations, especially close to the shore (CHAPTER I,
VI), and was responsible for exceptionally high nutrient concentrations measured temporarily at a station during a 24 h time series sampling (CHAPTER III).

Another factor determining the small-scale spatio-temporal distribution and sources of nutrients and OM are tidal dynamics, which were to a large part responsible for the dilution by seawater during high tide. The role of the tides is largest inside the estuarine lagoon, where salinity between 0 and 34 was observed (CHAPTER I, II, III, IV, V). Despite the micro-tidal regime, strong differences in nutrient concentrations were observed over a spring tidal cycle (CHAPTER III). Tidal dynamics were also responsible for a partial stratification in the estuarine lagoon with salt wedge character. Inflowing bottom water was usually of higher salinity, DO and pH and lower temperature and nutrient concentrations than the surface water, providing regular ventilation of the estuary (CHAPTER III). However, during strong rainfall, the tidal effects were of minor importance, due to the increased freshwater discharge.

**KEY FINDINGS related to objective I:**

- Estuarine and nearshore coastal waters of NE Hainan are considerably enriched in nutrients and OM, though there is a significant spatial and temporal variability.
- Precipitation is the predominant factor determining the sources, inputs, transport and biological processing of nutrients and OM. Tides and variable inputs associated to source-specific operation characteristics contribute to their local and short term variability.
- Agriculture effluents mainly affect the upper WWE, while effluents from aquaculture ponds are the main source of nutrients and OM in the estuarine lagoon of the WWE and coastal back-reef areas.
- Under low to moderate rain conditions, the export of anthropogenic nutrients from the WWE into coastal waters is restricted due to an efficient nutrient uptake by primary producers inside the estuarine lagoon. However, aquaculture ponds that release their effluents directly into coastal waters cause continuous nutrient enrichment exceeding a distance of 2500 m from the shore in the back-reef areas.
- Heavy precipitation causes pulsed exports of nutrients and OM derived from agriculture in the upper WWE into coastal waters, adding to the pond-derived nutrient enrichment. Exports are most likely enforced by anthropogenic alteration in water flow and the loss of mangroves that would reduce and/or retain nutrients and sediments.
3.2 Ecological consequences of anthropogenically altered biogeochemical fluxes for the estuarine and coastal system of Hainan

While in section 3.1, sources, pathways and processing of nutrients and OM were assessed from a pure biogeochemical point of view, the ecological relevance of their changes through urbanization, agriculture and pond aquaculture in the WWE and coastal back-reef areas is addressed in the following. The main factors involved and their causative relationship are summarized in a flow scheme in Fig. 11.

Fig. 11: Simplified flow scheme summarizing the main investigated factors involved in eutrophication of nearshore coastal waters, especially in back-reef areas of NE Hainan. Sources of nutrients and OM are indicated by red-framed boxes. Arrows define the direction of effect and the thickness of the arrow determines the significance of the effect. The according loading indicates, if the effect is positive (+ : the more the more / the less the less) or negative (- : the more the less / the less the more). Indirect factors that aggravate or mitigate the direct cause-effect relationship related to eutrophication are presented in green letters. POM refers here to dead OM.
3.2.1 Ecological consequences for the Wenchang/Wenjiao Estuary (WWE)

Temporal nutrient enrichment in the WWE derived from agriculture in the upper estuary and from aquaculture ponds in the estuarine lagoon is the cause for stimulation of a high phytoplankton biomass under low to moderate precipitation. This is indicated by high average chl a concentrations between 10 and 30 \( \mu g \ L^{-1} \) in the upper estuary and estuarine lagoon (CHAPTER I, V), which exceed those reported from many other estuaries (e.g. Chai et al., 2006 and references therein). These chl a concentrations were similar to those reported from estuaries that were suffering from eutrophication (e.g. Harding Jr. and Perry, 1997; Pereira-Filho et al., 2001; Paerl et al., 2003). Besides the high nutrient inputs, the phytoplankton biomass was likely supported by low allochthonous TSM and the increase of light penetration due to the higher transparency of entering salt water with the tides. The high primary productivity points to eutrophication in parts of the estuary. It appears to be most severe in the upper estuary, where the Secchi depth was 0.7 m and less, and bottom water DO concentrations were temporarily \(~2 \) mg L\(^{-1}\) (CHAPTER III), reflecting hypoxic conditions.

However, further towards the outlet, a larger Secchi depth of \( >1.5 \) m, elevated DO concentrations of \( >8 \) mg L\(^{-1}\) and lower chl a concentrations of \( <10 \) \( \mu g \ L^{-1} \) indicate rather unaffected conditions (CHAPTER I, III, V). Tidal flushing with nutrient-poor, oxygen-rich, saline water most likely mitigates the effects of nutrient enrichment by dilution (CHAPTER III). The observed salt wedge (high salinity of the bottom water) buffers the effects of oxygen consumption during OM recycling in the sediment, thereby ventilating the estuary. Another factor potentially mitigating the effects of estuarine eutrophication is the remaining mangroves that add to the estuarine filter function. However, their buffer capacity can be regarded as rather limited, as the size of the mangrove area has been drastically reduced.

Pulsed flushing as a result of heavy rains are the only events that intermit primary production in the upper estuary and lagoon (see 3.1.3), while processes usually occurring in this area are shifted to the outer estuary or coastal waters (see 3.2.2). Likely, these flushing events have a cleaning effect on the estuarine lagoon, as substances that have accumulated in the water column and/or surface sediments are exported (CHAPTER III, V). However, the high phytoplankton biomass in front of the estuarine lagoon that is stimulated due to nutrient exports and decreasing turbidity several days after rain events may be transported into the estuarine lagoon by decreased water runoff and increased tidal forcing, thereby amplifying eutrophication. Potential settling and decomposition of phytoplankton biomass in sediments of the
estuarine lagoon may even fuel the eutrophication effects. However, these estuarine processes subsequent to heavy precipitation could only be studied up to 12 days after the typhoon Kammuri (CHAPTER V).

The potential risk of harmful algae blooms developing in this context also needs further attention. They occur frequently in the South China Sea causing economic loss and impacts on human health (Wang et al., 2008b). One of the reasons for their occurrence in coastal waters is pulsed nutrient runoff due to enhanced river discharge (Hallegraeff, 1993; Anderson et al., 2002; Wang et al., 2008b). By that means, it is likely that harmful algae also reach the estuarine lagoon of the WWE several days after heavy rainfall. However, it is conceivable that in the upper estuary and the estuarine lagoon, harmful algae bloom occurrence is buffered by the Si(OH)₄ conditions. Although Si(OH)₄ concentrations in the WWE are below 190 μM, the global average in tropical regions (Jennerjahn et al., 2006), they contribute to a nitrogen/phosphorus/silica ratio sufficient for diatom growth (CHAPTER I, II; Billen and Garnier, 2007).

Overall, eutrophication in the WWE appears to be rather of local and/or temporal than of chronic concern up to now, despite considerable nutrient enrichment.

3.2.2 Ecological consequences for the nearshore coastal system with special emphasis on the seagrass meadows

**STIMULATION OF ALGAL PRODUCTIVITY AND RELATED CONSEQUENCES**

Higher average chl a concentrations of ~10 μg L⁻¹ close to the shore in the aquaculture-affected back-reef areas Qingge and Chang qi gang than at the control site Ye Lin (~6 μg L⁻¹) and than in oligotrophic reef areas worldwide (< 1 μg L⁻¹) indicate enhanced phytoplankton production as a consequence of nutrient enrichment (CHAPTER I). Furthermore, stimulation of the primary production caused by nutrients from pond effluents was indicated by large increases in chl a in the bioassays after incubation of offshore water close to shore at Qingge and Chang qi gang. In contrast, insignificant changes in chl a concentrations in the bioassays close to shore at the control site Ye Lin revealed little impact of land-based nutrients (CHAPTER VI). Despite lower chl a increases in the bioassays at the offshore stations of Qingge and Chang qi gang, these increases were still significant up to 1 and 2.5 km from the shore, respectively, indicating aquaculture effluents to cause water column eutrophication in the entire back-reef areas.
Also, epiphytic primary production was stimulated as revealed by very high epiphyte loads on seagrass leaves (Fig. 12). At some stations, epiphyte biomass even exceeded seagrass biomass and was much higher than values of ~200-350 mg epiphyte DW per g leaf DW reported in other studies (Frankovich and Fourqurean, 1997; Terrados and Medina Pons, 2008). Excessive epiphyte growth on seagrasses has often been reported to be a response to elevated nutrient concentrations in the water column (Tomasko and Lapointe, 1991; Neckles et al., 1993; Wear et al., 1999; Heck et al., 2000; Peterson et al., 2007). Therefore, the much higher epiphyte loads at Qingge and Chang qi gang compared to the control site Ye Lin reveal eutrophication at those sites related to the high nutrient fluxes from pond effluents. Epiphytes at Qingge and Chang qi gang were mainly filamentous, while in Ye Lin, they were dominated by calcareous organisms (Fig. 13; pers. observation). This further points to eutrophication, since a shift from calcareous to filamentous epiphytes in response to elevated water column nutrients has been observed in several field studies and fertilization experiments (Tomasko and Lapointe, 1991, Neckles et al., 1993, McGlathery, 1995; Short et al., 1995).

![Fig. 12: Epiphyte loads of *T. hemprichii* and *E. acoroides* along a distance gradient at the three study sites in July/August 2008 and March/April 2009. In Ye Lin, *E. acoroides* was absent.](image-url)
RESULTS & SYNOPTIC DISCUSSION

A massive chl a increase to values of 15-20 μg L⁻¹ in nearshore waters close to the outlet of the WWE several days after typhoon Kammuri indicates enhanced phytoplankton production as a result of nutrient export from the WWE (3.1.3; Fig. 10b; CHAPTER V). Strong river exports may even lead to algal blooms that can involve harmful species, as it has been reported from the area (Wang et al., 2008). Significant chl a increase of the bioassay at the 500 m station at Ye Lin, which was temporarily in reach of the river plume of the WWE, points to stimulation of primary productivity even after less intense rain events (CHAPTER VI). It is likely that rain-derived nutrient exports from the WWE, as well as from drainage channels and streams debouching into the back-reef areas, additionally trigger benthic and epiphytic primary production. Higher epiphyte loads in August 2008 than in April 2009 (Fig. 12) suggest that nutrient exports in the wake of typhoon Kammuri intensified the epiphyte growth. Thus, rain-derived pulsed nutrient exports trigger additional eutrophication effects in nearshore waters.

Elevated phytoplankton biomass in the water column most likely reduced the light penetration in nearshore coastal waters, as suggested by a horizontal visibility of <2 m and high TSM values from 20-50 mg L⁻¹ in the back-reef areas of Qingge and Chang qi gang close to shore. Moreover, seagrass leaves were shaded by the high epiphyte loads (Fig. 12). Several studies around the world have shown that stimulation of various forms of algae as a result of excess nutrient loading significantly reduce
seagrass growth and bed structure through competition for light (den Hartog, 1994; Duarte, 1995; Short et al., 1995; Wear et al., 1999; McGlathery, 2001). Thus, coastal eutrophication leads to a shift from nutrient limitation to light limitation of the seagrass production (Cloern, 2001).

There is a range of factors that may mitigate or aggravate the effects of nutrient-derived light stress to seagrasses: Tidal currents that transport nutrients and planktonic biomass offshore can mitigate shading effects. However, due to the partly enclosed morphology of back-reef areas, the effects of tidal mixing are limited. Furthermore, tidal forcing does not provide a direct relieve from epiphyte loads on seagrass leaves. Another factor that may be of advantage for seagrasses is the general low depth in the back-reef areas of usually <3 m. During low water spring tide, seagrasses are even exposed to air. Due to a low light absorbance by the shallow water column, seagrasses may compensate the light stress derived from overgrowth and/or shading by phytoplankton biomass.

Nevertheless, resuspension of soil and sediment particles transported into coastal waters from emptied ponds (CHAPTER I) and export of terrigenous matter with storm water runoff (CHAPTER V) most likely aggravate the light stress to seagrasses and coral reefs. Particles that get trapped in filamentous epiphytes of fouling algae on reefs even enforce the direct masking of seagrass leaves and coral colonies. However, the severest aggravating effect of eutrophication related to seagrass and coral shading in the back-reef areas of NE Hainan is likely the lacking top-down control on primary production by grazers. This is due to a very low abundance of primary consumers, including herbivorous fish and invertebrates (Scharfbillig, 2009), as a result of immense overfishing (Krumme et al., subm.). The effect of lacking grazers on epiphyte growth can even be stronger than that of elevated nutrient concentrations (e.g. Neckles et al., 1993; Heck et al., 2000). Also, grazing by zooplankton is incapable to control the nutrient stimulated phytoplankton biomass, as demonstrated by the phytoplankton bioassay (CHAPTER VI).

The combination of the different factors, thus, leads to shading of seagrasses and corals by direct overgrowth of algae and by high suspended matter in the water column, exposing these coastal habitats to a high mortality risk, especially those closest to the shore.
Inputs of particulates rich in OM from aquaculture effluents into coastal waters as well as decaying phytoplankton biomass derived from high in-situ production may alter the sediment biogeochemistry in coastal areas. This is additionally affected by the export of terrestrial OM from the WWE during rain and by decaying phytoplankton blooms in coastal waters stimulated by rain-derived nutrient supply.

Increased benthic recycling caused by POM inputs into the sediment augments bacterial biomass, which results in enhanced oxygen consumption. Thereby, sediments can turn anoxic. Under anoxic sediment conditions, sulphate reduction produces and accumulates hydrogen sulphide ($H_2S$) in the sediment porewater. $H_2S$ is highly toxic to plants when entering their tissues, even in relatively low concentrations of $10^{-3}$ to $10^{-5}$ mol L$^{-1}$ (Howarth and Teal, 1979; Raven and Scrimgeour, 1997). Sulphide invasion may therefore pose a serious problem to seagrasses rooted in sulphur-rich sediments. Though, seagrasses may be capable of reoxidizing sulphides (Holmer et al., 2005), given that photosynthesis allows to maintain a continuous oxygen supply to rhizomes and roots via the air-filled lacunae and to subsequently leak oxygen to the sediment (Pedersen et al., 2004; Borum et al., 2005).

The content of TN and $C_{org}$ (Tab. 4a) as well as AVS and CRS (Tab. 4b) in sediments in the back-reef areas was higher in July/August 2008 than in March/April 2009, reflecting higher OM input and sulphate reduction during the rainy season. This was likely because of a higher export from rivers and related decay of an enhanced phytoplankton biomass. The AVS pool, containing porewater $H_2S$ and $FeS$, is generally considered more reactive to changes in sulphate reduction rates than the CRS pool, consisting of $FeS_2$ and $S^0$ (Schippers and Jørgensen, 2002). Especially the relatively high AVS concentrations reveal that the seagrasses may be exposed to high $H_2S$ concentrations in the sediment porewater.

Determination of the sulphur isotopic composition ($\delta^{34}S$) in seagrass tissue has been identified as suitable tool to detect potential sulphide intrusion (e.g. Frederiksen et al., 2006). Due to discrimination against the heavier isotope during sulphate reduction, sulphide in the sediment porewater has a much lower $\delta^{34}S$ than seawater sulphate. The latter has a relatively constant $\delta^{34}S$ value of ~21‰ worldwide (Rees et al., 1978), whereas sediment sulphides may have $\delta^{34}S$ values as low as -27‰ in free sulphides or AVS and -42‰ in form of pyrite (Kaplan et al., 1963). $\delta^{34}S$ values were lower than 21‰ in all parts of $T. hemprichii$ from all the stations in NE Hainan. Therefore, significant sulphide intrusion into the seagrass tissue occurred and it was generally lower in
leaves than in roots and rhizomes, which are directly exposed to the sediment porewater. However, δ^{34}S values lower than 21‰ in seagrass leaves indicate that H_{2}S could not be oxidized completely in the roots and rhizomes, so that the leaf tissue was affected, reflecting an endangered state of the seagrasses.

Tab. 4: TN and Corg content (a) and AVS and CRS in the surface sediment at the three study sites close to shore (50 m distance) and far from shore (500 or 1000 m from shore) in July/August 2008 and March/April 2009.

<table>
<thead>
<tr>
<th>Location</th>
<th>Distance from shore [m]</th>
<th>TN in surface sediment [% sediment DW]</th>
<th>Corg in surface sediment [% sediment DW]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ye Lin</td>
<td>50</td>
<td>0.049 (±0.010)</td>
<td>0.025 (±0.004)</td>
</tr>
<tr>
<td>Ye Lin</td>
<td>500</td>
<td>0.025 (±0.002)</td>
<td>0.018 (±0.002)</td>
</tr>
<tr>
<td>Qingge</td>
<td>50</td>
<td>0.029 (±0.002)</td>
<td>0.024 (±0.008)</td>
</tr>
<tr>
<td>Qingge</td>
<td>1000</td>
<td>0.038 (±0.006)</td>
<td>0.026 (±0.002)</td>
</tr>
<tr>
<td>Chang qi gang</td>
<td>50</td>
<td>0.015 (±0.004)</td>
<td>0.014 (±0.004)</td>
</tr>
<tr>
<td>Chang qi gang</td>
<td>1000</td>
<td>0.032 (±0.007)</td>
<td>0.034 (±0.005)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>Distance from shore [m]</th>
<th>AVS [μM S (g wet wt)^{-1}]</th>
<th>CRS [μM S (g wet wt)^{-1}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ye Lin</td>
<td>50</td>
<td>1.91 (±0.79)</td>
<td>2.87 (±0.56)</td>
</tr>
<tr>
<td>Ye Lin</td>
<td>500</td>
<td>0.01 (±0.02)</td>
<td>0.32 (±0.05)</td>
</tr>
<tr>
<td>Qingge</td>
<td>50</td>
<td>0.32 (±0.33)</td>
<td>3.36 (±0.37)</td>
</tr>
<tr>
<td>Qingge</td>
<td>1000</td>
<td>0.55 (±0.37)</td>
<td>2.37 (±1.23)</td>
</tr>
<tr>
<td>Chang qi gang</td>
<td>50</td>
<td>0.26 (±0.19)</td>
<td>1.65 (±1.75)</td>
</tr>
<tr>
<td>Chang qi gang</td>
<td>1000</td>
<td>1.21 (±0.95)</td>
<td>3.76 (±1.68)</td>
</tr>
</tbody>
</table>

Although the highest OM contents and AVS concentrations were measured close to the shore at Ye Lin (Tab. 4b), the δ^{34}S values in the seagrass leaves at this station (Fig. 14) indicate the comparatively lowest sulphide intrusion into the seagrass leaves. This reveals the highest capacity of the seagrasses to oxidize H_{2}S in their below-ground tissues (roots and rhizomes), and thereby anticipating leaf tissue poisoning by H_{2}S. It is conceivable that seagrasses at Ye Lin perform a higher overall photosynthetic activity and oxygen translocation, whereas at Qingge and Chang qi gang, oxidation of H_{2}S may be restricted due to a reduced photosynthetic activity at these sites through shading by algae. It is likely that a higher patchiness at these sites even aggravates the reduced ability to oxidize H_{2}S, while seagrass meadows at Ye Lin benefit from their continuous bed character.
The high epiphyte loads and sulphide intrusion into seagrass leaf tissue indicate that the seagrasses in NE Hainan, which are exposed to pond effluents, are endangered because of a potentially lowered photosynthetic activity due to shading by algae, as well as by additional sulphide poisoning.

Fig. 14: δ³⁴S in *T. hemprichii* leafs, roots and rhizomes over distance from the shore (near= 50-100 m from shore, far=500-1000 m from shore) at the three study sites in March/April 2009.

**THE CURRENT STATE OF SEAGRASS MEADOWS**

Seagrass species abundance, shoot density and above and below-ground biomass are the common indicators for the performance of seagrass meadows (Kirkman, 1996; Wood and Lavy, 2001; Short et al., 2006). A much lower species abundance and shoot density (Fig. 15), as well as above- and below-ground biomass (Fig. 16) close to shore at Qingge and Chang qi gang than in Ye Lin indicate a lowered health state of the seagrasses at those sites. In addition, at Qingge and Chang qi gang, seagrasses did not occur in the first 50 m from the shore (Fig. 15). Seagrasses closer to shore at Chang qi gang and Qingge were only present in single patches and were restricted to the species *T. hemprichii* and *E. acoroides*, which are comparatively tolerant to e.g. reduced light and low salinity conditions (Bach et al., 1998; Vermaat et al., 1995). This reflects deteriorated growth conditions at those sites, implying that the high exposure to aquaculture effluents indeed causes degradation or even loss of seagrass.
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Fig. 15: Mean shoot density of the seagrass species over the distance gradients at the study sites Ye Lin, Qingge and Huiwen in July/August 2008 and March/April 2009 (n=30). Standard deviations are given for total seagrass shoot densities, and significantly higher total seagrass shoot densities over the distance gradient of each site are indicated with * (ANOVA and t-test; p<0.05).

Fig. 16: Mean values and standard deviation of total seagrass above-ground biomass (A.B.) and below-ground biomass (B.B.) at the three study sites over distance from the shore in July/August 2008 and March/April 2009 (n=5).
Seagrass shoot density and biomass increased in offshore direction starting from 250 m in Qingge and from 1000 m in Chang qi gang (Fig. 15, 16), which is concordant to improved environmental conditions further offshore as reflected by the lower nutrient and chl a concentrations (CHAPTER I, VI). However, at these distances from the shore, seagrasses did not show the good condition observed close to shore in Ye Lin, which is most likely due to the persistent effects of aquaculture effluents (CHAPTER VI). In contrast, considerably reduced species abundance, shoot density and biomass further offshore at Ye Lin compared to close to the shore indicates negative growth conditions due to the temporal exposure to the river plume of the WWE (CHAPTER VI). The temporal export of nutrient-, sediment- and OM-rich riverine water, thus, also delimits seagrass growth conditions.

A significantly lower shoot density and especially biomass in March/April 2009 than in July/August 2008 was likely associated with increased desiccation and temperature-related stress in the winter months, as also reported from other tropical seagrass meadows (Erftemeijer and Herman, 1994; Rasheed et al., 2008; Wirachwong and Holmer, 2010). However, it can also not be excluded that one or several extreme events, such as typhoon strikes and related consequences, might have been responsible for the enormous decline in shoot density in Ye Lin.

Overall, the seagrasses at the NE coast of Hainan appear to be seriously affected by eutrophication only maintaining a relatively healthy state at stations, which are not exposed to high nutrient and OM loads.

**SCENARIOS ON THE FUTURE STATE OF SEAGRASS MEADOWS**

The extension of seagrass meadows towards the shore is strongly limited by the unfavourable water column- and sediment- conditions associated with the effluent releases from ponds, whereas high nutrient loading derived by water export from the WWE delimits the offshore extension of the seagrasses at Ye Lin towards the reef crest.

Besides eutrophication, there is a range of other (anthropogenic) pressures that act upon the seagrasses. Seagrass meadows may directly be harmed by trampling from gastropod collectors, boat anchoring by fisherman and destructive fishery techniques, such as dynamite fishing, all applied in the back-reef areas of NE Hainan (pers. observation). These pressures may only be of local importance, though.
As everywhere on the planet, ocean-acidification may also affect the coral reefs and seagrass beds of Hainan. Progressing seawater acidification affects seagrasses by promoting a shift from calcareous epiphytes, which are usually found in oligotrophic seagrass beds, to filamentous epiphytes, due to dissolution of coralline algae (Martin et al., 2008). However, due to the distinct differences in epiphyte composition between the sites exposed to different nutrient inputs, eutrophication to date appears to have a higher effect than ocean-acidification. In the future, though, proceeding ocean-acidification may strongly contribute to the shading of seagrasses by filamentous algae.

In addition, the observed accelerated transport of material from rivers to coasts due to anthropogenic modifications in the coastal zone is responsible for an increased exposure of the coastal habitats to rain–derived river inputs. Besides fuelling eutrophication, these most likely also directly affect seagrass performance by exposing them to low saline and low pH water (CHAPTER I, V). Seagrasses are differently adapted to freshwater exposure, though generally they can only tolerate such conditions for some days (Lirman and Cropper Jr., 2003). Sedimentation is another factor associated with an elevated river export. Although seagrasses have species-specific tolerances to sediment loads (Bach et al., 1998; Cabaço et al., 2008), massive sediment burial generally causes seagrass loss (Campbell and McKenzie, 2004).

Impacts related to freshwater export are likely to increase in the future, because it is predicted that heavy rainfall associated with tropical storms will further increase in the course of man-made climate change (IPCC, 2011). Hainan’s coasts, being situated in the global centre of typhoon striking, will be extremely affected by the increased runoff and nutrient and OM export associated with these episodic events. Projected intensifying wind speeds during tropical storms (IPCC, 2011) may additionally pose the seagrasses to an enhanced risk of direct uprooting.

It has become clear that at the moment the seagrasses of Hainan are exposed to persistent eutrophication stress due to the continuous effluent inputs derived from aquaculture ponds, weakening their resilience towards external stresses. Therefore, it is likely that at some point in the future, the seagrasses will not be able to recover from extreme events, such as typhoons, especially if several storms affect the area within a short period of time. The complete loss of the seagrasses would eliminate their ecosystem functions and also remove their buffer function for the coral reefs, which would be similarly affected by the above mentioned pressures and may then disappear accordingly. Besides the gap, which would be torn in terms of ecosystem functioning, the lack of seagrasses and coral reefs would also lower the value of Hainan’s coasts as an attractive tourism destination, as it is currently increasingly promoted.
Thus, in order to support the resilience of the seagrass beds (and coral reefs) the management actions should be 1) to reduce the continuous nutrient inputs from aquaculture ponds and 2) to apply methods to reduce pulsed nutrient and OM exports from rivers and estuaries. If this is achieved, it is conceivable that seagrasses will subsequently extent in their range again and form coherent meadows.

**KEY FINDINGS related to objective II:**

- High primary production resulting from nutrient enrichment indicates that parts of the WWE are temporarily subject to eutrophication. Tidal mixing mitigates according effects of oxygen depletion, thereby reducing the negative ecological impact.
- The back-reef areas, which are exposed to nutrient- and OM-rich pond effluents or river exports, suffer from eutrophication, as indicated by a high primary production and sedimentary OM. While tidal mixing mitigates water column eutrophication, it is aggravated by low grazing on algae biomass due to overfishing, and by increased riverine exports.
- As a consequence, present seagrasses are threatened by light limitation due to excessive growth of benthic, epiphytic and planktonic algae, as well as by sulphide poisoning resulting from elevated OM recycling.
- Seagrasses meadows exposed to nutrient- and OM-rich pond effluents or river exports reveal a lower performance in terms of species richness, biomass and shoot density than moderately or unaffected sites.
- A thereby derived reduced resilience of the seagrasses may make them more susceptible to additional natural stressors e.g. typhoon events, which are predicted to intensify in the future. Seagrass meadows in NE Hainan are thus at high risk of die-off.
3.3 Implications of the results in the global context and for local management

3.3.1 Coastal pond aquaculture – an underestimated source of nutrients and organic matter to coastal waters

While the threat of eutrophication resulting from aquaculture has been recognized in small-scale, local studies, the majority of studies investigating large-scale eutrophication effects have mainly focused on agriculture as the main pressure. In global studies, nutrient inputs to the oceans was calculated by modelling approaches (e.g. Smith et al., 2003; Bouwman et al., 2009; Seitzinger et al., 2005, 2010). In these models, population growth and the runoff per area appear to be the most important input variables. Therefore, these studies only consider nutrients that are carried to the ocean with rain water runoff. However, the main part of the nutrients that are supplied to estuarine and coastal waters from aquaculture ponds is not transported by precipitation runoff. Instead, the water for the ponds originates from the sea/estuary, is pumped on land, and is enriched with land-derived nutrients. During pond drainage or meantime water exchange (CHAPTER I), the water is then released back to the sea/estuary. Thus, the global studies on nutrient inputs to the coasts via river exports did not account for these nutrients in their calculations. In many areas of the world, where there are no or only a few aquaculture ponds, nutrient inputs from aquaculture are likely negligible compared to those derived from agriculture and urban effluents. However, these calculations may lead to significant underestimation of nutrient input for areas with a high density of aquaculture ponds, like in many parts of SE Asia. While the highest current and predicted nutrient inputs to the ocean are in South Asia (Seitzinger et al., 2010), within these calculations the additional nutrient inputs from pond aquaculture are not even considered. This study demonstrates that in tropical areas, such as the investigated region, where pond aquaculture makes up a significant part of land use, simplified nutrient modelling via runoff does not represent the true land-based nutrient inputs to coastal waters. In order to receive a more realistic picture, it is necessary to add correction factors regarding additional water use by pond aquaculture to the models.

Results of this study also show that the impacts of effluents from agriculture and aquaculture on aquatic ecosystems are generally different: While effects of nutrient and OM from agriculture are mainly coupled with rain events and therefore only occur pulsed and mainly restricted to the rainy season, nutrient enrichment effects of aquaculture are evident all year round. Although there is not a continuous input of nutrients from effluents at a certain area, the whole ecosystem may be permanently
exposed to effluent inputs, due to the diffusive source character of aquaculture effluents. It is therefore inferred that eutrophication effects related to coastal aquaculture effluents are a permanent problem weakening the resilience of coastal ecosystems (see 3.2). As pond aquaculture production is predominantly situated in subtropical and tropical regions (FAO, 2010), it represents a considerable threat to the world’s coral reefs and seagrass meadows, which are of high global value (Costanza et al., 1997).

Furthermore, the continuous nutrient inputs bear a high potential for the occurrence of harmful algae blooms in nearshore coastal waters, due to their much higher contribution of nitrogen and phosphorus compared to silica. Anyhow, this relationship has not been sufficiently documented and should therefore be further investigated.

Overall, this implies that that the nutrient inputs from coastal aquaculture ponds and their consequences on the coastal ecosystems may have been underestimated in previous studies. Therefore, it is likely that in regions with significant aquaculture production, total land-based nutrient inputs and the risk of eutrophication are even higher than currently expected. The predicted further increase of pond aquaculture in many tropical and subtropical coastal areas as a result of population increase and declining natural fish stocks emphasizes the importance of these findings.

3.3.2 Socio-economic implications and management needs

Besides their negative effects on coastal habitats, high nutrient inputs to estuarine and coastal waters also have implications for people’s livelihood in the coastal zone. Pond aquaculture is directly depending on the quality of coastal waters as a source of intake water for the ponds. Deteriorated water quality in adjacent coastal waters has indirect consequences on aquaculture operation themselves by spreading of diseases and harmful algae species with intake water. This has become a worldwide increasing problem because of major crop losses, and has hampered the sustainable development of the aquaculture industry (Lin, 1989; Kautsky et al., 2000; Qi et al., 2004). A deteriorated water quality of adjacent water bodies also rises the costs for pond maintenance, including costs for electricity for paddle wheel aerators or filtration of the intake water (pers. comm. shrimp farmers). Furthermore, it enhances the need for pesticides and antibiotics, which again may have negative effects on the adjacent ecosystems, as well as on the consumers (Gräslund and Bengtsson, 2001; Holmström
et al., 2003). Excessive antibiotic and pesticide use in shrimp farms has e.g. led to import bans of the EU and the US for shrimp from Thailand and China.

In Hainan, prices for shrimp have decreased dramatically within the past ten years. Shrimps were exported to the US and Europe and up to 19 $ per kg shrimp could be gained, whereas in 2008/2009, shrimps and fish were mainly sold within China and prices varied from 2.50 to 4.70 $ per kg shrimp (mean: 4 $ kg\(^{-1}\); n=10; unpubl. data). This decrease in prices is mainly associated with reduced export possibilities because of excessive antibiotic and pesticide use as a result of a deteriorated water quality in the intake water of the ponds due to coastal eutrophication.

Pond aquaculture, as it is managed in many parts of the world, including Hainan, reflects an unprofitable venture that causes its own run-down by ecosystem degradation. In the future, aquaculture will be an important food production sector. However, without taking any measures its growth may be hampered due to increasing self-made pollution.

During the past decades, several studies aimed for improvement of the water quality in aquaculture ponds and their effluents. Governments of several countries established regulations in order to reduce the negative impact of aquaculture. However, it is rarely evaluated, if threshold values are met and in many cases governments do not manage shrimp and fish farming strictly (e.g. Biao and Kaijin, 2007). Technologies that reduce nutrient contamination on and from shrimp and fish ponds exist, though they are seldom employed. Easy and cheap water treatment can be achieved e.g. by culture with macroalgae, that filter the water and serve as additional biomass (e.g. Troell et al., 1999; Nelson et al., 2001; Jones et al., 2002). Also, natural stands of mangroves inside and around the ponds serve as water purifiers (Robertson and Phillips, 1995; Binh et al., 1997). These techniques have been implemented in selected farms in several countries. A few farms are also certified by eco-labels such as “International Federation of Organic Agricultural Movements (IFOAM)”, “Naturland”, “Global Aquaculture Alliance” that have strict and standardized production regulations and guarantee fish and shrimp to be raised in sustainable culture. There has been a heavily increasing demand for such products, especially in the US and Europe (FAO/NACA/UNEP/WB/WWF, 2006).

In Hainan, a few farms have started to apply integrated aquaculture practices, mainly polyculture of shrimp together with abalone and/or seaweed (Theodore, 2007); though, this has not been registered in the study area and none of the farms in Hainan is certified by an international eco-label. In our interviews, the majority of shrimp and fish pond owners stated that they would be open for further training and would like to
learn about better cultivation practices. This shows the potential for eco-friendly farming in the area. Treatment of aquaculture effluents would not only benefit the surrounding coastal habitats, but also the aquaculture industry by minimizing the risk of diseases and harmful algal blooms spreading with contaminated intake water. This in turn, would decrease the need for excessive application of antibiotics and pesticides, guaranteeing a better quality of the crops and potentially better prices. Besides that, the increasing tourism industry in Hainan would benefit from improved water quality of coastal waters. Apart from water treatment, farm placement in direct vicinity to back-reef areas should be avoided to sustain the health of coastal habitats and their associated communities.

**KEY FINDINGS related to the global context and local management:**

- Previous studies on global nutrient export have underestimated the role of coastal pond aquaculture as a nutrient source to the coastal ocean in terms of
  - an additional nutrient input into coastal waters independent from precipitation runoff
  - continuous eutrophication effects in the coastal zone in addition to pulsed effects
- In (sub)tropical areas with large-scale aquaculture, such as SE Asia, current and future nutrient inputs to the coasts may even be higher than widely expected
- The following management measurements are recommended:
  - to reduce direct nutrient and OM inputs into estuaries and coasts by e.g. treatment of urban effluents, optimization of fertilizer application on agriculture fields, reduction of water exchange in aquaculture ponds, polyculture and treatment of aquaculture effluents (e.g. with macroalgae)
  - to reduce the water transport from land to sea by e.g. reforestation of mangroves and river bed renaturation
  - to optimize the positioning of aquaculture ponds by e.g. removing aquaculture ponds from sensitive back-reef areas
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REFERENCES


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CHAPTER I

Effluent, nutrient and organic matter export from shrimp and fish ponds causing eutrophication in coastal and back-reef waters of NE Hainan, tropical China

by Lucia Herbeck, Daniela Unger, Ying Wu and Tim Jennerjahn

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Effluent, nutrient and organic matter export from shrimp and fish ponds causing eutrophication in coastal and back-reef waters of NE Hainan, tropical China

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Abstract

Global aquaculture has grown at a rate of 8.7% per year since 1970. Particularly along the coasts of tropical Asia, aquaculture ponds have expanded rapidly at the expense of natural wetlands. The objectives of this study were (i) to characterize the extent and production process of brackish-water pond aquaculture at the NE coast of Hainan, tropical China, (ii) to quantify effluent and organic carbon, nitrogen and phosphorus export from shrimp and fish ponds and (iii) to trace their effect on the water quality in adjacent estuarine and nearshore coastal waters harbouring seagrass meadows and coral reefs. During two expeditions in 2008 and 2009, we determined dissolved inorganic nutrients, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), chlorophyll a (chl a) and particulate organic matter (POM) in aquaculture ponds, drainage channels and coastal waters in three areas varying in extent of aquaculture ponds. From the analysis of satellite images we calculated a total of 39.6 km² covered by shrimp and fish ponds in the study area. Interviews with owners of ponds showed that there was no standardized production pattern evident concerning feeding management and water exchange. Nutrient and suspended matter concentrations were high in aquaculture ponds and drainage channels, even though varying considerably. The calculated annual export of total dissolved nitrogen (TDN) and particulate nitrogen (PN) from pond aquaculture into coastal waters was 599 and 1119 t yr⁻¹, respectively. High concentrations of dissolved inorganic nitrogen (DIN), phosphate and chl a at the majority of the coastal stations point at eutrophication of usually oligotrophic waters in the back-reef areas, especially close to shore. We suggest that the introduction of untreated aquaculture effluents into coastal waters
impairs the performance of seagrasses and coral reefs, particularly if released directly to back-reef areas, where estuarine retention and mixing with open ocean water is restricted.

Key words: aquaculture, shrimp and fish ponds, nutrient export, back-reef area, eutrophication, Hainan, China

1. Introduction

Aquaculture is with an average annual increase of 8.3% over the past 40 years the fastest-growing food-producing sector and set to overtake capture fisheries as a source of food fish. The per capita supply of food fish from aquaculture has already increased from 0.7 kg in 1970 to 7.8 kg in 2008 (FAO, 2010). Decreasing fish stocks in the oceans and rapid expansion of human population will most probably lead to an even increasing reliance on farmed seafood as source of protein (Naylor et al., 2000). China is by far the largest producer of aquaculture goods (32.7 million tonnes in 2008) accounting for 62% of global production in terms of quantity and 51% of global value (FAO, 2010). In the Asia-Pacific region, where 89% of the global aquaculture production takes place, culture in earthen ponds is the most important farming method for finfish and crustaceans in fresh- and brackish water (FAO, 2010). Brackish-water culture, which represented 7.7% of world production in 2008, accounted for 13.3% of total value, reflecting the prominence of relatively high-valued crustaceans and finfishes cultured in brackish water (FAO, 2010). Despite generating profits and income for local communities, aquaculture production bears a suite of environmentally adverse consequences.

Between 1980 and 2005, when aquaculture denoted the greatest increase, 20% of the world’s mangrove area was lost (FAO, 2007) and pond construction is cited as one of the major causes for this decline (Alongi, 2002; Chua et al., 1989; Páez-Osuna, 2001a). Conversion of mangrove area into pond area reduces the mangrove’s valuable ecosystem services (e.g. fish habitat, coastal protection, sediment trap) thereby affecting biodiversity and causing coastal erosion. Other impacts resulting from pond aquaculture are water pollution by excessive use of herbicides, pesticides and antibiotics, salinization, acidification, reduction of wild fish supplies through introduction of non-indigenous organisms, wild seed stock collection and inputs of wild fish for feed, as well as socio-economic consequences, such as marginalization of coastal...
communities and changes in traditional livelihoods (Dierberg and Kiattisimkul, 1996; Flaherty and Karnjanakesorn, 1995; Naylor et al., 2000; Páez-Osuna, 2001b; Phillips 1998; Primavera, 1997, 1998; Senarath and Visvanathan, 2001; Thongrak et al., 1997). One of the key environmental concerns about aquaculture is water degradation, due to discharge of effluents with high levels of nutrients and suspended solids into adjacent waters causing eutrophication, oxygen depletion and siltation (e.g. Burford et al. 2003).

To guarantee sufficient water quality for the animals raised, pond water is usually exchanged several times during the production cycle and nutrient-rich effluents are released into adjacent natural water bodies mostly without prior treatment. High concentrations of suspended organic solids, carbon, nitrogen and phosphorus in aquaculture effluents mainly originate from excess feeds or from excretion from the farmed animals (Burford and Williams, 2001). High stocking rates, low-grade food quality and low feed conversion rates promote high remineralisation within ponds. In the early stage of crop production, N-P-K fertilizers are often added to pond waters, in order to trigger growth of algae serving as feed for the young animals.

Tropical shallow coasts are often fringed by coral reefs and seagrass meadows, which are usually adapted to oligotrophic water conditions. They provide valuable ecosystem services, such as water filtration, coastal protection and nursery and feeding habitat for fish and other marine resources (Beck et al., 2001; Hemminga and Duarte, 2000). World-wide degradation of those habitats is mainly related to high nutrient inputs promoting vast growth of benthic algae, which may outcompete seagrass and corals (e.g. Hauxwell et al., 2001; Hughes, 1994; Silberstein et al., 1986). Little is known about the effects of pond aquaculture on the health of those habitats.

The majority of studies on pond aquaculture focus on water quality assessments in the ponds themselves or the quantification of effluent fluxes from intensively, semi-intensively and extensively managed shrimp and fish ponds (e.g. Alongi et al., 1999, 2000; Briggs and Funge-Smith, 1994; De Silva et al., 2010; Islam et al., 2004; Jackson et al., 2003; Páez-Osuna et al., 1997; Rivera-Monroy et al., 1999; Wahab et al., 2003). It has been shown in those studies that high loads of nitrogen, phosphorus and suspended solids are released from shrimp and fish ponds. However, the effects of these on water quality in the adjacent environment have seldom been studied. Impacts of aquaculture effluents on the water quality in coastal creeks have been addressed by a few studies, e.g. Biao et al. (2004), Burford et al. (2003), Costanzo et al. (2004) and Wolanski et al. (2000), who found elevated concentrations especially of dissolved nitrogen and chl a in outlet channels. However, only little is known on the effects of effluents from pond aquaculture on nutrient and chl a dynamics of the coastal seas.
China, one of the leading shrimp and fish producers, has no well-documented report on the effect of pond aquaculture on its coastal waters. The first comprehensive study about general operating characteristics of shrimp farming in China was presented by Biao and Kaijin (2007). Theodore (2007) studied ecological and socioeconomic characteristics of integrated aquaculture practices specific to north Hainan. However, cultivation practices and related effluent fluxes may vary between regions, e.g. due to climatic conditions, and therefore need to be evaluated locally.

Aim of this study was to determine the amount and composition of dissolved and particulate matter released from fish and shrimp ponds in NE Hainan, tropical China, and to assess the impact on the water quality of the receiving estuarine and nearshore coastal waters. Effluent, nutrient and particulate matter export from shrimp and fish farms was calculated from areal extent of ponds, operating characteristics and water quality parameters in pond effluents. We also determined dissolved constituents and chl a in three coastal areas varying in size of aquaculture cultivation area in their hinterland. This study is to our knowledge the first one that combines data on effluent fluxes from brackish-water pond culture with related effects on water quality in adjacent coastal waters harbouring coral reefs and seagrass meadows.

2. Materials and Methods

2.1 Study area

The study area is located at the NE coast of the island Hainan, South China, in the marginal tropics (Fig. 1a) and comprises a coastline of ~45 km. Coral reefs fringe parts of the coast in 0.5 to 4 km distance from the shore, and seagrass meadows occur in the back-reef areas (Fig. 1b). The area is subject to mixed semidiurnal microtides with a tidal range of about 0.5 and 1.5 m at neap and spring tide, respectively. The region is characterized by a tropical monsoon climate with a dry season from November to April and a rainy season from May to October. The total annual precipitation is 1500-2000 mm, of which 35-60% are related to typhoon-induced rainfall occurring mainly from July to September (Huang, 2003; Wang et al., 2008). Average air temperatures range between 14.6-20.8 °C in January and 25.2-33.1 °C in July.

2.2 Study sites and sampling time

This study focused on the three main sites of pond aquaculture production at the NE coast of Hainan (Fig. 1), the Wenchang/Wenjiao Estuary and adjacent coastal
zone (WWE), as well as the areas of Chang qi gan and Qingge including their back-reef areas.

The **Wenchang/Wenjiao Estuary (WWE; 19°35.9' N, 110°49.0' E)** is fed by two lowland rivers (Wenchang and Wenjiao) that drain agriculture areas of the coastal plain. They debouche into a shallow (mean depth: 3 m), kidney-shaped lagoon (Bamen Bay), which is connected to the sea via a narrow channel (max. depth = 10 m). In total, the estuary comprises an area of ~40 km². Since the 1960s, 73% of the fringing riverine mangrove along the estuary has been lost at the expense of aquaculture ponds with approximately 7.5 km² residual mangrove area in 2009 (Krumme et al., under review). In addition to ponds, approximately 0.05 km² are covered by floating net cages for fish cultivation (Krumme et al., under review). In the outer estuary, fringing coral reefs and species-rich seagrass meadows occur in south-eastern direction to the outlet of the estuarine lagoon.

**Chang qi gang** (19°27.2' N, 110°47.8' E) is the second largest aquaculture production area in NE Hainan. Ponds are mainly located around a tidal channel reaching ~6 km inland parallel to the coastline. Though, there are also ponds situated directly along the coastline, which release their effluents directly into the sea via artificial drainage channels. Shrimp and fish ponds cover major parts of the former mangrove area, of which approximately 85% had been lost since the 1960s with about 1.8 km² residual mangrove area in 2009 (Krumme and Herbeck, unpublished data) mainly near the outlet of the tidal channel. The reef crest is located ~3 km from the shoreline and the back-reef area comprises an area of ~23.2 km². Seagrass meadows dominated by the species *Thalassia hemprichii* and *Enhalus acoroides* occur within the back-reef area.

**Qingge** (19°19.7' N, 110°41.3' E) situated in the south of the study area comprises another important aquaculture production area, which now replaces former agricultural fields. Mangroves have never occurred in this area. The pond area is drained into the sea by a multitude of artificial channels. The reef crest is situated ~1 km offshore and the back-reef area comprises ~8.4 km². Seagrass meadows dominated by the species *Thalassia hemprichii* and *Enhalus acoroides* occur in the back-reef area.

Sampling took place in July/August 2008 (rainy season) and March/April 2009 (end of dry season). During both sampling campaigns major precipitation events occurred (Unger et al., subm. CSR, this issue), as first rain events had already set-in in the supposed dry season in March/April 2009.
2.3 Sampling and analysis

2.2.1 Spatial extent of shrimp and fish farms

The spatial extent of aquaculture ponds in the study area and specifically at the three study sites was determined from satellite images from 2009 (Geo Eye™, 2009), on which pond complexes were clearly visible (Fig. 2). Images were geo-referenced and groundtruthed with appropriate waypoints taken over the study area with a GPS, and digitized using ESRI ArcGIS 9.
Fig. 2: Satellite image (Geo EyeTM, 2009) showing aquaculture ponds at Qingge. White line at the bottom right site of the picture indicates waves breaking at the reef crest.

2.2.2 Operating characteristics of fish and shrimp farms

In order to collect information about operating characteristics of fish and shrimp farms in the selected areas, 18 and 41 interviews with randomly selected pond owners were carried out in 2008 and 2009, respectively. In total, there was a minimum of 15 interviews taken in each of the three study sites. Interviews were based on a semi-structured questionnaire with minutes translated and transcribed from memory. The questionnaire mainly focused on parameters needed to estimate the annual effluent export (e.g. number of crops per year, rate and quantity of water exchanged, pond depth), but also contained general questions related to stocking densities, feeding management, etc. In addition, specific information about ponds that were sampled for water (see below) were obtained regarding e.g. age of animals raised and days since last water exchange.

2.2.3 Water sampling and analysis

Water samples were collected from fish and shrimp ponds, drainage channels, estuaries/tidal inlets and coastal sites during several dates of the study period. Water from shrimp ponds (n=31), fish ponds (n=24) and drainage channels (n=59) was
collected by submersing a bottle from the pond/channel edge. Drainage channels were sampled 1-5 m before their discharge into the sea. Coastal waters were sampled randomly by boat along a land-sea gradient.

Water samples for nutrient analyses were filtered immediately after sampling through single use Sartorius Minisart® membrane filters (0.45 μm pore size) into PE bottles, which were rinsed three times with the filtered sampling water beforehand. Samples were preserved with a mercury chloride solution (50 μl of a 20 gL⁻¹ HgCl₂ solution added to 100 ml sample) and stored cool until analysis. For dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analysis, 10 ml of water was filtered through single use membrane filters into precombusted (5h, 450°C) glass ampoules. Samples were acidified to pH 2 with phosphoric acid, sealed and stored frozen until analysis. Water samples for chlorophyll a (chl a) and particulate matter determination, which were only collected from ponds and drainage channels, were stored cool and dark in PE tanks. Salinity (±0.1) and pH (±0.1) were measured with a WTW MultiLine F/Set3 multi-parameter probe before the water was filtered under constant pressure onto GF/F filters within the same day. Filters for particulate matter determination were dried at 40 °C, whereas filters for chl a determination were stored frozen until analysis within the following days.

Dissolved nutrients were analyzed using a continuous flow injection analyzing system (Skalar SAN++System). Nitrate+nitrite (NOₓ⁻), nitrite (NO₂⁻), phosphate (PO₄³⁻) and silicate (Si(OH)₄) were detected spectrophotometrically and ammonium (NH₄⁺) fluorometrically as a colored complex (Grasshoff et al., 1999). Determination limits were 0.08 μM, 0.03 μM, 0.06 μM, 0.07 μM and 0.19 μM for NOₓ⁻, NO₂⁻, NH₄⁺, PO₄³⁻ and Si(OH)₄, respectively, according to DIN 32645. The coefficient of variation of the procedure was <3.4%. Nitrate (NO₃⁻) was calculated as NOₓ⁻ - NO₂⁻. Concentration of dissolved inorganic nitrogen (DIN) is the sum of NO₃⁻, NO₂⁻, and NH₄⁺. DOC and TDN were measured simultaneously by the high temperature catalytic oxidation (HTCO) method using a Teledyne Tekmar Apollo 9000 Combustion TOC Analyzer at 680 °C (for samples collected in 2008) and a Shimadzu TOC-VPCH Total Organic Carbon Analyzer with a TNM-1 Total Nitrogen Measuring Unit combusting at 720 °C (for samples collected in 2009). No significant differences were found between measurements at both devices (M. Birkicht, pers. comm.). The relative deviation of the method was <2%. Dissolved organic nitrogen (DON) was calculated as TDN – DIN.

Concentrations of total suspended matter (TSM) were determined by weighing the dried filter, subtracting the original weight of the empty filter and dividing it by the respective volume of water filtered. Values given are the average of 2-3 filters. TSM on GF/F-filters was analyzed for total carbon (TC) and particulate nitrogen (PN) by high-
temperature combustion in a Carlo Erba NA 2100 elemental analyzer (Verardo et al., 1990). Particulate organic carbon (POC) was determined the same way after removal of carbonate by acidification with 1N HCl and subsequent drying at 40 °C. Measurements had a precision of 0.06% for POC and 0.02% for PN, based on repeated measurements of a standard (LECO 1012). Chl a concentrations were determined as indicator for phytoplankton biomass: Pigments were extracted from the filters in 10 ml 90% acetone at 4 °C in the dark for approximately 24 hours, and extracts were subsequently centrifuged at 60 rpm for three minutes. Chl a in the supernatant of samples collected in 2008 was determined with a Lovibond PC Spectro 1.0 photometer and calculated after Lorenzen (1967). Chl a in samples collected in 2009 was determined with a TURNER 10-AU field fluorometer after Arar and Collins (1997). Significant differences between standards measured with both devices could not be detected.

2.2.4 Data analysis

Suspiciously high concentrations of nutrients, DOC and DON from ponds and drainage channels were eliminated from data sets because we can not exclude the influence from factors such as high sulphide concentration, for example, which are likely to occur in drainage channels as a consequence of high organic matter degradation rates, and can disturb the photometric/fluorometric detection of dissolved nutrients (Grasshoff et al. 1999). Thus, calculated average concentrations for shrimp and fish ponds and especially in drainage channels represent conservative numbers.

The average annual export of effluents from shrimp and fish ponds was calculated for the different areas using the following equations:

\[
ES = c_s \times P \times a_s/100 \times d + i_s \times c_s \times z_s/100 \times P \times a_s/100 \times d
\]

\[
EF = c_f \times P \times a_f/100 \times d + i_f \times c_f \times z_f/100 \times P \times a_f/100 \times d
\]

\[
ET = ES + EF
\]

- **ES**: Effluent export from shrimp ponds [m³ yr⁻¹]
- **EF**: Effluent export from fish ponds [m³ yr⁻¹]
- **ET**: Total effluent export from aquaculture ponds [m³ yr⁻¹]
- **c_s**: number of shrimp crops per year
- **c_f**: number of fish crops per year
- **P**: pond area [m²]
- **a_s**: portion of shrimp ponds in area [%]
- **a_f**: portion of shrimp ponds in area [%]
- **d**: average pond depth [m]
i_s: events of partial water exchange in shrimp ponds during each crop  
i_f: events of partial water exchange in fish ponds during each crop  
z_s: portion of shrimp pond water exchanged [%]  
z_f: portion of fish pond water exchanged [%]  

The related annual export of dissolved and particulate matter was calculated for each area as the sum of average effluent export from shrimp ponds (E_s) and fish ponds (E_f) multiplied by average concentrations of dissolved and particulate matter in drainage channels.

The statistic tool of SIGMAPLOT 11.0 was used to perform the statistical analyses. The data was tested for normal distribution before choosing parametric or non-parametric statistical methods. The Pearson Product Moment Correlation analysis or Spearman Rank Order Correlation analysis was performed to test for significant correlations. Linear regression analysis was also performed. Kruskall-Wallis One Way Analysis of Variance on Ranks followed by Pairwise Multiple Comparison Procedure (Dunn’s method) was used to determine significant differences in water quality parameters between shrimp ponds, fish ponds and drainage channels. The Mann-Whitney t-test by Rank Sum was applied to detect significant differences between water quality parameters in the coastal zone between the rainy season 2008 and the dry season 2009.

3. Results

3.1. Spatial extent and operating characteristics of shrimp and fish farms in NE Hainan

In total, 39.6 km² are used for pond aquaculture in our study area (Tab. 1). The main production area is located in the lagoon of the Wenchang/Wenjiao Estuary (WWE), where 21.6 km² are used for aquaculture. Coastal fish and shrimp ponds that are situated directly along the shoreline make up 18.0 km², of which 8.7 km² are found in Chang qi gan and 2.4 km² in Qingge (Tab. 1).

Aquaculture development in NE Hainan started in the 1980s and is still increasing. The main shrimp species cultured are Litopenaeus vannamei (white shrimp), Penaeus chinensis (chinese shrimp) and Penaeus monodon (black tiger shrimp) and the main cultured fish species are Epinephelus awoar (banded grouper) and Epinephelus lancelatus (gentiana grouper). In 2009, the majority of ponds were used for shrimp cultivation (~60% on average), while fish cultivation (~40% on average) was less important. The respective share of each, however, varied regionally (Tab. 1).
and also temporally depending on market prices that were attained at a time. In Qingge, for example, shrimp culture was replaced by fish culture after import bans for shrimps from China, e.g. by the US in 2007. Three to four crops of shrimp are cultured per year, which have an average culture time of 70-80 days in summer and 90-120 days in winter. Some farms intermit their production during winter time (Dec-Feb). For fish culture, one crop is raised each year (culture time ~360 days). The ponds have an average size of 0.25 ha (ranging from 0.07-1.33 ha) and an average depth (d) of 1.7 m (ranging from 1.2 -2.0 m). 82% of the fish and shrimp farmers asked owned up to three ponds, but bigger farms with up to 18 ponds also exist (n=33).

From our interviews we can say that the majority of farms appear to be intensively managed, though cultivation practices varied considerably from pond to pond. Most ponds were equipped with paddlewheel aerators, which either work all day or during the night and a few hours during the day depending on the weather. The bottom of some ponds, especially those located above sea level, was covered by plastic sheets, in order to avoid seepage and to facilitate removal of accumulated organic matter after each production cycle. Larvae were mainly obtained from larvae-culturing factories in the area. Pond owners quoted that stocking densities of shrimp were between 49-300 individuals per m² (mean±SD= 135±66, n=10) and stocking densities were between 300–750 individuals per m² (n=5). Four different kinds of artificial feed pellets varying in size are used for the different age classes of shrimp and are often complemented by fish, fish eggs and other homemade feed. Artificial feed pellets are also fed to fishes <8cm and captured fish is used, when they are older. Shrimps are fed 2-4 times a day depending on age, whereas fish are usually fed between twice a day and once every two days depending on age. Farm owners reported to produce between 563 and 22500 kg shrimp per ha per cycle (n=10). This represents extremely high mean annual yields of 5.3 kg shrimp per m² ranging from 0.2-7.9 kg m⁻² yr⁻¹ with an average survival rate of ~50 % (n=10). Survival rates of fish are also 50% on average (10-70%). Though, survival rates can vary considerably from crop to crop and have been reported to be decreasing during the past years, especially for shrimp. Antibiotics, disinfectors and water conditioners are widely used in most farms. Especially during heavy rain events, such as typhoons, antibiotics and disinfectors are amply applied. Nevertheless, almost all farmers bewail increasing occurrence of diseases due to deteriorated quality of estuarine and coastal water that is taken to fill the ponds.

The frequency of water exchange varied according to production stage and instantaneous water quality. Pond water exchange is usually minimal during the first month of crop production and increases with maturity of the animals raised. On
average, 25% (z₃) of the shrimp pond water is exchanged 2.5 times (i₃) during each production cycle, whereas the same portion of fish pond water (z₄) is exchanged 20 times (i₄) during the production cycle. In addition to that, the ponds are drained completely during harvest procedure 3.5 times a year (c₃) in shrimp ponds and once a year (c₄) in fish ponds. Pond effluents are released into natural creeks or artificial drainage channels or pumped directly into estuarine or coastal waters without prior treatment. New water for the ponds is taken in from estuarine/coastal waters using pumping systems. Water intake only takes place during high tide, while effluents are released any time. In some farms, water is filtered and treated with bleaching powder as disinfectant before ponds are filled.

In terms of feeding, stocking densities, pond water treatment and water exchange rates, no consistent pattern in operating practices could be observed. All farmers appeared to rely on their own experience based on their technical skills and knowledge. All farmers stated that they are interested in training and advice for farming improvements.

Tab. 1: Spatial extent, contribution of fish and shrimp culture and average annual export of effluents and dissolved and particulate matter from pond aquaculture at the different study sites in NE Hainan.

<table>
<thead>
<tr>
<th></th>
<th>WWE</th>
<th>Chan qi gan</th>
<th>Qingge</th>
<th>Coastal pond aquaculture</th>
<th>Total pond aquaculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond area [km²]</td>
<td>21.6</td>
<td>8.7</td>
<td>2.4</td>
<td>18.0</td>
<td>39.6</td>
</tr>
<tr>
<td>Fish culture [%]</td>
<td>10</td>
<td>80</td>
<td>70</td>
<td>55</td>
<td>40</td>
</tr>
<tr>
<td>Shrimp culture [%]</td>
<td>90</td>
<td>20</td>
<td>30</td>
<td>45</td>
<td>60</td>
</tr>
<tr>
<td>Average export of aquaculture effluents [10⁶ m³ yr⁻¹]</td>
<td>210</td>
<td>88</td>
<td>24</td>
<td>180</td>
<td>391</td>
</tr>
<tr>
<td>Average annual export loads from pond aquaculture [t yr⁻¹]:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP*</td>
<td>25</td>
<td>11</td>
<td>3</td>
<td>22</td>
<td>47</td>
</tr>
<tr>
<td>DIN</td>
<td>194</td>
<td>81</td>
<td>22</td>
<td>166</td>
<td>362</td>
</tr>
<tr>
<td>DON</td>
<td>147</td>
<td>60</td>
<td>16</td>
<td>123</td>
<td>268</td>
</tr>
<tr>
<td>PN</td>
<td>599</td>
<td>251</td>
<td>68</td>
<td>514</td>
<td>1119</td>
</tr>
<tr>
<td>TN</td>
<td>920</td>
<td>385</td>
<td>104</td>
<td>789</td>
<td>1718</td>
</tr>
<tr>
<td>DOC</td>
<td>1103</td>
<td>462</td>
<td>125</td>
<td>945</td>
<td>2059</td>
</tr>
<tr>
<td>POC</td>
<td>3908</td>
<td>1638</td>
<td>441</td>
<td>3350</td>
<td>7295</td>
</tr>
<tr>
<td>TOC</td>
<td>5011</td>
<td>2100</td>
<td>566</td>
<td>4295</td>
<td>9354</td>
</tr>
</tbody>
</table>

* refers to PO₄³⁻-phosphorus only
3.2 Water quality in shrimp ponds, fish ponds and drainage channels

An average pH around 8.3 and average salinities of 12.8 in shrimp ponds and 20.3 in fish ponds (Tab. 2) signify that the ponds are filled with estuarine/marine waters and confirm brackish water pond culture to be the common cultivation practice in the area. A lower pH in drainage channels (7.8 on average) indicates that these may also be fed with freshwater from the upper watershed (especially during and after rainfall). An average salinity of 17.2 in the drainage channels, which usually do not receive inflow from marine water, designates supply of salinity-rich pond effluents.

Very high average concentrations of nutrients and dissolved organic matter were found in waters of shrimp and fish ponds and drainage channels (Tab. 2). Especially average ammonium concentrations were high, ranging from 42.9 μM in fish ponds to 55.9 μM in drainage channels, and accounted for 60-85% of the DIN (Fig. 3). DIN concentrations in shrimp and fish ponds correlated significantly with age (in days) of the shrimps (p<0.05; corr. coefficient: 0.55) and fishes (p<0.05; corr. coefficient: 0.54) cultured. DON concentrations in fish ponds were significantly lower than in shrimp ponds and drainage channels (p<0.05) and accounted only for 25% of the TDN in fish ponds compared to 45% in shrimp ponds and drainage channels. Average DOC concentrations in shrimp ponds of 747 μM were almost twice as high as in fish ponds and drainage channel. Average phosphate concentrations in ponds and drainage channels ranged between 3.8 μM and 6.5 μM. Silicate concentrations in drainage channels of 54.5 μM were significantly higher than in shrimp and fish ponds (p<0.05).

In drainage channels, average concentrations of TSM (343 mgL⁻¹), POC (17 mgL⁻¹) and PN (2.8 mgL⁻¹) were higher than in fish and shrimp ponds. PN and POC concentrations were almost the same in fish and shrimp ponds (1.8 mg L⁻¹ and 11.0 mg L⁻¹), whereas TSM concentrations were higher in fish ponds (172 mgL⁻¹) than in shrimp ponds (133 mgL⁻¹). Average chl a concentrations ranged from 64 – 84 μgL⁻¹ and were highest in shrimp ponds (Tab. 2). Average molar C/N ratios of particulate matter (POC/PN) were significantly higher in drainage channels (9.9) than in fish ponds (7.8) and shrimp ponds (7.5), whereas average C/N-ratios of dissolved organic matter (DOC/DON) were significantly lower (p<0.05) in drainage channels (9.0) than in fish ponds (17.0) and shrimp ponds (12.1). Average inorganic N/P-ratios (DIN/PO₄³⁻) were 16.6 (fish ponds), 20.3 (drainage channels), and 24.5 (shrimp ponds).

Concentrations of all parameters measured in the respective fish and shrimp ponds and in drainage channels varied considerably, as indicated by the large range and a standard deviation, which even exceeded average values in some cases (Tab. 1). Due to the high variability within the three groups, only few significant differences between fish ponds, shrimp ponds and drainage channels could be detected.
Tab. 2: Water quality in shrimp ponds, fish ponds and drainage channels

<table>
<thead>
<tr>
<th></th>
<th>Salinity</th>
<th>pH</th>
<th>PO$_4^{3-}$</th>
<th>Si(OH)$_4$</th>
<th>NO$_3^-$</th>
<th>NO$_2^-$</th>
<th>NH$_4^+$</th>
<th>DIN</th>
<th>DON</th>
<th>DOC</th>
<th>TSM</th>
<th>PN</th>
<th>POC</th>
<th>Chl a</th>
</tr>
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<tbody>
<tr>
<td><strong>Shrimp ponds</strong></td>
<td>Mean</td>
<td>12.8</td>
<td>8.2</td>
<td>3.8</td>
<td>21.4</td>
<td>17.4</td>
<td>6.1</td>
<td>46.4</td>
<td>76.7</td>
<td>58.3</td>
<td>746.8</td>
<td>133.0</td>
<td>1.8</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2.0-23.1</td>
<td>7.6-9.5</td>
<td>0.9-14.3</td>
<td>0.2-80.8</td>
<td>0.0-147</td>
<td>0.0-36.8</td>
<td>0.8-429</td>
<td>0.8-456</td>
<td>23.8-106</td>
<td>258-1363</td>
<td>16.5-574</td>
<td>0.1-7.5</td>
<td>0.6-45.4</td>
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CHAPTER I

3.3 Export of effluents and dissolved and particulate matter from shrimp and fish ponds

Based on the presented data, we calculated that a total of $391 \times 10^6$ m$^3$ of effluents are released without any prior treatment from shrimp and fish ponds in the study area every year (Tab. 1). 54% of these effluents are initially drained into the Wenchang/Wenjiao Estuary, whereas the remaining 46% are directly exported to coastal waters via small drainage channels. Effluent export from the study sites Chang qi gang and Qingge accounted for 49% and 13% of the direct export to coastal waters, respectively. In the study area, 9354 t yr$^{-1}$ total organic carbon (TOC), 1718 t yr$^{-1}$ total nitrogen (TN) and 47.3 t yr$^{-1}$ dissolved phosphate are exported (Tab. 1) from total pond aquaculture. Particulate matter accounted with 65% PN and 78% POC for the major part of the TN and TOC, respectively.

3.4 Water quality in receiving estuarine and nearshore coastal waters

In estuarine waters of the WWE and Chang qi gang, the salinity was generally lower than in coastal waters, and nutrient concentrations and phytoplankton biomass were mostly high with concentrations >10 μM DIN, >1 μM PO$_4^{3-}$ and >3 μg L$^{-1}$ chl a (Fig. 4). Nutrient dynamics of the WWE are described in detail in Liu et al. (2011). Water quality parameters in nearshore coastal waters were comparable at all three study sites (Fig. 4, 5). In each coastal area, lowest salinities and highest concentrations of dissolved nutrients and organic matter were found within 100 m distance from the
shore and there was a general trend of rising salinity and decreasing concentrations of dissolved nutrients, organic matter and chl a in offshore direction (Fig. 5). There was a linear relationship of salinity with silicate ($r^2=0.77$, $p<0.001$) and nitrate ($r^2=0.68$, $p<0.001$), but not with phosphate and ammonium (Fig. 6). A strong spatial and temporal variability in all parameters was observed with temporal variability visible on a seasonal scale (rainy season 2008 vs. dry season 2009; Fig. 4, 5), as well as on a day-to-day scale.

Due to enhanced estuarine mixing, the range of salinity variability in the coastal waters of WWE and Chang qi gang (8-34 and 13-33, respectively) was higher than in Qingge (19-34; Fig. 4a, 6). Salinity values <15 were recorded during the rainy season in 2008 and were associated with a typhoon event (Herbeck et al., 2011). Silicate concentrations, which were usually relatively low (0-25 μM), were also higher in WWE and Chan qi gang (up to 76 μM) than in Qingge during single sampling events of the rainy season (Fig. 6a).

At all coastal sites, phosphate concentrations (Fig. 4b, 5a, 6b) were significantly higher ($p<0.05$) during the dry season in March/April 2009 (0.8 – 3.7 μM) compared to the rainy season in July/August 2008 (0 – 1.8 μM). In WWE and Qingge, phosphate concentrations declined in offshore direction, whereas values stayed on a relatively constant level over the distance transect in Chan qi gang. Phosphate concentrations were also high at high salinities (Fig. 6b).

Concentrations of dissolved inorganic nitrogen (Fig. 4c, 5b) were high close to the shore at some sampling events (up to 31 μM) and decreased in offshore direction (correlation with $p<0.05$ except Chang qi gang in 2008). DIN was dominated by ammonium making up 45-65% of the DIN pool (Fig. 3). While nitrate+nitrite concentrations tended to decline with higher salinities, this was not the case for ammonium concentrations (Fig. 6c, d). Also DON concentrations (Fig. 5c) were high, especially in Qingge and Chang qi gang, where concentrations >150 μM were measured over the whole distance gradient. DON concentrations did neither correlate with distance nor with salinity ($p>0.05$).

Concentrations of DOC (Fig. 5d) were significantly higher ($p<0.05$) at all coastal sites during the dry season in March/April 2009 (156 – 1718 μM) compared to the rainy season in July/August 2008 (42 – 1077 μM). Chl a concentrations were up to 38 μgL$^{-1}$ directly adjacent to the shore, but decreased in offshore direction and were <3 μgL$^{-1}$ at most stations (Fig. 4d, 5e). Chl a concentrations correlated positively with concentrations of salinity, DIN, NH$_4^+$, NO$_3^-$, and Si(OH)$_4$ in WWE and Huiwen ($p<0.05$). In Qingge, significant correlations of chl a were only found with salinity and NO$_3^-$ (2009) and TDN and DOC (2008).
Fig. 4: Concentration of salinity (a), PO₄³⁻ (b), DIN (c) and chl a (d) in estuarine and nearshore coastal waters in July/August 2008 and March/April 2009.
Fig. 5: Concentrations of PO$_4^{3-}$ (a), DIN (b), DON (c), DOC (d), and chl a (e) over a distance gradient from the shore in nearshore coastal waters at the three study sites in July/August 2008 and March/April 2009. Significant Spearman Rank Order Correlations are indicated with + for 2008 and * for 2009.
4. Discussion

4.1 Water quality in shrimp ponds, fish ponds and drainage channels

The water quality in drainage channels was comparable to fish and shrimp ponds, which confirms pond effluents to be their primary source of water. Very high average concentrations of nutrients and dissolved and particulate organic matter in shrimp ponds, fish ponds and drainage channels reflect high inputs and remineralisation processes. Ammonification appears to be especially high resulting in the high ammonium concentrations in ponds and drainage channels, while comparatively lower nitrate concentrations in ponds can be referred to a low abundance of nitrifiers in pond sediments, which may be inhibited by pond conditions (Burford and Longemore, 2001). In contrast to earlier findings that ammonium is the dominant N species (Lorenzen et al., 1997), we found DON to be the dominant dissolved N component in shrimp ponds, as also observed by Jackson et al. (2003). Higher concentrations of DON and DOC in shrimp compared to fish ponds indicates
dissolution of greater amounts of feed in the water of shrimp ponds. Reasons for that may be the addition of homemade feed, such as low quality local fish and eggs, to shrimp ponds, which are frequently used in Chinese farms and are often instable in water getting rather remineralized than consumed (Biao and Kajin, 2007). More frequent feeding of shrimp compared to fish may also cause larger amounts of feed to remain in excess resulting in enhanced leaching of dissolved organic matter from food particles in shrimp ponds. Since DON is only slowly utilized by bacteria in shrimp pond water, it can accumulate over the crop cycle (Burford and Williams, 2001). High chl a concentrations together with a C/N ratio of particulate matter close to the Redfield ratio (6.6) indicate phytoplankton as the primary suspended matter source in shrimp and fish ponds, as was also inferred by Jackson et al. (2003). Enhanced concentrations of POC and PN with an elevated C/N ratio compared to shrimp and fish ponds and similar chl a levels indicate additional contribution of OM-rich pond sediments in drainage channels. Those are resuspended in the water during pond drainage at harvest. Also nutrient concentrations tended to be higher in drainage channels compared to ponds, which may be driven by further remineralisation processes of organic matter in the channels. Since drainage channels receive effluents from several ponds, they contain effluents of different nutrient concentrations. The variability in water quality parameters observed in drainage channels is thus directly linked to the different production stages and farm management of neighbouring ponds of an area. The high variability in the water quality parameters between the respective shrimp and fish ponds is concordant with results of other studies reporting on substantial variability in water parameters of pond effluents within and between days, and is largely due to the different production states of the ponds during water sampling (e.g. Burford et al., 2003; Jackson et al., 2003). The observed increase of nutrient concentrations in shrimp and fish ponds with animal age is consistent with results of other studies (e.g. Biao et al., 2004; Burford et al., 2003; Hopkins et al., 1993). It can be related to enhanced ammonia excretion by larger animals and to increased remineralisation resulting from higher food application towards the end of the cultivation period. Moreover, parts of the variability can be due to the time of sampling; Nutrient concentrations are generally lower after water exchange and increase over time. The high variability in water quality is probably further driven by the observed heterogeneity in operating characteristics of the respective farms in the area. Despite the high variability, average nutrient concentrations (especially NH₄⁺ and DON) in aquaculture ponds and especially in drainage channels can be regarded as very high. In 95 % of the drainage channels sampled, nutrient concentrations exceeded the maximum allowed concentrations (DIN: 7.1 μM; PO₄³⁻: 0.48 μM) for inlet and outlet rivers in China (SEPA, 1997).
4.2 Export of effluents and dissolved and particulate matter from shrimp and fish ponds

The major part of carbon and nitrogen exported to estuarine and coastal waters occurs in solid form with POC and PN accounting for 78% and 65% of the TOC and TN, respectively. Total phosphorous (TP) was not measured, but according to other studies, which showed that dissolved phosphate accounts for less than 15% of the TP in shrimp pond effluents (e.g. Funge-Smith and Briggs, 1998; Islam et al., 2004), most phosphorous can be expected to be associated with particles. This supports the assumption that exported matter from aquaculture ponds mainly consists of phytoplankton and organic matter-rich particles, which are further recycled in the receiving natural waters. But also the flux of dissolved matter with aquaculture effluents was very high (Tab. 1).

There are few data available about export rates from other pond aquaculture areas. Biao and Kaijin (2007) reported that 658 t of nitrogen and 307 t of phosphorus were released with shrimp sewage in 1998 in Fujian Province, which has a coastline of 2 120 km. Our estimates from a much smaller area in NE Hainan (coastline ~ 45 km) exceed those loads by far, which indicates that NE Hainan is a globally significant area of aquaculture production. De Silva et al. (2010) estimated 31 602 t of nitrogen and 9893 t of phosphorus to be discharged from pond-based striped catfish production in 2007 in the Mekong Delta comprising an area of approximately 70 km². These estimates are substantially higher than ours. However, they were calculated from nutrient models based on nutrients added as feed and removed by fish harvest, which did not take into account loss terms, such as uptake by phytoplankton and other biota, denitrification and volatilization of ammonia within the ponds and drainage channels. Thus, they do not represent the actual net N and P loads released, as calculated in our approach.

Export yields of TN from aquaculture ponds in NE Hainan was in between those of other intensive shrimp ponds and higher than export rates from ponds of semi-intensive and extensive shrimp production reported by other studies worldwide (Tab. 3). Under the assumption that dissolved inorganic phosphate accounts for 15% of the TP, we arrive at extrapolated TP export rates of about 8 t km⁻² yr⁻¹, which correspond to those reported in the literature (Tab. 3). Annual export of N and P in Hainan is enhanced relative to other regions by the fact that there are up to four crops of shrimp raised, while in many regions of the world only two crops are harvested each year (e.g. Briggs and Funge-Smith, 1994; Islam et al., 2004; Páez-Osuna et al., 1997). It is likely that the major part of the nutrients and organic matter is released into the environment at the time of harvest due to the high concentration at the late production stage, the
fact that the ponds are completely emptied during harvest, and the additional export of suspended organic matter-rich pond sediments. Therefore, nutrient and organic matter export is increased disproportionally with each crop raised.

As in every nutrient budget, there are uncertainties when calculating fluxes and yields, which may introduce large errors. The variety of methods used to calculate export yields impairs comparison between studies and areas. Our calculations of annual effluent export can be regarded as conservative, as we excluded extremely high values from the calculations of nutrients and organic matter exports. Furthermore, our calculations consider minimal water exchange from ponds and do not include additional water exchange related to e.g. excessive rain water flushing.

Irrespective of potential errors, our results reveal a substantial supply of organic carbon, nitrogen and phosphorus to a relatively small coastal area of NE Hainan. This is of particular importance, since aquaculture ponds predominantly replaced former mangrove areas, which usually function as a sink for land-derived nutrients. Instead, aquaculture-derived N and P loads are since then being released from a land area, where originally few nutrients were released from, turning it from a net nutrient sink or weak source into a very strong net nutrient source. Therefore, the additional inputs of N and P from aquaculture production represent a considerable human intervention in a tropical coastal zone.

Tab. 3: Comparison of nitrogen and phosphorus export from aquaculture ponds in NE-Hainan to that of ponds for intensive, semi-intensive, and extensive shrimp production world wide. Yields are based on total nitrogen and total phosphorus if not stated else.

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<td>[t km(^{-2}) yr(^{-1})]</td>
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\(^a\) refers to PO\(_4\)\(^3-\)phosphate only  
\(^b\) refers to DIN-nitrogen only
4.3 Effects on water quality and trophic status of receiving estuarine and nearshore coastal waters

High concentrations of nutrients, dissolved organic matter and chl a close to the shore at all three coastal study sites (Fig. 4, 5) reflect considerable impact from land. Concentrations of nutrients and dissolved organic matter tend to decrease in offshore direction as a matter of dilution with oceanic water and uptake by primary producers. A similar composition of DIN species in coastal waters and pond waters and drainage channels (Fig. 3) indicates nitrogen to mainly originate from aquaculture effluents. While silicate and nitrate+nitrite concentrations, which are typically not enhanced in aquaculture effluents, are low at high salinities, concentrations of ammonium and phosphate can be strongly enhanced in high salinity coastal waters (Fig. 6). Despite a generally large variability, phosphate even displayed maximum values at high salinity. This reflects direct injection of phosphate- and ammonium-laden water from aquaculture drainage channels into high salinity coastal waters being responsible for the observed high concentrations of phosphate and DIN in back-reef waters. Chl a concentrations were also high close to the shore due to introduction of phytoplankton-rich aquaculture effluents and nutrient-stimulated productivity of coastal waters decreasing in offshore direction with decreasing nutrient availability.

There was a considerable spatial and temporal variability in nutrient, dissolved organic matter and chl a concentrations in coastal waters, especially close to the shore (Fig. 5). This variability is mainly driven by temporally and spatially varying export of aquaculture effluents to coastal waters via drainage channels. Since production in ponds is usually not synchronized, water exchange is random depending on e.g. production stage of the respective ponds. The introduction of nutrient-rich effluents from only a single pond drained at a time may cause a disproportional local increase of nutrient concentrations in the receiving water body. Therefore, nutrient concentrations in coastal waters vary according to numbers of ponds in the area releasing water at a time.

Also, tidal dynamics may influence nutrient and suspended matter concentrations, which is most evident at sites with strong land-derived inputs. Krumme at al. (under review) observed a tidally driven variability of nutrient concentrations at a station in ~1.5 km distance from the outlet of the WWE with nitrate+nitrite ranging from 0.1-1.8 μM, ammonium from 0.3-5.3 μM, phosphate from 0.3-1.0 μM and silicate from 3.1-6.0 μM. This reveals that even in a micro tidal area, tidal currents have a great effect on nutrient concentrations and dispersal.

Furthermore, rain events can cause an additional introduction of nutrients because drainage channels may carry floodwater derived from the hinterland that is
enriched in nutrients during and after rain events besides aquaculture effluents. The effects of continuous aquaculture inputs versus pulsed rain inputs are reflected in the amount and composition of nutrients in coastal waters. Strong rain falls, especially those associated with typhoon Kammuri in August 2008, were responsible for low salinities in back-reef areas between 2 and 5, where salinities are usually >20 (Fig. 4a, 6). High concentrations of silicate and nitrate+nitrite, but not of ammonium and phosphate, at low salinities (Fig. 6) in coastal waters indicate that rain events are mainly responsible for the introduction of nitrate and silicate into coastal waters with floodwater. Nitrate probably originates from atmospheric inputs from rain and leaching of fertilizers from agricultural fields, while silicate concentrations can be attributed to increased weathering during rainfalls (Herbeck et al., 2011). Thus, during rain events, aquaculture effluents are not the exclusive source for elevated DIN concentrations in the coastal zone.

Concentrations of most parameters displayed little seasonal variation, which is most probably related to the fact that there were significant rain events during both sampling seasons. Average PO$_4$$^{3-}$, DOC and DON concentrations were higher during March/April 2009 than in 2008. This is likely due to the fact that rain falls were the first after the dry season probably leaching significant amounts of nutrients from the hinterland that have accumulated over the dry season. This ‘first-flush’ effect was also observed in other areas with the onset of the rainy season (e.g. Eyre and Balls, 1999; Boonphakdee and Fujiwara, 2008).

According to categories established for coastal waters of the Baltic (DIN>2.1 μM; Håkanson, 1994) and of the eastern Mediterranean Sea (DIN>0.4 μM; Karydis, 1996), DIN concentrations rank almost all the stations in the category of eutrophic or hypertrophic waters. The same is true for phosphate concentrations, which for the majority of stations exceeded 1.1 μM defined as the threshold value for TP classifying waters as eutrophic (Smith et al., 1999). Concentrations of <2 μM DIN and <0.5 μM PO$_4$$^{3-}$ are reported for the majority of other tropical back-reef and seagrass areas (e.g. Szmant, 2002). Chl a concentrations were at most stations between 1 and 3 μg L$^{-1}$, which refers to mesotrophic waters according to the classification by Håkanson (1994) for the Baltic Sea. In coral reef areas worldwide, however, chl a concentrations are usually <1 μg L$^{-1}$, while values around 1 μg L$^{-1}$ are found in sewage contaminated reef areas (e.g. Furnas et al., 1990; Liston et al. 1992; Otero and Carbery, 2005; Van Duyl et al., 2002). According to these literature values, our data indicate strong eutrophication effects in coastal waters of NE Hainan.

Although no water quality data are available for the period before pond aquaculture started, original concentrations should have been significantly lower. This
assumption is supported by Feng (1996, cited in Biao and Kajin, 2007), who found values of chemical oxygen demand (COD), active phosphate and ammonium in the Bohai Sea, where large-scale shrimp farming is conducted, to be 3.7, 7.8, and 2.4 times higher compared to values before the development of aquaculture production. In our study area, the reef forms a barrier that hampers water exchange with the coastal ocean. Therefore, residence time of nutrient-rich effluents in back-reef areas is prolonged in comparison to open shelves increasing exposure of seagrass and corals to high nutrient and organic matter concentrations. This may trigger enhanced productivity not only of phytoplankton, but also of macroalgae and epiphytic algae communities, which shade seagrasses and corals and deteriorate their health (e.g. Hauxwell et al., 2001; Hughes, 1994; Silberstein et al., 1986). We observed thick epiphytic mats on seagrass leaves and corals, which reflect the nutrient enrichment in coastal waters. Therefore, the placement of ponds in Qingge and Chang qi gang appears especially harmful, since the enclosed character of the back-reef areas worsens negative effects of aquaculture effluents.

Even though the nutrient and organic matter flux from aquaculture ponds in WWE was 2.3 and 8.9 times higher than in Chang qi gang and Qingge, respectively, concentrations of dissolved nutrients in the coastal zone of the WWE were similar or lower than in Chang qi gang and Qingge. This is most probably due to the efficient filtering capacity of the estuarine lagoon of the WWE, where parts of nutrients from aquaculture effluents are transferred into biomass and retained, thereby reducing nutrient export to coastal waters (Herbeck et al., 2011). However, after strong rain events, the estuarine lagoon gets flushed by fresh water resulting in an immediate export of nutrients and organic matter from aquaculture effluents and other land-based sources into coastal waters (Herbeck et al., 2011; Krumme et al., under review). Strong rain events should also enhance export of nutrients into the coastal zone from coastal aquaculture production, such as in Chang qi gang and Qingge, as drainage channels may carry both, floodwater derived from the hinterland and aquaculture effluents. Comparing the three investigated areas, it can be concluded that aquaculture effluents initially released into waters of the estuarine lagoon in WWE have less effects on the water quality of coastal waters than effluents from ponds in direct vicinity to the shore in Chang qi gang and Qingge, as parts of the nutrients are retained at least during dry conditions.
5. Summary and Conclusions

High amounts of aquaculture effluents rich in dissolved inorganic and organic matter were released from aquaculture ponds at the NE coast of Hainan concentrated in a relatively small geographical area. These inputs cause eutrophic conditions in the adjacent coastal waters. Concentrations decreased in offshore direction but exceeded threshold values for good water quality even at most remote stations. Despite highest aquaculture production area and amounts of effluents released in the watershed of WWE, nutrient concentrations in adjacent coastal waters were not elevated compared to coastal sites of Qingge and Chang qi gang having smaller pond areas in their hinterland. This is attributed to the fact that effluents from WWE are released into an estuarine lagoon, which functions as a filter reducing nutrient export under dry weather conditions, whereas at Qingge and Chang qi gang, aquaculture effluents are released directly to coastal waters. Aquaculture production in direct adjacency to back-reef areas, such as in Qingge and Chang qi gang, may be especially harmful to seagrass meadows and coral reefs, due to limited water exchange resulting in eutrophication and, subsequently, shading and competition pressure on these valuable habitats from an increased algae biomass. In order to conserve the natural services provided by the coastal habitats, effluents should be treated before release into natural water bodies and pond aquaculture next to back-reef areas should generally be relinquished.

Acknowledgements

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The impact of anthropogenic activities on nutrient dynamics in the tropical Wenchanghe and Wenjiaohe Estuary and Lagoon system in East Hainan, China

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The impact of anthropogenic activities on nutrient dynamics in the tropical Wenchanghe and Wenjiaohe Estuary and Lagoon system in East Hainan, China

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Abstract

Biogeochemical observations were carried out in the Wenchanghe and Wenjiaohe Estuary, Bamen Bay and Gaolong Bay during 2006-2009 to understand the nutrient dynamics of these areas and their relationship with the sustainability of the ecosystems in the coastal areas of Eastern Hainan Island and its adjacent South China Sea. Nutrients in river/estuary waters, groundwater, aquaculture effluents and rainwater samples were analyzed using spectrophotometry. Nutrient levels in the tropical Wenchanghe and Wenjiaohe show a wide range of variation depending on the system, nutrient element and season. These two rivers are enriched with DIN and depleted in PO4 3- with the DIN:PO 4 3- ratios varied from 60 to 411. In the rivers, TDP was mainly composed of DOP, representing ~65%. DON accounted for 40% of TDN in the Wenchanghe and 76% of that in the Wenjiaohe. Dissolved silicate levels in the Wenjiaohe and Wenchanghe were lower than average levels in tropical systems. Nutrients in the Wenchanghe and Wenjiaohe show a wide range of variation depending on the system, nutrient element and season. These two rivers are enriched with DIN and depleted in PO4 3- with the DIN:PO 4 3- ratios varied from 60 to 411. In the rivers, TDP was mainly composed of DOP, representing ~65%. DON accounted for 40% of TDN in the Wenchanghe and 76% of that in the Wenjiaohe. Dissolved silicate levels in the Wenjiaohe and Wenchanghe were lower than average levels in tropical systems. Nutrients in the Wenchanghe and Wenjiaohe Estuary behave either conservatively or non-conservatively depending on the element being considered and the season. Based on observations of nutrients in various aquatic environments, a simple steady-state mass-balance box model was employed to assess nutrient budgets in the estuary system. Nutrients in the studied system were mostly from riverine input, groundwater discharge and aquaculture effluents. The nutrients exported in the studied system are largely confined to the immediate estuaries. The typhoon-induced runoff of terrestrial rainwater can not only increase nutrient inputs to the coastal ecosystem but can also result in nutrient imbalance, affecting phytoplankton production and

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composition.

**Keywords:** nutrients, sources; budget, Wenchanghe and Wenjiaohe Estuary, lagoon, South China Sea

1. Introduction

Riverine transport is a principal pathway of particulates and dissolved elements from land to sea. Estuaries modify riverine nutrient fluxes to the sea significantly through biogeochemical processes (Liu and Zhang, 2004; Liu et al., 2009; Soetaert et al., 2006). Small and midsized rivers, which are more easily affected by natural and environmental changes than the major river systems, can still contribute to substantial changes in the ecosystems in coastal ocean (Jennerjahn et al., 2004). Dramatic increases in the delivery of riverborne nutrients and changes in nutrient ratios owing to anthropogenic activities are known to result in eutrophication, which modifies the aquatic food webs and causes severe hypoxic events in coastal environments (Turner and Rabalais, 1994; Turner, 2002; Liu et al., 2009; Diaz and Rosenberg, 2008).

Hainan Island, situated in the southern part of China in the South China Sea (SCS), is abundant in tropical ecosystems, such as mangroves and coral reefs, and has a surface area of 33920 km² and population of 750×10⁴ inhabitants. A tropical monsoonal insular climate prevails in Hainan Island, with northerly winds in the winter and southerly winds in the summer (Su, 2004). However, since the 1970s, many fringing reefs have been destroyed by tourism and for high-grade lime and cement purposes, which has resulted in an approximately 100-meter retreat of the coastal zone (Chen and Teng, 1996). Since the 1980s, the mangrove area has been reduced; 2×10⁵ m² of the whole Hainan Island mangrove ecosystem has been changed to a manmade shrimp ecosystem (Jin et al., 2008). Due to climate change and anthropogenic activities, such as intense agricultural, fishing and tourism activities, the tropical ecosystems of Hainan Island are facing serious problems, such as changes in land use, destruction of mangroves, aquaculture effluents, overfishing, use of illegal fishing techniques (i.e., dynamite and cyanide), sedimentation, waste discharge and fertilizer and pesticide use, which are threatening more than 90% of the sensitive ecosystems of the island (Jin et al., 2008; Gong et al., 2008).

This study presents the results of biogeochemical observations in the Wenchanghe and Wenjiaohe Estuary and Lagoon system during 2006-2009. Nutrients in river/estuary waters, groundwater, aquaculture waters and rainwater samples were collected and analyzed. The results of this study have led to a better understanding of the nutrient dynamics in the region and their relationship with the sustainability of the ecosystems in the coastal areas of Eastern Hainan Island and the adjacent coastal waters of the SCS.
2. Materials and methods

2.1 Study area

Qinglan Lagoon, situated in Wenchang City, which is in the northeastern part of Hainan Island, is famous for its abundant mangroves, coral reefs and the longest fringing reef with a surface area of 40 km² in history, which has now been reduced to approximately 20 km², and has annual average rainfall of 1740.5 mm and evaporation of 1115.7 mm (Wang, 2002). Qinglan Lagoon is composed of two bays: the internal Bamen Bay and the outer Gaolong Bay (Wang et al., 2006). The Wenchanghe and Wenjiaohe empty into Bamen Bay from the west and east (Fig. 1) with a drainage area of 380.9 km² and 522.0 km², length of 37.1 km and 56.0 km and freshwater discharge of 9.09 m³ s⁻¹ and 11.6 m³ s⁻¹, respectively (Zeng and Zeng, 1989). Approximately 82% of the total annual water flow in these rivers occurs during the wet season from May to October. Bamen Bay is a shallow water of body with a water depth of approximately 1.0 m in the center, 2-3 m in the southwest and at the Qinglan tidal inlet, and less than 1.0 m off the Wenjiaohe Estuary. Bamen Bay is characterized by an irregular diurnal tide and mixed tide, with a diurnal tide for 15-18 days followed by a semidiurnal tide for approximately 11 days (Wang, 2002). Gaolong Bay connects the Qinglan tidal inlet in the southeast of Bamen Bay and joins the SCS in the south (Fig. 1). Flourishing mangroves develop from Wenchanghe to the northern part of Bamen Bay and Qinglan tidal inlet, and coral reefs develop outside the Qinglan tidal inlet. However, the mangrove and coral reef ecosystems are subjected to deterioration caused by anthropogenic activities (Jin et al., 2008).
Fig. 1: (a) Locations of the stations for the cruises in the Wenchanghe and Wenjiaohe Estuary and Lagoon system from 2006 to 2009, which shows the sampling periods of December 2006, August 2007, July-August 2008 and March-April 2009 in the estuary. (b) The sampling stations in the submarine groundwater (left, •: 2007; ○: 2008; ▲: 2009) and aquaculture effluents (right, ○: 2008; ▲: 2009). In addition, two anchor stations (★) were observed over 25 hours in Bamen Bay and the Qinglan tidal inlet in 2008, and one anchor station was observed each day over the investigation periods in Gaolong Bay in 2007, 2008 and 2009. (c) The tracks of the drift observations in the Qinglan tidal inlet and Gaolong Bay in 2008. The tracks for each drift observation were identified with progressive vectors (arrows) to show the track directions.
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2.2 Sampling

Field observations were undertaken in the Wenchanghe and Wenjiaohe Estuary during 2006-2009 (specifically, December 5-16, 2006, August 12-30, 2007, July 23 -August 15, 2008, March 26 -April 15, 2009) and included fresh water to marine water with salinities of 30-34. The stations occupied in the cruises are presented in Fig. 1. The investigations were carried out in the estuary three times in the 2008 (two before the typhoon and one after) and twice in the 2009 cruises. In the eastern part of Bamen Bay, sampling is quite difficult because the Wenjiaohe mouth develops a subaqueous delta with a water depth less than 1.0 m and because fishing nets is widespread. As a result, samples in the eastern part of Bamen Bay are scarce. In August 2008, two anchor stations, which were located in Bamen Bay and the Qinglan tidal inlet (Fig. 1), were observed over 25 hours. In Gaolong Bay, one anchor station was observed each day at 8 A.M. in August 2007 and at high tide over the investigation periods in the 2008 and 2009 cruises (Fig. 1). In addition, drift experiments of approximately 8 hrs were conducted in the Qinglan tidal inlet and Gaolong Bay (Fig. 1) to follow the water mass movement in 2008. The drift was a cross sail at a 0.6 m depth attached to a buoy by a nylon cable. A thin bamboo pole fixed to the top of the buoy carried a flag. The trajectory of the drift was recorded with a Global Positioning System (Model: Explorist 500, US). Water samples were taken adjacent to the drift every 20 minutes for drift Dr2 and every 30-60 minutes for drifts Dr4 and Dr5 (Fig. 1). Rainwater, groundwater and aquaculture effluent samples around the estuaries were also collected during the expeditions (Fig. 1).

The temperature and salinity of the water were measured in situ with a WTW MultiLine F/Set3 multiparameter probe and a CTD (Alec Electronics AAQ1183). In the field observations, water samples were collected with 2-L polyethylene bottles attached to a glass-fiber-reinforced fishing pole, except when the water depth was greater than 3 m, a 10-L Niskin bottle was used to collect near-bottom water. After collection, we filtered the samples through 0.45-μm cellulose acetate filters that had been cleaned with hydrochloric acid (pH=2) and rinsed with Milli-Q water before use. The filtrates were fixed using saturated HgCl2 and stored in the dark. In the land laboratory, the filters were dried at 50 °C and weighed again to determine the suspended particulate matter.

2.3 Chemical analysis

The nutrients were analyzed using an autoanalyzer (Model: Skalar SANplus) and manual methods (Liu et al., 2005). The analytical precision of NO2-, NO3-, NH4+, PO43- and Si(OH)4 were 0.01 μM, 0.06 μM, 0.09 μM, 0.03 μM and 0.15 μM, respectively (Liu et al., 2009). The concentration of dissolved inorganic nitrogen (DIN) is the sum of NO3-, NO2- and NH4+. The total dissolved nitrogen (TDN) and phosphorus (TDP) were decomposed to NO3- and PO43-, respectively, with a boracic acid-persulfate oxidation solution and were measured
using an autoanalyzer (Liu et al., 2009). The analytical precision of TDN and TDP were 0.68 
\(\mu\)M and 0.02 \(\mu\)M, respectively. The concentration of dissolved organic nitrogen (DON) and
dissolved organic phosphorus (DOP) is the difference between TDN and DIN and TDP and
\(\text{PO}_4^{3-}\), respectively. The samples were analyzed in duplicate, and the precision for DON and
DOP were 15\% and 5\%, respectively.

2.4 Estuarine nutrient budgets-LOICZ approach

We adopted a box model devised by Land Ocean Interactions in the Coastal Zone
(LOICZ) (http://nest.su.se, Gordon et al., 1996) to construct nutrient budgets from
non-conservative distributions of nutrients and water budgets, which in turn were
constrained by the salt balance. In this model, an estuary is treated as a single box, which is
well mixed both vertically and horizontally, and is assumed to be at a steady state. This
model has been extensively used and described in many sources (Liu et al., 2005, 2007,
2009). Briefly, the water mass balance was estimated using the following equation:

\[
V_R = V_{in} - V_{out} = -V_Q - V_P - V_G - V_W + V_E \quad (1)
\]

where \(V_R\) is denoted as the residual flow, which is equal to the net input of
freshwater, and \(V_Q, V_P, V_E, V_G, V_W, V_{in}, V_{out}\) are the mean flow rate of the river water,
precipitation, evaporation, groundwater, waste water, advective inflow and outflow of water
from the system of interest, respectively. Letting salinity be 0 for fresh water \((V_Q, V_P\) and \(V_E)\),
the salt balance in the system of interest, therefore, can be derived:

\[
V_X (S_I - S_2) = S_R V_R \quad (2)
\]

where \(S_R = (S_I + S_2)/2\), \(S_I\) and \(S_2\) are the mean salinities in the system of interest and
the adjacent system, respectively, and \(V_X\) is the water exchange flow or mixing flow between
the system of interest and adjacent system. The total water exchange time \(\tau\) of the system
of interest can be estimated from the ratio \(V_S/(V_R + V_X)\), where \(V_S\) is the volume of the
system.

Non-conservative fluxes of nutrient elements \(\Delta Y\) can be derived based on water
budgets and nutrient concentrations:

\[
\Delta Y = \Sigma \text{outflux} - \Sigma \text{influx} = V_R C_R + V_X C_X - V_Q C_Q - V_P C_P \quad (3)
\]

where \(C_Q, C_1, C_2, C_P, C_R\) and \(C_X\) denote the mean element concentration in the river
runoff, system of interest, adjacent ocean system, precipitation, residual-flow boundary
\((C_R = (C_1 + C_2)/2)\) and mixing flow \((C_X = C_1 - C_2)\), respectively. A negative or positive sign of
\( \Delta Y \) indicates that the system of interest is a sink or a source, respectively.

3. Results

3.1 Hydrographic character

As the investigated estuary system is in a tropical region, the water temperature varied from 22 to 34 °C, with higher values in the summer than in the winter or spring. The salinity in Bamen Bay changed significantly with the tide, with values of 20-30 in Dec 2006, 10-30 in Aug 2007, 1-25 in Jul-Aug 2008 and 10-25 in April 2009. During the 2008 cruise, Typhoon Kammuri passed the northern part of Hainan Island, and low salinity was only observed near the Wenchanghe and Wenjiaohe mouth before the typhoon, while a salinity of approximately 1 was observed in Gaolong Bay after the typhoon.

Statistical analysis based on the time-series data collected at the Qinglan tidal inlet over the last 40 years indicated that the air temperature has increased, especially since 2000 at a rate of approximately 0.30 °C yr\(^{-1}\). Accordingly, the water temperature has increased at a rate of 0.014 °C yr\(^{-1}\). The annual rainfall in Qinglan slightly increased with an increase rate of approximately 4 mm yr\(^{-1}\). Although as shown in Fig. 2, salinity slightly decreased at a rate of 0.025 yr\(^{-1}\), and tide levels slightly increased, we should note that they may be affected by differences on sampling periods. In addition, the changes in water temperature and salinity are not statistically significant. The ecological environment of Qinglan Lagoon may be affected by climate change.
3.2 Nutrients in the rivers

The nutrient concentrations in the Wenchanghe and Wenjiaohe varied with season and were affected by rainfall and anthropogenic activities, such as fertilizer application and wastewater discharge. The concentrations of nutrients in these two rivers are generally enriched with DIN and Si(OH)₄ and depleted in PO₄³⁻, similar to the other rivers in the temporal and subtropical regions of China (Liu et al., 2009). The concentrations of dissolved inorganic nitrogen (NO₃⁻, NO₂⁻, NH₄⁺) and dissolved silicate in the Wenchanghe are 1.4-2.1 times higher than those in the Wenjiaohe. While DON concentrations in the Wenjiaohe are 1.7 times higher than in the Wenchanghe, the concentrations of phosphate, DOP and TDP were comparable in the Wenchanghe and Wenjiaohe (Tab. 1). The nutrient transport fluxes from rivers into the estuary system are estimated by the product of the nutrient concentrations and long-term average freshwater discharges of the Wenchanghe and Wenjiaohe (Tab. 1).
Tab. 1: Concentrations of nutrients (μM), the percentage of DON in TDN and DOP in TDP, the molar ratios of DIN/DIP and Si/DIN (A) and annual average nutrient fluxes (10⁶ mol yr⁻¹) (B) in the Wenchanghe (WC) and Wenjiaohe (WJ) in 2006 to 2009

| Time | River | NO₃⁻ | NO₂⁻ | NH₄⁺ | PO₄³⁻ | Si(OH)₄ | TDN | DON | TDP | DOP | DON/TDN | DOP/TDP | DIN/DIP | Si/DIN |
|------|-------|------|------|------|-------|---------|------|-----|-----|-----|-------|---------|---------|---------|-------|
| 2006 | WC    | 44.4 | 1.30 | 3.83 | 0.12  | 157     | 66   | 17  | 0.69| 0.57| 25    | 83      | 411     | 3.2     |
| 2007 | WC    | 68.4 | 4.23 | 56   | 1.06  | 176     | 142  | 13  | 5.9 | 4.9 | 9     | 82      | 122     | 1.4     |
| 2007 | WJ    | 42.6 | 5.12 | 28   | 0.71  | 148     | 111  | 36  | 2.03| 1.32| 32    | 65      | 106     | 2.0     |
| 2008 | WC    | 63.8 | 2.17 | 12.5 | 0.54  | 153     | 103  | 24  | 1.53| 0.99| 24    | 65      | 145     | 1.9     |
| 2008 | WJ    | 17.5 | 1.20 | 1.13 | 0.23  | 108     | 70   | 50  | 3.03| 2.8 | 71    | 92      | 85      | 5.4     |
| 2009 | WC    | 82   | 2.68 | 13.7 | 0.76  | 172     | 152  | 53  | 0.98| 0.22| 35    | 23      | 129     | 1.7     |
| 2009 | WJ    | 90   | 2.86 | 3.92 | 1.00  | 98      | 156  | 59  | 1.51| 0.50| 38    | 33      | 97      | 1.0     |

Panel B: Nutrient fluxes

| River | NO₃⁻ | NO₂⁻ | NH₄⁺ | PO₄³⁻ | Si(OH)₄ | TDN | DON | TDP | DOP | DON/TDN | DOP/TDP | DIN/DIP | Si/DIN |
|-------|------|------|------|-------|---------|------|-----|-----|-----|-------|---------|---------|---------|-------|
| WC    | 18.5±4.5 | 0.74±0.35 | 6.18±6.73 | 0.18±0.11 | 47.1±3.2 | 33.2±11.2 | 7.7±5.2 | 0.66±0.71 | 0.48±0.62 | 25.5±9.6 |
| WJ    | 18.3±13.5 | 1.12±0.72 | 3.97±5.30 | 0.24±0.14 | 43.0±9.7 | 41.1±15.9 | 17.7±4.3 | 0.80±0.28 | 0.56±0.42 | 23.4±14.5 |
| Sum   | 36.9±14.2 | 1.86±0.80 | 10.2±8.6 | 0.42±0.18 | 90.1±10.2 | 74.2±19.4 | 25.4±6.8 | 1.46±0.76 | 1.04±0.75 | 48.9±17.4 |
3.3 Nutrients at anchor stations

The nutrient concentrations in Bamen Bay in 2008 changed 2-10 times, except ammonium changed 7-16 times and nitrate changed 68-140 times. Nutrient levels had maximum values at low tide and low values at high tide, except for certain phosphorus species (phosphate, DOP and TDP). These results indicate that various nitrogen species (nitrate, nitrite, ammonium, TDN and DON) and dissolved silicate in Bamen Bay are affected by land sources. Generally, nutrient concentrations in the surface waters were higher and had larger differences than in the near-bottom waters (Fig. 3). However, in the Qinglan tidal inlet, nutrient concentrations changed less than 7 times, except 9-16 times for DOP, which are narrower than in Bamen Bay. All the nutrient concentrations in the near-bottom waters were comparable to or higher than those in the surface waters (Fig. 3). The nutrient concentrations did not vary with tide, except the dissolved silicate concentrations had maximum values at low tide, similar to those in Bamen Bay.

In Gaolong Bay, the nutrient concentrations changed 2-62 times depending on the season, element and tide but did not show a variation pattern similar to those above (Fig. 4). Generally, dissolved inorganic nitrogen (NO$_3^-$, NO$_2^-$, NH$_4^+$) changed in a wide range and was affected by anthropogenic activities, such as fertilizer use and wastewater discharge (Liu et al., 2009). Various phosphorus species (phosphate, DOP and TDP) changed within a narrow range and were affected by the adsorption/desorption of phosphate to/off of the suspended particulate matter. The typhoon in August 2008 increased surface runoff; accordingly, the salinity decreased, and the nutrient concentrations increased (Fig. 4).
Fig. 3: Variations of nutrients at the anchor stations in Bamen Bay (left) and the Qinglan tidal inlet (right) in 2008; the tide (cm) during the investigation period was provided (bottom).
Fig. 4: Variations of nutrients at the anchor stations in Gaolong Bay during the 2007 (a), 2008 (b) and 2009 (c) cruises.
3.4 Nutrients during drift observations

The drift observations were mainly made during the ebb tide; the drift flowed from the Qinglan tidal inlet to Gaolong Bay and was stranded several times; only three drift observations were successful. Drift 2 was during the ebb tide with salinity less than 6, indicating that freshwater flows into the Gaolong Bay. Drift 4 was during the ebb tide, except for the last two samplings, while drift 5 was during the flood tide (Fig. 5). During the whole observation, there was a remarkable correlation between tide and salinity ($R=0.894$, $p=0.001$). When plotting the nutrient concentrations versus salinity for the whole observation, remarkable correlations were observed between the nutrients (i.e., $\text{NO}_3^-$, $\text{NO}_2^-$, $\text{NH}_4^+$, DIN, TDN and $\text{Si(OH)}_4$) and salinity ($R=0.86-0.98$, $p=0.001$), especially between phosphorus species ($\text{PO}_4^{3-}$ and TDP) and salinity, with $R=0.56$ at $p=0.01$, and between DOP or DON and salinity, with $R=0.45-0.47$ at $p=0.05$ (Fig. 5). This indicates that the nutrient concentrations changed depending on water mass, estuary processes, biological activities and anthropogenic activities (Liu et al., 2005).
y = -1.6419x - 12.457
R = 0.894, N=21, p=0.001
Fig. 5: (a) Changes in the tide (cm) and salinity with time during three drift observations and the tide vs. salinity during the whole observation, (b) nutrient concentrations ($\mu$M) vs. salinity during the whole observation and the molar ratios of nutrients vs. salinity.

\begin{align*}
&\text{NO}_3^- (\mu\text{M}) \quad y = -0.9729x + 34.404 \quad R^2 = 0.9509 \\
&\text{PO}_4^{3-} (\mu\text{M}) \quad y = -0.0096x + 0.97 \quad R^2 = 0.3158 \\
&\text{NO}_2^- (\mu\text{M}) \quad y = -0.0466x + 2.0377 \quad R^2 = 0.8641 \\
&\text{NH}_4^+ (\mu\text{M}) \quad y = -0.6671x + 24.917 \quad R^2 = 0.7473 \\
&\text{DOP} (\mu\text{M}) \quad y = -0.0096x + 0.97 \quad R^2 = 0.3158 \\
&\text{DON} (\mu\text{M}) \quad y = -1.9659x + 91.956 \quad R^2 = 0.8578 \\
&\text{TDP} (\mu\text{M}) \quad y = -2.8951x + 108.08 \quad R^2 = 0.9187 \\
&\text{H}_4\text{SiO}_4 (\mu\text{M}) \quad y = -1.6042x + 66.499 \quad R^2 = 0.8418 \\
&\text{DIN/PO}_4^{3-} \quad y = -0.0023x + 1.8331 \quad R^2 = 0.0047 \\
&\text{NO}_3^-/(\text{NH}_4^+ + \text{DON}) \quad y = -0.0136x + 0.661 \quad R^2 = 0.7518
\end{align*}
3.5 Longitudinal profiles of nutrients in the estuary

Nutrients in the estuaries behave either conservatively or non-conservatively depending on the chemistry of nutrient elements, estuarine circulation system and season (Liu et al., 2009). It appears that river water stages related to the dry and flood seasons, \textit{in situ} biological uptake and regeneration and phosphate adsorption/desorption are important factors affecting nutrient distributions in the estuary (Zhang, 1996).

The concentrations of $\text{NO}_3^-$ appeared to be subjected to regeneration in Winter 2006 and Spring 2009 and removal in the summers of 2007 and 2008. Ammonium, nitrite and DON undergo regeneration in the estuary (Fig. 6a and 6b). This indicates that dissolved inorganic and organic nitrogen were regenerated from organic matter degradation and that denitrification processes may take place in the estuary in the summer. $\text{N}_2$ was produced from $^{15}\text{NO}_3^-$-added sediment incubation experiments in the estuary (Song G.D., personal communication). The molar ratios of $\text{NO}_3^-$:\(\text{NH}_4^++\text{DON}\) decreased with an increase in salinity, indicating the dominance of regenerated nitrogen towards the ocean (Fig. 5b and 6e). Both $\text{PO}_4^{3-}$ and DOP have desorption/adsorption behaviors of/onto suspended particles and are affected by the degradation of organic matter and anthropogenic activities in the estuary (Fig. 6c), as in other major Chinese estuaries (Chen et al., 1985; Liu and Zhang, 2004; Zhang et al., 1997, 2007). The concentrations of $\text{Si(OH)}_4$ appeared to be largely subjected to a simple estuarine dilution in December 2006 and March-April 2009, as in other major Chinese estuaries (Zhang, 1996; Liu et al., 2009), while dissolved silicate undergoes depletion in the estuary under salinity less than 20 in the summer (Fig. 6d). In the studied estuary, the $\text{DIN:PO}_4^{3-}$ molar ratios show a decreasing trend with an increase in salinity, except those molar ratios in August 2007 were scattered, while the $\text{Si(OH)}_4:\text{DIN}$ molar ratios were higher than or comparable to 1, except for those in August 2007, indicating that the characteristics of tropical rivers are generally enriched with dissolved silicate (Fig. 5b and 6e).
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Fig. 6: Plots of nitrate and ammonium (a), nitrite and DON (b), phosphate and DOP (c), dissolved silicate vs. salinity along the longitudinal profile of the estuary (d) and molar ratios of nutrients vs. salinity (e) in 2006 to 2009.
3.6 Nutrients in the groundwater

The nutrient concentrations in the groundwater during the investigated periods changed 4-3000 times depending on the element, site and season. Dramatic variations of the nutrient concentrations are related to rock type and agricultural activities (irrigation and fertilizer usage) (Liu GQ et al., 2007). Compared to other aquatic environments (river, rain and aquaculture waters), groundwater is enriched with $\text{NO}_3^-$, $\text{PO}_4^{3-}$ and $\text{Si(OH)}_4$ and has average levels for the other elements and with very high $\text{DIN:PO}_4^{3-}$ and $\text{Si(OH)}_4$:DIN molar ratios (Tab. 2). DON represented 1-69% of TDN, with an average of 14±17%, and DOP accounted for 5-75% of TDP, with an average of 30±23%.

Based on the naturally occurring $^{226}\text{Ra}$ isotope measured during the 2007 and 2008 cruises, the submarine groundwater discharge into the estuary system was calculated as $3.2 \text{ m}^3 \text{s}^{-1}$ (Ni Su, personal communication). The nutrient transport fluxes from submarine groundwater into the estuary system were estimated by the product of the nutrient concentrations and submarine groundwater discharge (Tab. 2).

3.7 Nutrients in the rainwater

The nutrient concentrations in the rainwater changed depending on rain events, element and season. The rainwater is generally enriched with $\text{NH}_4^+$, $\text{NO}_3^-$ and DON and depleted in $\text{PO}_4^{3-}$, DOP and $\text{Si(OH)}_4$, with very high $\text{DIN:PO}_4^{3-}$ and very low $\text{Si(OH)}_4$:DIN molar ratios (Tab. 2). DON represented <63% of TDN, with an average of 32±24%. The contribution of DON in TDN in the rainwater is comparable to the global average of 30% (Duce et al., 2008). With respect to TDP, DOP accounted for 11-89%, with an average of 50±11%, which is comparable to that in other major Chinese rivers (47%; Liu et al., 2009).

There are still no data published to our knowledge for dry and wet nutrient depositions in Hainan. Based on rainwater samples collected on the top of the hotel near Gaolong Bay during the cruises, the wet depositional fluxes of nutrients were determined from the average nutrient concentration and rainfall rate (long-term average annual rainfall of 1740±431 mm) (Tab. 3).
Tab. 2: Concentrations of nutrients (μM), the percentage of DON in TDN and DOP in TDP, the molar ratios of DIN/DIP and Si/DIN in groundwater in Wenchang (A) and nutrient transport fluxes (10^6 mol yr⁻¹) from groundwater into the estuary system (B).

### Panel A: Nutrient concentrations and composition

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**Panel B: Nutrient transport fluxes**

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Tab. 3: Concentrations of nutrients (μM), the percentage of DON in TDN and DOP in TDP, the molar ratios of DIN/DIP and Si/DIN in each rain event during investigation periods in Wenchang (A) and atmospheric depositions (10^6 mol yr⁻¹) (B)

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<td>±0.66</td>
<td>±0.28</td>
<td>±0.01</td>
<td>±0.00</td>
<td>±0.38</td>
</tr>
</tbody>
</table>
3.8 Nutrients in the aquaculture waters

Shrimp and fish aquaculture are very popular in Bamen Bay and the coastal areas of East Hainan. Approximately 21.6 km² are covered by shrimp and fish ponds, and their effluents are drained directly into the estuary without any prior treatment. Aquaculture waters are generally enriched with almost all nutrient elements (Tab. 2). DON represented 25%-98% of TDN, with an average of 72±30%. With respect to TDP, DOP accounted for 8-59%, with an average of 37±20%. Nutrient concentrations in aquaculture waters changed, depending mostly on production stage, water exchange and feeding management of the respective ponds. The total volume of aquaculture effluents drained into the estuary was estimated at 209.68×10⁶ m³ yr⁻¹ (Herbeck et al., unpublished data). This estimation was based on pond surface area (21.6 km²), average water depth in the ponds (1.7 m) and average water exchange rates of the shrimp and fish ponds. Nutrient fluxes transported from aquaculture effluents were estimated by the average nutrient concentrations in pond waters and total effluent inputs into the estuary, shown in Tab. 4.
Tab. 4: Concentrations of nutrients (µM), the percentage of DON in TDN and DOP in TDP, the molar ratios of DIN/DIP and Si/DIN in aquaculture waters in Wenchang (A) and nutrient fluxes (10^6 mol yr⁻¹) from aquaculture effluents into the estuary system (B)

<table>
<thead>
<tr>
<th>Date</th>
<th>Station</th>
<th>Pond</th>
<th>NO₃⁻</th>
<th>NO₂⁻</th>
<th>NH₄⁺</th>
<th>DIP</th>
<th>Si(OH)₄</th>
<th>TDN</th>
<th>DON</th>
<th>TDP</th>
<th>DOP</th>
<th>DIN</th>
<th>DON/TDN</th>
<th>DOP/TDP</th>
<th>DIN/DIP</th>
<th>Si/DIN</th>
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</thead>
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<tr>
<td>2008-8-2</td>
<td>S-3</td>
<td>Shrimp</td>
<td>15.1</td>
<td>181</td>
<td>157</td>
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<td>58</td>
<td>473</td>
<td>121</td>
<td>7.7</td>
<td>0.62</td>
<td>353</td>
<td>0.25</td>
<td>0.08</td>
<td>50</td>
<td>0.2</td>
</tr>
<tr>
<td>2008-8-4</td>
<td>S-1</td>
<td>Shrimp</td>
<td>5.49</td>
<td>0.23</td>
<td>7.16</td>
<td>0.36</td>
<td>82</td>
<td>71.2</td>
<td>58</td>
<td>0.62</td>
<td>0.26</td>
<td>12.9</td>
<td>0.82</td>
<td>0.43</td>
<td>36</td>
<td>6.4</td>
</tr>
<tr>
<td>2008-8-4</td>
<td>S-2</td>
<td>Shrimp</td>
<td>8.44</td>
<td>127</td>
<td>117</td>
<td>11.1</td>
<td>23.8</td>
<td>383</td>
<td>130</td>
<td>12.3</td>
<td>1.22</td>
<td>253</td>
<td>0.34</td>
<td>0.10</td>
<td>23</td>
<td>0.1</td>
</tr>
<tr>
<td>2009-3-27</td>
<td>WJ-SP-1</td>
<td>Fish</td>
<td>0.01</td>
<td>0.31</td>
<td>0.32</td>
<td>0.49</td>
<td>138</td>
<td>40</td>
<td>39</td>
<td>1.05</td>
<td>0.56</td>
<td>0.64</td>
<td>0.98</td>
<td>0.53</td>
<td>1</td>
<td>217</td>
</tr>
<tr>
<td>2009-3-27</td>
<td>WJ-SP-2</td>
<td>Shrimp</td>
<td>1.59</td>
<td>0.30</td>
<td>7.05</td>
<td>0.66</td>
<td>29.5</td>
<td>65</td>
<td>56</td>
<td>0.99</td>
<td>0.33</td>
<td>8.94</td>
<td>0.86</td>
<td>0.33</td>
<td>14</td>
<td>3.3</td>
</tr>
<tr>
<td>2009-3-27</td>
<td>WJ-SP-3</td>
<td>Shrimp</td>
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<td>77</td>
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<tr>
<td>2009-4-1</td>
<td>BM-SP-1</td>
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<td>2.57</td>
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<td>1.58</td>
<td>0.96</td>
<td>0.18</td>
<td>0.6</td>
<td>3.3</td>
</tr>
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<td>2009-4-1</td>
<td>BM-SP-2</td>
<td>Shrimp</td>
<td>0.01</td>
<td>0.08</td>
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<td>141</td>
<td>27.2</td>
<td>26</td>
<td>0.66</td>
<td>0.39</td>
<td>0.81</td>
<td>0.97</td>
<td>0.59</td>
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<tr>
<td>2009-4-1</td>
<td>BM-SP-3</td>
<td>Shrimp</td>
<td>1.56</td>
<td>0.24</td>
<td>1.08</td>
<td>1.10</td>
<td>5.64</td>
<td>45</td>
<td>42</td>
<td>2.26</td>
<td>1.16</td>
<td>2.88</td>
<td>0.94</td>
<td>0.51</td>
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</tr>
</tbody>
</table>

Panel B: Nutrient fluxes

<table>
<thead>
<tr>
<th></th>
<th>NO₃⁻</th>
<th>NO₂⁻</th>
<th>NH₄⁺</th>
<th>PO₄³⁻</th>
<th>Si(OH)₄</th>
<th>TDN</th>
<th>DON</th>
<th>TDP</th>
<th>DOP</th>
<th>DIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.48</td>
<td>7.25</td>
<td>7.00</td>
<td>0.55</td>
<td>13.0</td>
<td>28.1</td>
<td>12.4</td>
<td>6.8</td>
<td>0.13</td>
<td>15.7</td>
</tr>
<tr>
<td>Stdev</td>
<td>±2.17</td>
<td>±14.5</td>
<td>±12.5</td>
<td>±0.81</td>
<td>±10.9</td>
<td>±35.4</td>
<td>±8.2</td>
<td>±0.86</td>
<td>±0.08</td>
<td>±27.7</td>
</tr>
</tbody>
</table>
4. Discussion

4.1 Biogeochemistry of nutrients in the rivers

Nutrient levels in the tropical Wenchanghe and Wenjiaohe show a wide range of variation depending on the system, nutrient element and season. According to the classification of the pollution index based on the river DIN and PO$_4^{3-}$ concentrations devised by Smith et al. (2003), DIN concentrations of these two rivers are at levels between those of the average global conditions (52 $\mu$M) and polluted water (110 $\mu$M), except for one cruise in the Wenjiaohe with a DIN level near that of clean water (Tab. 1). The excess riverine DIN is derived from extensive agricultural land leaching, municipal sewage discharge, and groundwater and aquaculture water discharge over the drainage basins of these rivers. The NH$_4^+$ contribution to the DIN concentration is relatively high, up to 44-88% in the August 2007 cruise, and is related to the application of inorganic N fertilizer (ammonium bicarbonate) in the drainage basins and to wastewater discharge. The PO$_4^{3-}$ concentrations in the Wenchanghe and Wenjiaohe are at levels between the pristine level (0.5 $\mu$M) and clean water level (1.4 $\mu$M) according to the global river data (Smith et al., 2003). The lower PO$_4^{3-}$ concentrations in the rivers are quite universal in other Chinese rivers and are related to their adsorption onto particulates (Liu et al., 2009) because suspended particulate matters are abundant in the river waters (7-44 mg l$^{-1}$). Therefore, the DIN:PO$_4^{3-}$ ratios are quite high, varying from 60 to 411. The DIN:PO$_4^{3-}$ ratios in these two rivers are higher than in any other river in the world (Billen and Garnier, 2007). Very high ratios of DIN:PO$_4^{3-}$ might also result from very low phosphate values.

Although dissolved organic nutrients tend to be less bioavailable than dissolved inorganic forms, the form of the nutrient may determine the impact on receiving coastal marine ecosystems, including the loss of habitat and biodiversity, low dissolved oxygen conditions, and an increase in the frequency and severity of harmful algae blooms (Cotner and Wetzel, 1992; Seitzinger et al., 2005). There is growing evidence that phytoplankton can use both phosphate and DOP (Cotner and Wetzel, 1992; Huang et al., 2005), and DON is implicated in the formation of some coastal harmful algal blooms (Berg et al., 1997; Seitzinger et al., 2002). In the Wenchanghe and Wenjiaohe, DOP concentrations were higher than those of phosphate, representing ~65% of TDP. With respect to TDN, DON accounted for 40% of TDN in the Wenchanghe and 76% in the Wenjiaohe. This indicates that transports of dissolved organic N and P, especially P, to the SCS are important and may determine the impact on receiving coastal marine ecosystems.

Dissolved silicate is mainly delivered via weathering, which is constrained by the interaction of tectonic activities, rock type and climate. Dissolved silicate levels in the rivers are, in general, higher in the warm and wet south than in the cold and dry north of China (Liu et al., 2009), with higher erosion rates in the south of China than in the north of China. The
total weathering rate in Chinese river watersheds is obviously higher than those of other major world watersheds (Li, 2003). Dissolved silicate levels in the investigated region are lower than in other rivers in Hainan, such as the dissolved silicate concentration of 327 μM in the Wanquanhe (Li, R.H. et al., in preparation), while dissolved silicate levels in the Wenchanghe are comparable to other tropical rivers in the world, such as the Wonokromo and Porong Rivers (180 μM) in eastern Java, Indonesia (Jennerjahn et al., 2004).

4.2 Nutrient transports in the lagoon

As current data are available for the two anchor stations in Bamen Bay and Qinglan tidal inlet, where the nutrients were measured, nutrient fluxes can be obtained by multiplying the velocity and nutrient concentrations, as was done in the Yalujiang Estuary (Liu and Zhang, 2004) and Jiaozhou Bay mouth (Liu et al., 2007). Temporal nutrient transports are separated into east-westward and north-southward direction transports because the anchor station in Bamen Bay is in northeast of the Bay. At the anchor station within the bay, nutrients in the surface layer were mainly transported westward and southward, except phosphate and TDP, which were mainly transported westward and northward. At the near-bottom layer, nitrate, nitrite, TDN, DON and dissolved silicate were mainly transported westward and southward, while ammonium, phosphate, TDP and DOP were mainly transported eastward and northward (Tab. 5). On average, nitrogen and silicon were mainly transported westward and southward, except for ammonium, while phosphorus and ammonium were mainly transported eastward and northward.

At the anchor station in the Qinglan tidal inlet, nitrite, ammonium, TDN and DON in the surface layer were mainly transported westward and northward, while nitrate, phosphate, TDP, DOP and dissolved silicate in the surface layer were mainly transported westward and southward. For the near-bottom layer, nitrite was mainly transported westward and northward; ammonium, nitrate, dissolved silicate, phosphate, TDN and DON were mainly transported eastward and northward, while TDP and DOP were mainly transported westward and southward (Tab. 5). For the vertical average, nutrients were mainly transported westward and northward, except TDP and DOP were mainly transported westward and southward.

Nutrients within Bamen Bay were transported out of the bay, except ammonium and phosphorus, which were regenerated in the estuaries, while nutrients in the Qinglan tidal inlet were mainly transported into the bay, except for TDP and DOP. This is related to the northward transport of waters, which was longer in duration than the southward transport at the anchor stations within the bay and in the Qinglan tidal inlet. On average, in the investigated region, the flood tide was approximately 1.4-fold longer in duration than the ebb tide. During the observations at both anchor stations, the flood tide was 2-fold longer in duration than the ebb tide.
Tab. 5: Transports of velocity (cm s^{-1}) and nutrients (mmol m^{-2} s^{-1}) along east-westward (E-W) and north-southward (N-S) directions at anchor stations within Bamen bay and in the Qinglan tidal inlet. Both eastward and northward transports are indicated with a positive value and both westward and southward by a negative value.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Direction</th>
<th>Bamen Bay-surface</th>
<th>Bamen Bay-bottom</th>
<th>Qinglan tidal inlet-surface</th>
<th>Qinglan tidal inlet-Bottom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity</td>
<td>W-E</td>
<td>-43.4</td>
<td>30.0</td>
<td>-107.3</td>
<td>130.0</td>
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<tr>
<td>Velocity</td>
<td>S-N</td>
<td>-144.7</td>
<td>108.7</td>
<td>-113.9</td>
<td>162.0</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>W-E</td>
<td>-45.7</td>
<td>14.0</td>
<td>-50.7</td>
<td>29.6</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>S-N</td>
<td>-107.6</td>
<td>33.6</td>
<td>-63.3</td>
<td>37.0</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>W-E</td>
<td>-0.37</td>
<td>0.16</td>
<td>-0.59</td>
<td>1.24</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>S-N</td>
<td>-0.81</td>
<td>0.59</td>
<td>-0.62</td>
<td>1.69</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>W-E</td>
<td>-2.76</td>
<td>-4.90</td>
<td>-1.23</td>
<td>0.27</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>S-N</td>
<td>0.49</td>
<td>0.52</td>
<td>-1.85</td>
<td>0.32</td>
</tr>
<tr>
<td>Si(OH)$_4$</td>
<td>W-E</td>
<td>-1680</td>
<td>501</td>
<td>-1331</td>
<td>1054</td>
</tr>
<tr>
<td>Si(OH)$_4$</td>
<td>S-N</td>
<td>-3585</td>
<td>1348</td>
<td>-1457</td>
<td>1261</td>
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<tr>
<td>PO$_4^{3-}$</td>
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<tr>
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<td>0.53</td>
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<td>TDP</td>
<td>W-E</td>
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<td>-0.68</td>
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<td></td>
<td>DOP W-E</td>
<td></td>
<td>DOP S-N</td>
<td></td>
<td>TDN W-E</td>
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<tr>
<td>-----</td>
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</tr>
<tr>
<td></td>
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<td>0.11</td>
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</tr>
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<td>0.14</td>
<td></td>
<td>0.11</td>
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<tr>
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<tr>
<td></td>
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<td></td>
<td>0.21</td>
<td></td>
<td>-3.4</td>
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</table>
4.3 Water and nutrient budgets

The LOICZ biogeochemical budget approach was used to evaluate the relative importance of external nutrient inputs versus the physical transports and internal biogeochemical processes within a body of water (Gordon et al., 1996; Liu et al., 2009). In this study, the Wenchanghe and Wenjiaohe Estuary and Bamen Bay system was treated as a single box, which was well mixed both vertically and horizontally and assumed to be at a steady state. Freshwater input to this studied system included \(287 \times 10^6\) m\(^3\) yr\(^{-1}\) and \(366 \times 10^6\) m\(^3\) yr\(^{-1}\) of river discharge from the Wenchanghe and Wenjiaohe, respectively, with a total river water discharge of \(653 \times 10^6\) m\(^3\) yr\(^{-1}\) (\(V_Q\)), \(33.3 \times 10^6\) m\(^3\) yr\(^{-1}\) of precipitation (\(V_P\)) and \(96.5 \times 10^6\) m\(^3\) yr\(^{-1}\) of submarine groundwater discharge (\(V_G\)), which was reduced by \(21.4 \times 10^6\) m\(^3\) yr\(^{-1}\) of evaporation (\(V_E\)). Based on the monitoring data, domestic and industrial wastewater discharges directly into the bay were \(14.7 \times 10^6\) m\(^3\) yr\(^{-1}\) (\(V_W\)) (Hainan Provincial Marine Environment Monitor Center, Personal communication; Wang, D.R., unpublished data). In addition, aquaculture effluents entering the studied system were estimated at \(210 \times 10^6\) m\(^3\) yr\(^{-1}\) (Lucia S. Herbeck et al., in preparation). From the water mass balance, the net water exchange (\(V_R\)) from the studied system to the SCS has a residual flow of \(986 \times 10^6\) m\(^3\) yr\(^{-1}\). Based on the salt balance, the water exchange flow from the SCS to the studied system (\(V_X\)) was \(1564 \times 10^6\) m\(^3\) yr\(^{-1}\). The volume of studied system (\(V_S\)) is \(54.39 \times 10^6\) m\(^3\), and the total water exchange time (\(\tau\)) of the studied system can be estimated at 7.8 days from the ratio \(V_S/(V_R+V_X)\).

Nutrient transport fluxes from rivers, rainwater, submarine groundwater and aquaculture water into the studied system in this study were based on the investigations during 2006-2009, as aforementioned (Tab. 1-4, 6). The nutrient concentrations in domestic and industrial wastewater were estimated based on observations of a polluted river, Longtouhe, which is in Wanning City and empties into Xiaohai Lagoon with a black color and foul odor. Nutrient concentrations in this river were similar to those in the wastewater effluent of sewage treatment plants near Jiaozhou Bay (Liu et al., 2005). The nutrient transports by wastewater discharge were estimated from the products of nutrient concentrations in the Longtouhe and wastewater discharge, shown in Tab. 6. Considering the nutrient inputs, the dissolved silicate and various nitrogen species load in the studied system were mainly from rivers, followed by submarine groundwater discharge and aquaculture effluents; the phosphorus load in the studied system was also mainly from the river input, aquaculture effluents and submarine groundwater discharge, in which the contribution of aquaculture effluents was higher than the submarine groundwater discharge.

Based on the observations in 2006-2009, the concentrations of nutrients in this studied system and its adjacent sea were obtained and used to construct nutrient budgets. The nutrient budgets for this estuary are provided in Tab. 6. The residual flow transports nutrients off the estuary and is much less than the total input. The mixing flow transports large
amounts of nutrients off the estuary, except for TDP and DOP. The model shows that this studied system is a sink of nutrients, which are buried into the sediments or are transformed into other forms, such as uptake by phytoplankton and mangroves. The nitrogen budget is consistent with the nutrient uptake and transformation processes, such as denitrification and primary production, existing in the system (Song G.D., personal communication). Nutrient fluxes at the sediment-water interface incubated in mangrove regions show that mangrove sediments are sinks of nitrogen and phosphorus (Deng K., personal communication), similar to those observations in the mangrove areas of the Red River Estuary in Vietnam and Phuket, Thailand (Kristensen et al., 2000; Wosten et al., 2003). Nutrient fluxes out of this studied system to the SCS can be estimated as a sum of the net residual flux (C_RVR) and mixing flux (C_XV_X) (Liu et al., 2009). These fluxes are 1.2-2.6 times higher than those of the riverine inputs, except DON, DOP and TDP (Tab. 6), indicating that estuarine processes (such as scavenging, regeneration and wastewater discharge) increased the riverine fluxes. Moreover, the molar ratios of DIN:PO_4^{3-} were 29-186 in all the external nutrient inputs to the studied system and were 71-124 in the output waters to the SCS. They significantly exceed the Redfield ratio (16:1), indicating that the nutrient transports may affect the coastal ecosystem of the SCS, especially in the winter, when the primary production in east Hainan is the highest (Gao and Wang, 2008).
Tab. 6: Nutrient budgets (10^6 mol yr⁻¹) in the Wenchanghe and Wenjiaohe Estuary and Bamen Bay system. In the table, positive values indicate transport into the studied system; negative data show export of nutrients from the system. Note: C= nutrient concentrations, 1 = Wenchanghe and Wenjiaohe Estuary and Bamen Bay system, 2 = South China Sea.

<table>
<thead>
<tr>
<th></th>
<th>NO₃⁻</th>
<th>NO₂⁻</th>
<th>NH₄⁺</th>
<th>PO₄³⁻</th>
<th>Si(OH)₄</th>
<th>TDN</th>
<th>DON</th>
<th>TDP</th>
<th>DOP</th>
<th>DIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>River input</td>
<td>36.9±14.2</td>
<td>1.86±0.80</td>
<td>10.2±8.6</td>
<td>0.42±0.18</td>
<td>90.1±10.2</td>
<td>74.2±19.4</td>
<td>25.4±6.8</td>
<td>1.46±0.76</td>
<td>1.04±0.75</td>
<td>48.9±17.4</td>
</tr>
<tr>
<td>Atmospheric deposition (surface area = 19.14 km²)</td>
<td>0.86±0.21</td>
<td>0.01±0.00</td>
<td>0.67±0.17</td>
<td>0.01±0.00</td>
<td>0.04±0.01</td>
<td>2.67±0.66</td>
<td>1.13±0.28</td>
<td>0.02±0.01</td>
<td>0.01±0.00</td>
<td>1.54±0.38</td>
</tr>
<tr>
<td>Aquaculture effluents</td>
<td>1.48±2.17</td>
<td>7.25±14.5</td>
<td>7.00±12.5</td>
<td>0.55±0.81</td>
<td>13.0±10.9</td>
<td>28.1±35.4</td>
<td>12.4±8.2</td>
<td>0.68±0.86</td>
<td>0.13±0.08</td>
<td>15.7±27.7</td>
</tr>
<tr>
<td>Groundwater discharge</td>
<td>38.4±33.3</td>
<td>0.08±0.18</td>
<td>0.65±1.19</td>
<td>0.48±0.88</td>
<td>32.7±14.0</td>
<td>40.4±33.4</td>
<td>3.84±3.42</td>
<td>0.54±0.98</td>
<td>0.06±0.11</td>
<td>36.5±31.7</td>
</tr>
<tr>
<td>Wastewater discharge</td>
<td>0.82±0.25</td>
<td>0.10±0.03</td>
<td>0.12±0.04</td>
<td>0.01±0.00</td>
<td>2.87±0.86</td>
<td>1.34±0.40</td>
<td>0.30±0.09</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
<td>1.04±0.31</td>
</tr>
<tr>
<td>Input</td>
<td>78.5±36.3</td>
<td>9.30±14.5</td>
<td>18.6±15.2</td>
<td>1.46±1.21</td>
<td>138.7±20.5</td>
<td>146.7±52.4</td>
<td>43.1±11.2</td>
<td>2.74±1.51</td>
<td>1.28±0.77</td>
<td>103.7±45.5</td>
</tr>
<tr>
<td>Concentration (C₁)</td>
<td>24.3±25.1</td>
<td>2.00±1.39</td>
<td>15.8±14.9</td>
<td>0.50±0.35</td>
<td>68.0±45.5</td>
<td>64.6±40.5</td>
<td>22.9±14.7</td>
<td>0.85±0.32</td>
<td>0.36±0.19</td>
<td>42.2±32.7</td>
</tr>
<tr>
<td>Concentration (C₂)</td>
<td>4.98±6.43</td>
<td>0.52±0.53</td>
<td>5.70±4.51</td>
<td>0.25±0.22</td>
<td>16.1±15.8</td>
<td>19.2±13.2</td>
<td>8.02±3.77</td>
<td>0.48±0.30</td>
<td>0.23±0.11</td>
<td>11.2±11.2</td>
</tr>
</tbody>
</table>
Residual flow (=VR\times C_R, V_R=986\times 10^6 \text{ m}^3 \text{ yr}^{-1}, C_R= (C_2+C_1)/2)

|        | -14.5±10.8 | -1.24±0.73 | -10.6±7.7 | -0.37±0.21 | -41.4±23.8 | -41.3±21.0 | -15.2±7.5 | -0.66±0.22 | -0.29±0.11 | -26.3±17.0 |
|        | -14.5±10.8 | -1.24±0.73 | -10.6±7.7 | -0.37±0.21 | -41.4±23.8 | -41.3±21.0 | -15.2±7.5 | -0.66±0.22 | -0.29±0.11 | -26.3±17.0 |

Mixing exchange (=V_X\times (C_2-C_1), V_X= 1564\times 10^6 \text{ m}^3 \text{ yr}^{-1})

|        | -30.3±22.7 | -2.32±1.74 | -15.8±11.9 | -0.39±0.29 | -81.2±60.9 | -71.0±53.2 | -23.3±17.5 | -0.59±0.44 | -0.20±0.15 | -48.4±36.3 |
|        | -30.3±22.7 | -2.32±1.74 | -15.8±11.9 | -0.39±0.29 | -81.2±60.9 | -71.0±53.2 | -23.3±17.5 | -0.59±0.44 | -0.20±0.15 | -48.4±36.3 |

Output

|        | -44.8±25.2 | -3.56±1.89 | -26.4±14.1 | -0.76±0.36 | -122.6±65.4 | -112.3±57.2 | -38.5±19.0 | -1.25±0.49 | -0.49±0.19 | -74.7±40.1 |
|        | -44.8±25.2 | -3.56±1.89 | -26.4±14.1 | -0.76±0.36 | -122.6±65.4 | -112.3±57.2 | -38.5±19.0 | -1.25±0.49 | -0.49±0.19 | -74.7±40.1 |

Sink/source

|        | -33.7±15.7 | -5.74±4.08 | 7.83±5.56 | -0.70±0.41 | -16.1±4.0 | -34.4±13.1 | -4.52±1.36 | -1.49±0.80 | -0.79±0.37 | -28.9±14.2 |
|        | -33.7±15.7 | -5.74±4.08 | 7.83±5.56 | -0.70±0.41 | -16.1±4.0 | -34.4±13.1 | -4.52±1.36 | -1.49±0.80 | -0.79±0.37 | -28.9±14.2 |
4.4 The influence of the typhoon on nutrient transports in the estuary and its ecological significance

Hainan Island is influenced by the East Asian monsoon and is among the most typhoon-affected areas in China (Ren et al. 2002). On average, 2.5 typhoons directly hit Hainan Island annually, and an additional 4.4 typhoons influence Hainan Island; typhoon-induced rainfall accounts for 36% of total annual rainfall (Wang, 1985). During the investigated periods, Typhoon Kammuri passed the northern part of Hainan Island on August 7, 2008, which resulted in rainfall at Wenchang of over 200 mm (China Meteorological Forum’s Archiver: http://www.cmabbs.com/archiver/?tid-12991-page-8.html).

Low salinity (less than 5) water was collected from near the Wenchanghe and Wenjiaohe mouths to the Gaolong Bay. The salinity of the Qinglan tidal inlet decreased from ~30 before the typhoon to 13 after the typhoon. The concentrations of dissolved inorganic nutrients increased by 6-16 times, except for phosphate, while the total and dissolved organic N and P increased by nearly 5 times due to the typhoon (Fig. 4b). Based on these observations, the nutrient levels in rainwater were lower than in the other waters, such as river water and groundwater, except for nitrate and ammonium levels, which were higher in rainwater than in aquaculture water. This indicates that the typhoon rainfall increased nutrient levels in the investigated regions as a result of extensive agricultural land leaching and aquaculture effluents, while the concentration of phosphate only slightly increased after typhoon-related adsorption onto particulates as the suspended particulate matter content increased from 7-24 mg L\(^{-1}\) to 50 mg L\(^{-1}\) due to the typhoon (Fig. 4b).

Daily average rainfall represents 0.2% of the volume of the studied estuary system for normal rain events. However, rainfall can represent 10% of the volume of the studied estuary system for normal typhoon events (rainfall 300 mm) and can be up to nearly 30% for violent typhoon events such as Typhoon Faye, which landed between Wenchang and Haikou, with rainfall of 777 mm per day, on September 7, 1963 (Wang, 1985). In the meantime, although instantaneous freshwater discharge data are not available, freshwater discharge should be two to three times the long-term average value. The total water exchange time of the studied system will decrease from 7.8 days for a normal rain event to 2.6 days for a normal typhoon (rainfall 300 mm) and 1.2 days for violent typhoon events (rainfall 777 mm). The topography of Hainan Island includes highlands at the center surrounded by lowlands. The Wenchanghe and Wenjiaohe originate from the center of Hainan Island. They are short rivers and can quickly accumulate rainwater after a typhoon, thus forming high flood peaks, and can transport freshwater into the SCS in a short time. Although the distributions of nutrient concentrations versus salinity in the studied system were not significantly changed after the typhoon, the subsequent terrestrial rainwater runoff with enriched nutrients was transported to the SCS (Fig. 4b and 6). Typhoon rain, therefore, is more able to influence offshore water than normal rain discharge, which tends to be
confined to the coastal shelf (Zheng and Tang, 2007).

The typhoons that affect Hainan Island mainly occur from May to November, and especially in August and September (Wang, 1985; Ren et al., 2002). Moreover, the summer upwelling in the east of Hainan Island is a common seasonal phenomenon during June–September, with the upwelling center of Qinglan Bay (Jing et al., 2009). Both the typhoon-induced terrestrial rainwater runoff and the upwelling-introduced nutrients into the euphotic zone from below will lead to a phytoplankton bloom east of Hainan Island in the summer. Due to anthropogenic activities, such as tourism and the construction of the space center in Wenchang City, the tropical ecosystems of the studied estuary are facing more serious problems. The coastal environmental in the east of Hainan Island should be given more attention.

Both the increased delivery of nutrients and their imbalance are known to be major threats to coastal zone ecosystems, causing severe eutrophication problems, such as harmful algal blooms and hypoxia (Turner and Rabalais, 1994; Liu et al., 2009). The indicator of coastal eutrophication potential (ICEP) of terrigenous nutrient inputs is addressed based on the flux of nitrogen (N-ICEP) or phosphorus (P-ICEP) delivered in excess over silica and is expressed in terms of carbon equivalence (Garnier et al., 2010 and references therein). For the studied estuary, based on calculations from the DIN, DIP, TDN, TDP and DSi fluxes, riverine input and wastewater discharge show both negative N- and P-ICEP values, indicating that silicon is not limiting over N and/or P, such that the diatoms are expected to overcome any harmful algal bloom development. Atmospheric deposition, aquaculture effluents and groundwater discharge show negative or very low P-ICEP values but positive N-ICEP values, indicating that P limitation should prevent the growth of non-diatoms once the diatom bloom has depleted all the available silicon despite the presence of excess nitrogen. The typhoon-induced terrestrial rainwater runoff, therefore, can not only increase nutrient inputs to the coastal ecosystem but can also result in nutrient imbalance, affecting phytoplankton production and composition.

5. Conclusions

This work conducted biogeochemical observations on nutrient dynamics in a river/estuary and lagoon system. Nutrient levels in the tropical Wenchanghe and Wenjiaohe show a wide range of variation depending on the system, nutrient element and season. DIN concentrations in these two rivers are generally at levels between those of average global conditions and those of polluted waters that are affected by extensive agricultural activities, urbanization and anthropogenic activities. \( \text{PO}_4^{3-} \) concentrations in these rivers are at levels between pristine and clean water levels according to the global river data related to the
adsorption onto particulates. Therefore, the N:P ratios are highly varied from 60 to 411. In these rivers, DOP represented ~65% of TDP, while DON accounted for 40% of TDN in the Wenchanghe and 76% in the Wenjiaohe. This result indicates that the dissolved organic N and P transports to the SCS are important and may determine the impact on receiving coastal marine ecosystems. Dissolved silicate levels in these rivers are at lower than average levels in tropical systems.

Nutrients in the Wenchanghe and Wenjiaohe Estuary behave either conservatively or non-conservatively depending on the element and season. Based on the observations of the nutrients in rivers, estuaries, rainwater, groundwater and aquaculture effluents, a simple steady-state mass-balance box model was employed to assess nutrient budgets in the studied system. Nutrient sources in the estuary system were primarily from riverine input, followed by groundwater discharge and aquaculture effluents. The nutrients exported in the studied system are largely confined to the immediate estuaries. Typhoon-induced terrestrial rainwater runoff can not only increase nutrient inputs to the coastal ecosystem but can also result in nutrient imbalance, affecting phytoplankton production and composition.

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CHAPTER III

Tide- and rainfall-induced variations of physical and chemical parameters in a mangrove-depleted estuary of East Hainan (South China Sea)

by Uwe Krumme, Lucia Herbeck and Tianci Wang

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Tide- and rainfall-induced variations of physical and chemical parameters in a mangrove-depleted estuary of East Hainan (South China Sea)

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Abstract

The estuarine dynamics favoring the coexistence of mangroves, seagrass and corals at small river mouths are often poorly understood. We characterize the tidal, day/night and rainfall-induced short-term dynamics in salinity, pH, dissolved oxygen (DO), water transparency, surface currents and dissolved nutrients (NO$_3^-$, NH$_4^+$, PO$_4^{3-}$, Si(OH)$_4$) of the Wenchang/Wenjiao estuary (East Hainan). Samples were taken at three fixed sites along the estuary during 24 hour spring tide cycles in different seasons. Salinity, DO, water transparency and pH generally increased seawards while nutrients decreased. All parameters varied with the tidal cycle, partially in interaction with the diel cycle. Nutrients usually fluctuated inversely with water level. Stratification was strong. Inflowing bottom water was of higher salinity, DO and pH and lower temperature and nutrient concentrations than the surface water, providing regular ventilation of the estuary. Under dry weather conditions, the brackish-water lagoon functions as an effective filter of nutrients and sediments. Presently, the runoff from common intense rains in the watershed affects the estuary with little time delay due to terrestrial deforestation, channelization and loss of mangrove area. The frequency, strength and duration of intermittent estuarization of the back-reef areas have likely increased in the past and deteriorate present seagrass and coral health.

Keywords: nutrients; mixed semidiurnal microtides; stratification; reef lagoon; coastal zone; estuaries; Hainan; Wenchang River Estuary
1. Introduction

Mangroves, seagrass beds and coral reefs are widely recognized for their high productivity, rich biodiversity and various ecosystem services (e.g. Harborne et al., 2006). These tropical coastal habitats can co-occur in close vicinity when land-based input of freshwater, nutrients and sediment is minor, or restricted to pulsed events. This is often the case when smaller tropical rivers meet the sea (e.g. Wolanski et al., 2001; Wolanski, 2007). Despite river input, intact mangroves feature mechanisms, such as lateral trapping, biological flocculation (e.g. Fortes, 2001; Wolanksi et al., 2001), or circulation patterns prevail (e.g. Kitheka, 1997), that mitigate eutrophication and siltation of the nearshore area so that seagrasses and corals can co-exist with nearby riverine mangroves on the long run.

However, many smaller estuaries have been increasingly degraded due to human interventions, and nearshore habitats are adversely impacted. The risk of nearshore habitat degradation depends on the interactive effect of several factors, e.g. the level of exposure to pollution, tides or local currents (Fabricius, 2005). Microtidal areas (<2 m tidal amplitude) are considered to be more vulnerable to eutrophication due to longer residence times and reduced pollutant removal than areas with larger tides (Monbet, 1992).

Nevertheless, the tides play a key role in estuarine short-term dynamics, even in microtidal estuaries. Despite of the limited size of smaller estuaries, their water properties can be extremely dynamic, both in space and time (e.g. Wolanski, 1994; Victor et al., 2006; Wolanski, 2007). The rise and fall of the sea level influence changes in physical and chemical parameters, which in turn influence phytoplankton production dynamics and, thus nutrient uptake conditions in estuaries (e.g. Lucas and Cloern, 2002). Knowledge of the differential processes during all tidal stages is critical for understanding the various estuarine processes. Investigation of tidally-driven dynamics is also paramount to establish reasonable sampling designs for long-term monitoring programs and to avoid mixing of spatial with temporal variations in the interpretation of snapshot data.

The South China Sea is part of the mega-diverse tropical Western Pacific. The coasts are dominated by mixed semidiurnal tides, mostly with <2 m tidal amplitude. There are few studies that have comprehensively investigated tide-related dynamics of physico-chemical parameters and nutrients in smaller estuaries of the region (e.g. Shasmudin and Ambak, 1983). Many estuaries along the South China Sea coastline are subject to various human stressors (Morton and Blackmore, 2001; Burke et al., 2002). In the northern South China Sea they suffer particularly from severe mangrove
loss due to conversion into rice fields or aquaculture ponds (Li and Lee, 1997). Most seagrass meadows and nearshore reefs are in an alarming state (Burke et al., 2002). Moreover, the estuaries of the northern South China Sea are regularly affected by typhoons during the summer monsoon season (Wang et al., 2008), which can lead to prolonged deterioration of nearshore water properties (Herbeck et al., 2011).

Smaller estuaries typically have a restricted estuarine volume, although high, pulsed runoff from monsoon-related precipitation events can occur (Vijith et al., 2009). Under pristine or less impacted conditions, these episodic events do not harm the co-existence and health of tropical coastal ecosystems. However, given the elevated levels of anthropogenic disturbance of tropical estuaries in the northern South China Sea, the question arises on the driving forces that have ensured the co-existence of tropical marine habitats near river mouths and how these mechanisms perform under altered conditions.

This study aims to characterize tidally-driven dynamics in physico-chemical parameters and dissolved nutrients during spring tide cycles along a moderately-sized, shallow estuary of a lowland river at the East coast of Hainan (tropical China) using sampling campaigns from different years under different rainfall regimes. Three specific questions were posed with regard to physico-chemical parameters and nutrient concentrations: (1) how do the parameters differ among and how do they vary during spring tide cycles at the three estuarine sites (horizontal scale)? (2) For a given estuarine site, are there differences between surface and bottom water characteristics (i.e. vertical scale)? (3) What is the influence of pulsed runoff on estuarine dynamics? The likely consequences of these dynamics for the status of the estuary and its adjacent nearshore habitats are discussed.

2. Material and methods

2.1 Study area

Hainan is the largest island in the South China Sea. The Wenchang/Wenjiao estuary (WWE) is located on the east coast of Hainan in the marginal tropics (N 19°36’ E 110°49’). Two lowland rivers (Wenchang and Wenjiao), drain agriculture areas of the coastal plain and enter a shallow (mean depth: 3 m), kidney-shaped lagoon (Bamen Bay). The lagoon is connected to the sea via a narrow inlet channel with a maximum depth of about 10 m; the northernmost tip of the inlet channel (~5 m deep) extends close to the mouth of the Wenchang River (Fig. 1). The Wenchang River passes the county capital Wenchang (55,800 inhabitants in 2009) before entering the lagoon in the
north-west. It brings untreated urban effluents, sediments and governs the freshwater supply of the system. The Wenjiao River at the north-east of Bamen Bay is dammed. Due to a lack of reliable discharge data, we estimated a residence time $T$ of water in the estuary of 5.6 days using the empirical formula of Uncles et al. (2002); $T = 0.23 (\text{MSTR})^{0.4} (\text{TL})^{1.2}$, where MSTR is the mean spring tidal range (1.65 m) and TL is the distance from the mouth to the tidal limit of the Wenchang River (17 km). The removal of protective lowland rainforests in North-East Hainan took already place between the 10th and 14th century for agricultural purposes so that 1997 only 4% of the primary forest of Hainan was left (Huang, 2003). Some physical characteristics of the estuary are summarized in Tab. 1.

Tab. 1: Some physical characteristics of the Wenchang/Wenjiao river estuary, East Hainan, China. Area and length values without a source are own estimates based on GIS analysis (visual interpretation) of a remote sensing image (Geoeye-1 Geo PSM 4-bands image on September 22, 2009 with 0.6 m resolution).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Wenchang River</th>
<th>Wenjiao River</th>
<th>Entire estuary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>37.1 km</td>
<td>56 km</td>
<td></td>
</tr>
<tr>
<td>Catchment area*</td>
<td>380.9 km$^2$</td>
<td>522 km$^2$</td>
<td>902.9 km$^2$</td>
</tr>
<tr>
<td>Mangrove area</td>
<td></td>
<td>812 ha</td>
<td></td>
</tr>
<tr>
<td>Aquaculture pond area in former mangroves</td>
<td></td>
<td>2156 ha</td>
<td></td>
</tr>
<tr>
<td>Surface area with respect to mean sea level</td>
<td></td>
<td>2389 ha</td>
<td></td>
</tr>
<tr>
<td>Area covered by floating net cages</td>
<td></td>
<td>4.8 ha</td>
<td></td>
</tr>
<tr>
<td>Estuarine volume (for 4 m water depth)</td>
<td></td>
<td>$100 \times 10^6$ m$^3$</td>
<td></td>
</tr>
</tbody>
</table>

*: Zeng and Zeng, 1989

73% of the fringing riverine mangrove has been lost since the 1960s; aquaculture ponds now cover 2156 ha of the former mangrove area around the estuary (Tab. 1; Fig. 1). The relationship between estuarine open water area (at mean sea level) and mangrove area changed from 1:1.24 in the 1960s to 1:0.27 in present times. Nevertheless, the WWE still houses the most speciose and largest mangrove area of Hainan (Li and Lee, 1997). The former mangrove area has not only lost its ecological function as filter and sediment trap but, in addition, is a major source of untreated effluents. Wastewater also enters from the port of Qinglan (Fig. 1) and feces and excess feed are released from hundreds of floating net cages containing live fishes and invertebrates that are mainly lined up opposite to the port of Qinglan. Degraded seagrass beds and fringing reefs occur in the outer estuarine zone.
More than 70 % of the annual precipitation (1500 to 2000 mm) occurs from May to October during the South-west monsoon (hot and rainy period). Though, there is a considerable inter-annual variability. Often the bulk of the annual precipitation occurs during few, heavy rainfall, which can be separated by week-long rainless periods. 35 to 60 % of the annual precipitation is linked to typhoons during the South-west monsoon (Huang 2003).

The east coast of Hainan is subject to mixed semidiurnal microtides. At spring tides, it experiences two high tides and two low tides of different size every lunar day, in the sequence “lower low water” (LW; late afternoon or in the early night), “lower high water” and “higher low water” (at night), and “higher high water” (HW; usually between the early morning and midday). We concentrate on the difference between LW and HW because differences between the lower high tide and the higher low tide were usually negligible. As a consequence, flood tide (14-15 hs) and ebb tide (7-8 hs) are very asymmetric in length. Spring tidal ranges are around 1.6 m but can occasionally

---

**Fig. 1**: Wenchang/Wenjiao estuary (East Hainan) in the northern South China Sea (arrow in a). Location of three fixed sampling sites in the upper (A), middle (B) and outer (C) estuary (b); map from satellite image analysis of the estuary (Geoeye-1 Geo PSM 4-bands image on September 22, 2009 with 0.6 m resolution). Approximate bathymetry based on >250 depth measurements taken throughout the estuary during neap tides.
exceed 2 m. At neap tide, the tide is semidiurnal with tidal ranges usually <1m and a
daytime and a nightly high-water.

2.2 Study sites

Sampling was carried out at three fixed sites in the upper, middle and outer
estuary (Fig. 1). The upper estuarine site (A) was located in the lower reaches of the
Wenchang River (width at the site: ca. 100 m, maximum depth of the river thalweg at
lower LW: 3-4 m). It is part of largest contiguous mangrove area of the estuary with
more than 10 tree species. The mangrove is, however, partially degraded due to active
and abandoned aquaculture ponds with dams and semi-natural narrow creeks for
water supply and wastewater drainage. The substrate in the river is muddy with coarse
gravel eroded from the dams and isolated rocks.

The middle estuarine site (B) was located in the shallow open lagoon
(maximum depth at the main channel at lower LW: 5-6 m). The western site is cut-off
from regular tidal inundation by aquaculture ponds, while mangrove stands still fringe
the eastern site (at least 10 species) and some larger patches of outcropping bedrock
occur there.

The outer estuarine site (C) was part of a shallow back-reef area (about 1 m
water depth at lower LW). The largest coconut plantations of Hainan form the
hinterland of the beach. The substrate is basically sand with coral rubble. Patches of
seagrass (up to six species, but mainly Thalassia hemprichii) grow on slightly elevated
platforms that are partially exposed to air during spring low-tides. Seagrass cover is
highly variable in space and among years, ranging from 0 to 100 %. The fringing reef is
highly degraded due to habitat destruction (bomb and cyanid fishing), sedimentation,
and overexploitation (own observations; Hutchings and Wu, 1987; Fiege et al., 1994).
Coral distribution is largely restricted to a narrow stretch along the reef front, where
wave action removes sediments (own observation). At ebb tides, parts of the plume
from the lagoon pass this site eastwards in alongshore direction and flow towards the
fringing reef (Ye, 1988; own observations). The distance from upper to middle and
middle to outer estuarine site was ca. 5.2 km and 7.8 km, respectively.

2.3 Sampling

Given the microtidal regime, sampling was carried out during spring tides, to
capture the maximum tidal oscillation and the period of greatest water exchange with
the mangrove. During six consecutive spring tide days, each site was sampled
continuously during 24 hours from an anchored boat with one repetition during the next
spring tide. Replicate spring tide sampling was carried out in 2007 (August 25-30; September 06-11), 2008 (July 29-August 04; August 14-19), and 2009 (March 29/30; April 11-16). A temporary research ban from Chinese authorities disallowed the scheduled sampling between March 31 and April 03, 2009.

No rainfall data are available for the Wenchang/Wenjiao region. Daily precipitation data from the nearest weather stations in Haikou (ca. 60 km linear distance to the northwest) and Qionghai (ca. 50 km to the southwest) (Climate Center, Utah State University, http://climate.usurf.usu.edu/products/data.php?tab=gsod) did not match the local precipitation events in the Wenchang/Wenjiao area and are therefore not shown. The sampling in 2007 was carried out during a dry period of the summer monsoon. Nameable precipitation only occurred during the final sampling at the upper estuarine site in September 10/11. Data of spring tide cycles from 2008, which were taken before and after the passage of typhoon Kammuri, are analyzed in detail in Herbeck et al. (2011) and Krumme and Wang (unpublished results). The designated winter monsoon sampling in March/April 2009 coincided with the first heavy rainfall period in the study area.

The following abiotic parameters were measured at the water surface every 30 min: The water level (±2 cm) was determined with a tidal gauge and HOBO water level data logger (Onset Computer Corporation). Salinity (using the practical salinity scale; ±0.1), water temperature (±0.1 °C) and pH (±0.1) were measured with a WTW-multi parameter probe (LF197 equipped with the sonde WTW Tetracon 325). For determination of dissolved oxygen (DO; ±0.02 mg l\(^{-1}\)) a portable dissolved oxygen meter (HachLange HQ40d) was used. Surface water samples for probe measurements were taken with a bucket. Surface current speed (cm s\(^{-1}\)) was determined by measuring the time needed for the tidal current to stretch a 10 m long tape attached to a weight with a buoy. Current direction was determined by bearing of the direction along the stretched tape with a compass. Each current velocity was converted into the distance the water traveled within the 30-min time interval [m s\(^{-1}\) × 60 s × 30 min]. The sum of the distances at flood and ebb tide intervals were used to generate an estimate for the mean net tidal excursion at spring tides. The tidal excursion is the total distance traveled by a water particle from low water slack to high water slack and vice versa. In addition to surface water measurements, every hour bottom water salinity, temperature, DO content, and pH were determined ca. 0.5 m above the ground using the Ayin bottom water sampler (Krumme et al., 2010). Briefly, the Ayin bottom water sampler is an empty sample bottle, which is lowered upside down using a weight attached to the neck of the bottle. After touchdown, the bottle is turned in situ above the ground via a rope which runs through a loop at the weight and is attached to the bottom
of the bottle. Once the air in the bottle is replaced by water, the bottle is lifted. Vertical Secchi depth was also measured hourly during daylight hours (06:00–18:00).

Water samples for analysis of dissolved nutrients were taken every three hours. Samples were filtered immediately through single-use membrane filters (0.45 μm pore size) into PE bottles, which were rinsed three times with the filtered sampling water before filling. Samples were preserved with a mercury chloride solution (50 μl of a 20 g l⁻¹ HgCl₂-solution added to 100 ml sample) and stored cool until analysis. Dissolved nutrients were detected spectrophotometrically (NOₓ⁻, PO₄³⁻, Si(OH)₄⁻) and fluorometrically (NH₄⁺) as a colored complex (Grasshoff et al., 1999) using a continuous flow analyzing system (Skalar SAN⁺⁺System). The percental differences of the annual performance evaluation for NOₓ⁻, NH₄⁺, PO₄³⁻ and Si(OH)₄⁻ were 0.5, -1.7, -1.1, and 1.8, respectively, using standards from OSIL Nutrient Standard Solutions. Determination limits were 0.08 μM, 0.06 μM, 0.07 μM and 0.19 μM for NOₓ⁻, NH₄⁺, PO₄³⁻ and Si(OH)₄⁻, respectively, according to DIN 32645. The coefficient of variation of the procedure was <3.4%.

3. Results

3.1 Physical parameters

The mean spring tidal range (absolute water level rise from lower LW until higher HW) at the upper, middle and outer estuarine site was 169 cm (range: 138 - 220), 169 cm (range: 130 - 200), and 152 cm (range: 1.35 - 1.77), respectively. Overall, salinity, DO content, and pH increased towards the coast (Fig. 2). Surface water temperature displayed no consistent longitudinal gradient but was clearly influenced by season with lower temperatures in March/April (ca. 26°C) than in June-September (ca. 30°C). Water transparency also increased towards the sea, however, since the outer estuarine site was very shallow, vertical Secchi depth readings usually equaled water depth and did not cover the potential visibility range, which usually exceeded 2 m.
Fig. 2: Medians with 25-75% quartile and min-max of surface salinity, dissolved oxygen content, pH, water temperature, and Secchi depth for each spring tide cycle sampled at the upper (grey), middle (dotted) and outer (white) estuarine site in 2007, 2008, and 2009. n.d.: no data.
During the spring tide cycles, physical parameters usually oscillated regularly with the tide irrespective of season and sometimes values also varied in interaction with the diel cycle (Fig. 3). At the upper estuarine site, surface and bottom water
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Salinity usually co-varied with water level, except after strong rainfall as witnessed during the tidal cycle in April 2009, when surface salinities stayed permanently low (Fig. 3a). Despite a water depth of only 2 to 4 m, the water column was highly stratified with bottom water salinities often being >10 units higher than the salinity at the surface. Surface water temperatures were always highest around midday and the early afternoon and lowest in the morning. After sunrise, water temperature often continued to fall and only rose with a time delay. At night, surface water temperatures could intermittently be colder than the more saline bottom water (not observed during the tidal cycle shown in Fig. 3a, but in the first and second 2007 and in the first 2008 tidal cycle; see Electronic supplementary material 1). DO contents co-varied with water level and were usually higher at the bottom. Maximum values always occurred around the higher HW. This was the only period when DO at the surface could exceed values at the bottom, likely due to increased in situ phytoplankton activity in the upper water column during maximum insolation (see Electronic supplementary material 1). This effect is not visible in Fig. 3a because run-off from a thunderstorm at the beginning caused a rapid decrease and permanently low water transparencies throughout the tidal cycle. The pH was stratified with higher pH values at the bottom. Higher values were recorded during the higher HW period, when the landward excursion of the tide reached its maximum. Secchi depth was usually lowest at lower LW and highest after the higher HW at the onset of ebb tide. The current was bi-directional, upstream (20°) at flood and downstream (195°) at ebb tides. Flood current speeds reached a maximum of 0.3 ms⁻¹, while ebb tide current speeds could exceed 0.6 ms⁻¹. Around slack lower LW in the upper and middle estuarine site there was outflowing surface water despite rising water levels, indicating that at early flood tide marine water entered the system along the bottom.

In the middle estuarine site, salinities also co-varied with water level (Fig. 3b). The water was also stratified, often in the range of 5 units. Around slack lower LW, salinities always displayed an overall cycle minimum, likely due to water flowing off the Wenchang River and temporally influencing the lagoon. Bottom water temperatures were usually 1.5°C cooler than surface water temperatures. Lowest bottom water temperatures were recorded during the higher HW (maximum intrusion of the tide). Surface water temperatures were lowest in the morning and reached a maximum in the afternoon during lower LW. DO contents were usually higher at the bottom than at the surface, especially during the higher HW (maximum intrusion of the tide). Surface DO contents were usually highest at late ebb tide after the ebb tide minimum in Secchi depth and when lower LW still occurred at daylight; DO then decreased through the night. Intermittently, lower DO values around midday were likely due to photoinhibition.
pH co-varied with the changes in DO. Secchi depth was usually lowest at lower LW and highest after the higher HW at the onset of ebb tide. The currents were also bi-directional, upstream at flood (325°) and downstream (120°) at ebb tide, and of similar strength as at the upper estuarine site.

In the outer estuarine site, salinity also co-varied with water level (Fig. 3c). Another maximum at lower LW was likely due to evaporation given water depths <1 m and maximum daily water temperatures during this tidal stage. The site was characterized by the smallest range of salinity values during tidal cycles, i.e. relatively high and stable salinities over time. Yet, the water column of the back-reef area displayed a minor stratification, with slightly higher salinities (<1 unit) and lower temperatures (<1°C) close to the ground as evidenced by samples taken manually with the sample bottle a few centimeters above the substrate. This was not always detectable in our standard measurements, which were taken ca. 0.5 m above the ground. Water temperatures usually varied inversely to water level. Intense rains from 19:00 to 21:00 on Mar 29 caused a delayed fall in surface salinity and a rise in water temperature. DO contents were extremely high (>10 mg l⁻¹) during the lower LW phase or even increased after sunset with slightly rising water levels. Minimum values were recorded in the morning and increased again with a time delay when the higher HW occurred around sunrise. The range in DO contents was the greatest of all estuarine sites. Again, pH co-varied with the changes in DO but displayed a low pH-spike due to a second thunderstorm between 05:00 and 06:00 on Mar 30. Secchi depth was always equal to water depth, except for periods of stronger ebb tide currents or wave-induced sediment resuspension. The currents were usually alongshore (340° at flood and 130° at ebb tide), generally weak, and rarely exceeding 0.2 ms⁻¹. However, wind and waves could significantly alter surface current directions at this site.

The mean net tidal excursion based on surface current measurements at the upper, middle and outer estuarine site was 7.3 km downstream (±2.6 SD), 3.6 km downstream (±4.7 SD), and 0.9 km westward (±0.6 SD), respectively. This indicates that the lagoon was clearly an ebb-dominated system at spring tides.

### 3.2 Chemical parameters

Nutrient concentrations of NO₃⁻, NH₄⁺, PO₄³⁻ and Si(OH)₄ generally decreased towards the sea, by at least one order of magnitude (Fig. 4).
Fig. 4: Medians with 25-75% quartile and min-max of surface $\text{NO}_x^-$, $\text{NH}_4^+$, $\text{PO}_4^{3-}$ and $\text{Si(OH)}_4^-$ for each spring tide cycle sampled at the upper (grey), middle (dotted) and outer (white) estuarine site in 2007, 2008, and 2009. *: Effluents from the cleaning of a nearby shrimp pond. n.d.: no data.
Fig. 5: Examples of spring tidal variations in chemical parameters at the three fixed estuarine sites (a-c) of the Wenchang/Wenjiao estuary (East Hainan) in Mar/Apr 2009. Grey columns indicate night. Filled symbols: surface water; open symbols: bottom water 0.5 m above the ground. Heavy rainfall in (a) occurred from 16:15 - 16:45 h, and in (c) from 19:00 - 21:00 h, and from 05:00 - 06:00 h.
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The concentrations of nutrients usually showed a clear tidal signature in surface and bottom water, irrespective of season (Fig. 5; see also Electronic supplementary material 2). At the upper and middle estuarine sites, \( \text{NO}_x^- \), \( \text{NH}_4^+ \), \( \text{PO}_4^{3-} \) and silicate were...
inversely related to water level with mostly lowest concentrations around the higher HW (maximum intrusion of the tide) and highest concentrations at lower LW (Fig. 5a, b; 6). Concentrations in the surface water were usually higher than in the bottom water. After thunderstorms, as it was the case in April 2009 in the upper estuarine site (Fig. 5a), surface water concentrations of NO$_3^-$ and Si(OH)$_4^-$ did not vary with the tide. Fluctuations in PO$_4^{3-}$ concentrations were inconsistent and negligible.

At the outer estuarine site, nutrient concentrations fluctuated at low levels (often around the detection level) and tide-induced patterns were less consistent compared to the other sites (Fig. 5c). The bottom water at the outer estuarine site was not sampled given the small water depth. The range in nutrient concentrations between higher HW and lower LW decreased towards the coast (Fig. 6). The ranges were greatest for NO$_3^-$, NH$_4^+$ and Si(OH)$_4^-$.

4. Discussion

The WWE is a relatively small, shallow, but strongly stratified system with considerable horizontal dynamics despite microtides. The three sampling sites displayed distinct physico-chemical characteristics despite spatial proximity. The difference of the water bodies between the sampling sites was generally visible by eye: the water was brown in the upper, green in the middle and blue in the outer estuarine site (Fig. 7).

Major changes in water parameters of the system occurred on two predictable time scales: tidal/diel (i.e. differences between lower LW and higher HW/differences between day and night) and seasonal (i.e. differences in dry vs. wet season, e.g. in water temperature, precipitation); and on one unpredictable time scale: hours to days (i.e. changes in water parameters induced by heavy rainfall due to thunderstorms or typhoons).

4.1 Short-term variations: ecosystem effects and functions

The WWE is a tide-dominated system with a pronounced salt wedge extending >10 km into the shallow lagoon and lower reaches of the Wenchang River. This is facilitated by the northward extension of the inlet channel (Fig. 1) and dredging of the channel south of Qinglan harbor. The intrusion of cooler, more saline bottom water with higher DO and lower nutrient concentrations than the surface water caused regular co-varying fluctuations in physical and chemical parameters over spring tide cycles. The greatest effect of the marine intrusion occurred around the peak of the landward
excursion of the tide, i.e. the higher HW during morning hours (e.g. maxima in salinity, Secchi depth and minima in nutrient concentrations at the upper and middle estuarine site; Figs. 3, 5, 6). This suggests that the main effect of the rising tide is the dilution of the sea-going river water containing nutrients and sediments (Liu et al., 2011). Tide-related variations in nutrient concentrations were greatest in the upper estuarine site where freshwater and brackish-marine water directly meet (Fig. 6). To avoid the mixing of local temporal variation with spatial variation in larger-scale surveys, sampling activities in the extreme estuarine transitional zones should be standardized to fixed tidal stages, e.g. around LW or HW. The lower HW or higher LW at night did result in no or only minor oscillations of physico-chemical parameters. Ye (1988) analyzed the tide-related variation in current patterns and salinity at the mouth of the WWE and also found that salinity was positively related with tidal height, but no other water parameters were measured. Ye (1988) already observed the ebb-dominance of the system and highlighted the funnel effect of the inlet with average current velocities of 0.9 ms⁻¹ that ensures the maintenance of a self-scouring tidal channel. In contrast to our results, Ye (1988) found no evidence of stratification. The regular inflow of water of marine origin along the bottom effectively buffers the lagoon against adverse effects of discharges from urban, agricultural and aquaculture sources. The ebb-dominance, the salt wedge and the intermediate residence time suggest that the lagoon is well-flushed and therefore a relatively robust system despite large-scale mangrove loss. The resilience of the system may be exemplified by beds of oysters and other bivalves that still abound in the low intertidal and shallow subtidal zone of the middle and upper estuary; though specimens are mostly small due to intense collection by locals during spring low tides.

The inverse relationship between nutrient concentrations, salinity and water level in the upper and middle estuarine site suggests that nutrient-rich surface flow (from the river and aquaculture pond discharges) and pore water flow (from the water table of surrounding mangroves and ponds) influenced the lagoon and lower reaches of the river during late ebb tide. Lara and Dittmar (1999) and Dittmar and Lara (2001) showed that the outflow of nutrient- and organic matter-rich pore- and seepage water from the mangrove sediment into channel water in north Brazil led to characteristic tidal signatures with maximum concentrations during low tide. Akamatsu et al. (2009) also found an inverse relationship between nutrient and organic matter concentrations and tidal height and highlighted the importance of groundwater flow for the nutrient budget in a mangrove system in Japan. However, Pratihary et al. (2009) showed for the Modavi estuary, West India that the role of estuarine sediments changed between pre-monsoon and monsoon season. While the estuarine sediments served as a net source
of dissolved inorganic nitrogen (mainly $\text{NH}_4^+$) during marine dominated dry season conditions, it acted as a net sink during the near freshwater conditions of the wet season. Spatial variability in sediment types in the Wenchang/Wenjiao estuary (mud vs. sand) may also influence the importance of porewater release. Herbeck et al. (2011) suggest that the typhoon-induced resuspension of estuarine sediments and associated release of nitrate and ammonium from sediment pore water into the water column was probably negligible compared to the external nutrient inputs (leaching from soils, aquaculture, rain). Further research on the role of the sediments in the biogeochemistry of the Wenchang/Wenjiao estuary is certainly warranted.

The green color of the water, a rich nutrient supply, an intermediate residence time of 5.6 days and a relatively wide euphotic zone suggest that the lagoon is the major area of nutrient uptake with continuously high phytoplankton abundances supporting the artisanal fishery (Krumme et al., CSR, in revision). Under dry weather conditions, the lagoon functions as an effective filter of nutrients and sediments (Liu et al., 2011) as suggested by low nutrient concentrations and clear-water conditions of the back-reef area during all tidal stages. At spring tides, phytoplankton production is restricted to the period from higher HW in the morning (lower nutrient concentrations) to lower LW in the evening (higher nutrient concentrations). The spring tide oxygen cycle suggests that phytoplankton activity is usually greatest during late ebb tide in the afternoon (Fig. 3). In another estuarine system of the South China Sea, the Sungai Ibai estuary, Terengganu (Malaysia), inflow of marine water during flood tide led to higher salinities, lower nutrient concentrations (similar to the WWE) and lower photosynthetic rates at high tide compared to low tide (Shasmudin and Ambak, 1983).

At the upper estuarine site of the WWE very high nutrient concentrations were temporarily detected in July 2008 when effluents from the cleaning of a nearby shrimp pond passed the study site around high tide (Figs. 4, 6). The fact that nutrient concentrations in the upper estuarine site were in the range of those during the witnessed event of shrimp pond cleaning suggests that aquaculture effluents enter as a non-point source and are a major source of nutrient input into the lagoon. The high level of dissolved nutrients was, however, not linked to high in-situ phytoplankton activity because surface oxygen levels were relatively low. Light limitation due to the high turbidity (low Secchi depths in brown water) and the increased mixing during spring tides may be the major reason for this. Surface oxygen concentrations peaked only around the high HW when high insolation and weaker currents coincided, i.e. the phytoplankton population had a longer residence time in the narrow euphotic zone (Monbet, 1992).
A major shortcoming of our study is the lack of sampling at neap tides. However, the higher nutrient concentrations at lower LW in the WWE and data from Herbeck et al. (2011) suggest that at neap tides the estuary is characterized by overall higher nutrient concentrations than spring tides due to reduced mixing with marine waters and likely more favorable conditions for phytoplankton production.

It was striking that the DO content at the outer estuarine site peaked hours after sunset (sometimes at 22:00) with values >11 mg l⁻¹ or remained extremely high after sunset. This phenomenon recurred in all but two occasions: after a typhoon in 2008 (Krumme and Wang, unpublished results) and after the first heavy monsoon rains in April 2009 with maximum Secchi depths of only 1.6 and 2.0 m, respectively. Except for Mazda et al. (1990), we did not find accounts of a similar phenomenon from other back-reef areas in the literature.

Four primary producers could potentially be involved in the extended release of DO after sunset in the back-reef area: 1) phytoplankton; 2) macroalgae; 3) seagrass; or 4) the group of benthic microalgae (covering almost the entire substrate in the back-reef areas as turf algae on widespread coral rubble and epiphytes of seagrass leaves). Phytoplankton can be rejected as a potential source of DO because it has no potential to store oxygen for delayed release (e.g. Monbet, 1992). Furthermore, in situ oxygen consumption experiments in 2009 showed that the oxygen production of phytoplankton immediately decreased with sunset (D. Maier, ZMT, unpubl. results). Macroalgae are rare and can therefore also be excluded as potential sources of DO. Seagrass could potentially be involved when abundant, but the phenomenon occurred even in March 2009 (Fig. 3) when seagrass cover at the near surroundings of the sample site was <5%. Hence, we consider the group of benthic microalgae, which flourish in great abundance on coral rubble and, where present, on seagrass leaves, as the most likely producers causing the delayed release of oxygen. During the afternoon lower LW period (with elevated water temperatures and salinities) we could observe air bubbles adhered to the dense cover of turf microalgae/epiphytes, which sporadically rose to the water surface. It is proposed here that oxygen from the afternoon photosynthesis stored in the bubbles was successively dissolved in the water column due to rising water pressure at flood tide after sunset. Thereby, high DO contents were sustained throughout the early night. We interpret the delayed oxygen release as a sign of degradation of the back-reef habitat, which is likely due to excess nutrient input and overexploitation of grazing organisms, such as snails, sea urchins or fishes. Specific studies have to test these hypotheses.
4.2 Influence of heavy rainfall and ecosystem implications

Our data from dry summer, pre- and post-typhoon and first heavy rains in 2007, 2008 and 2009, respectively, allow for a first evaluation of the influence of heavy rainfall on estuarine dynamics and a characterization of the present ecological status of the estuarine and nearshore system (Figs. 2, 4).

Due to the nature of a relatively small catchment area and short river length, the discharge of the WWE does not integrate rainfall events over a larger region but fluctuates in response to local rainfall events. During the summer monsoon, there is no continuously elevated river discharge. The river influence decreases remarkably during week-long dry periods (e.g. in 2007). Once heavy rains fall, the river discharge increases and physico-chemical parameters change immediately throughout the estuary and at the coast. Pulsed runoff seems to be typical for monsoonal estuaries (Wolanski, 1994; Vijith et al., 2009). However, the immediate propagation of stronger rainfall in the watershed to the nearshore zone is likely atypical and a sign of a degraded ecosystem. Fig. 3a demonstrates the immediate effect of thunderstorm-induced precipitation in the watershed of the WWE from 16:15-16:40 at April 13, 2009 for the upper estuarine site. Surface salinity, water temperature, pH and Secchi depth decreased within hours after the rain events. Secchi depth (~0.2 m) and surface salinity (~1 psu) remained low for the remainders of the observations (April 14). Our sampling at the outer estuarine site a day later (April 15/16) showed lower salinities, water temperatures, higher nutrient concentrations and an altered DO cycle compared to the sampling in March 29/30 (Figs. 2, 3, 6). Similar, though stronger and longer-lasting effects were detected with typhoon-induced intense rains in 2008 (Fig. 4; Herbeck et al., 2011). Our assumption of an immediate change of estuarine parameters following rainfall is backed by a third observation: First local rains after a dry period that preceded our final 2007 sampling on Sep. 10/11 at the upper estuarine site also led to e.g. low Secchi depths, surface salinities and water temperatures throughout the tidal cycle (Fig. 2, see Electronic supplementary material 1). This suggests that nowadays the runoff from common intense rains in the watershed affect the estuarine and nearshore system with little time delay and clearly detectable abiotic signals. The satellite image scene in Fig. 7 illustrates the short-cut between the watershed and the nearshore zone. A plume leaves the Wenchang River after a rainfall event at ebb tide. The image gives an idea on how easy a greater discharge may dominate the entire lagoon and affect the nearshore zone through the inlet channel.
Fig. 7: Satellite image scene of the Wenchang/Wenjiao estuary after a rainfall event at ebb tide (Geoeye-1 Geo PSM 4-bands image on Oct 05, 2009). Arrows indicate the plume of the Wenchang River water (**), the plume of the lagoon at the mouth of the inlet channel (**) and parts of this plume in the back-reef area (*).

The most likely reasons for the prompt discharge after any type of heavy rainfall to the nearshore zone are deforestation, channelization, loss of floodplains and mangroves, and the decoupling of remaining mangroves from regular tidal inundations by dams of aquaculture ponds. Mangroves are effective sinks of fine sediment and local fishermen state that decades ago the lagoon was deeper and the water was clearer (Krumme et al., CSR, in revision). In a stratified estuary such as the WWE, each spring flood tide the lighter surface water is transported laterally into the mangrove. The dense mangrove forest reduces current velocities and suspended sediment is deposited (Furukawa et al., 1997; Wolanski et al., 2001), mostly within 50 m from a river edge (Victor et al., 2004). Other important hydrodynamic effects, such as lateral trapping, biological flocculation or self-scouring are also negatively influenced when mangrove area is lost (Wolanski et al., 2001; Wolanski, 2007). It is noteworthy that adverse effects of runoff are presently mainly due to the Wenchang River and would be even more severe if the larger Wenjiao River was not dammed.

Substantial modifications are ongoing or planned for the WWE, mainly linked to the construction of China’s fourth space center in the Wenchang county (for example, regular deepening of the channel, construction of a mole (Figs. 1, 7), but also to the government’s plan to turn Hainan into an “international tourist destination”. Given these
scheduled large-scale changes on the coast, the present work also serves as reference against which the effects of future changes can be assessed.

5. Conclusions

A horizontal gradient in physico-chemical parameters was persistent over time: Salinity, DO content, water transparency and pH increased towards the coast, while nutrient concentration decreased. All physical parameters varied with spring tide-induced changes in water level, partially in interaction with the diel cycle. Patterns were site-specific and were significantly altered by rainfall. Nutrient concentrations usually varied inversely with water level (question 1). The upper estuarine site exhibited the greatest changes in physico-chemical parameters during tidal cycles, which suggest that standardization of sampling is of particular importance in the lower reaches of rivers.

The WWE is a relatively small, shallow, strongly stratified tide-dominated system. Cooler, more saline bottom water with higher DO content and lower nutrient concentrations enter the estuarine lagoon through the inlet channel during flood tides (question 2). Regular tide-induced marine intrusion ventilates the benthos and buffers the system against eutrophication and facilitates buoyancy trapping of sediments in the remaining mangroves.

Since a relatively small catchment area and small estuarine volume coincide with very episodic rainfall, the system is characterized by pulsed runoff events, which is however, typical for monsoonal estuaries. Yet, it is atypical that runoff from smaller local thunderstorms causes immediate changes in all physico-chemical parameters through the lagoon to the nearshore reef habitats within hours (question 3).

The frequency, strength and duration of events, in which turbid, brackish water reaches the reef habitats, may have increased in the past and contribute to the ongoing degradation of the neashore seagrass and coral reef habitats (pers. observations; Hutchings and Wu, 1987; Fiege et al., 1994). The short-cut between the drainage basin and nearshore zone are likely due to deforestation, channelization in the watershed and substantial loss of fringing mangroves in the estuary. The degraded conditions of the estuary, its mangroves and the seagrass and reef habitats in the nearshore region are likely to deteriorate when nutrient and sediment input are not reduced, e.g. by rehabilitation of mangroves and riparian vegetation (Wolanski, 2007).
Supplement 1 Spring tidal variations in salinity, water temperature, dissolved oxygen concentrations and Secchi depth at the upper estuarine site of the Wenchang/Wenjiao estuary (East Hainan) in the first (a) and second (b) expedition in August/September 2007. Grey columns indicate night. Filled symbols: surface water; open symbols: bottom water 0.5 m above the ground. Rainfall preceded the second expedition.
Supplement 2 Examples of spring tidal variations in chemical parameters at three fixed estuarine sites (a–c) of the Wenchang/Wenjiao estuary (East Hainan) in August 2007. Grey columns indicate night. Open symbols: bottom water 0.5 m above the ground (only available for the upper estuarine site).

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References


CHAPTER IV

Sources, transformation and fate of particulate amino acids and hexosamines under varying hydrological regimes in the tropical Wenchang/Wenjiao Rivers and estuary, Hainan, China

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Sources, transformation and fate of particulate amino acids and hexosamines under varying hydrological regimes in the tropical Wenchang/Wenjiao Rivers and estuary, Hainan, China

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Abstract

The small tropical Wenchang and Wenjiao Rivers on the island of Hainan, tropical China, are affected by effluents from municipal sewage, aquaculture and agriculture and by contrasting hydrological regimes related to monsoon and tropical storms. In order to obtain information on the sources, transformation and fate of organic matter (OM) we investigated the amount and composition of amino acids and hexosamines as well as the carbon isotope composition in suspended particulate matter (SPM) from the Wenchang/Wenjiao Estuary. SPM was collected along the salinity gradient starting from the river sites, along the lagoon-shaped Bamen Bay to coastal waters during four sampling campaigns between 2006 and 2009. SPM concentrations ranged between 4.7-58.2 mg L\textsuperscript{-1}. Apart from highest values after heavy rain events in spring and summer, SPM showed little seasonal variation, but increased with salinity. From SPM POC\% (1.2-20.9%), C/N (4.9-16.5), \(\delta^{13}\text{C}_{\text{org}}\) (-31.5 to -19.5‰), the molar composition and content of amino acids and hexosamines (8.2-156.2 mg g\textsuperscript{-1} dry weight) and by comparison with sediments, mangroves, soils and plants we are able to show that soil-derived material, freshwater plankton and marine plankton were the major sources of suspended OM. High POC and amino acid contents were related to primary production sustained by dissolved nutrients to a large extent stemming from municipal and aquaculture effluents. Principal component analysis showed that the
suite of biogeochemical parameters measured clearly depicts the terrestrial vs. marine origin and the freshness/reactivity of OM. The four groups of samples resulting from cluster analysis were basically related to varying hydrological regimes. With respect to the sources, degradation and fate of particulate OM the major factors were: (i) the year round input of labile, amino acid rich riverine OM matter at the freshwater dominated sites, (ii) high input of degraded soil OM after heavy rains with dispersal throughout the estuary and export to the adjacent coastal area, (iii) significant production of labile marine OM especially during summer inside the bay and the (iv) dominance of refractory marine OM during winter and spring season and in the offshore region. While a major part of the fresh OM fueled by anthropogenic nutrients appears to be stored or recycled, inside the bay, periodic torrential rainfalls can lead to a pulsed export of this OM to the coastal area where it may adversely affect seagrass meadows and coral reefs.

**Keywords:** suspended particulate matter; amino acids; hexosamines; factor analysis; mangrove; stable carbon isotopes; Wenchang/Wenjiao Estuary

1. Introduction

Over the past decades intense land use change, agriculture and population growth have significantly altered river fluxes of carbon, nutrients and sediments (Smith et al., 2003; Syvitski et al., 2005). The impacts on receiving estuaries and coastal waters are, among others, shifts in plankton species composition and the enhancement of primary production, potentially leading to intensified organic matter respiration and the formation of oxygen deficient zones (Diaz and Rosenberg, 2008; Zhang et al., 2010). Watersheds and coastlines of China are no exception to these global trends and are heavily affected by human alterations. Anthropogenic impacts comprise rising nutrient loads of rivers and estuaries from fertilizer application, rapid development of aquaculture facilities and urbanisation (Li et al., 2002; Liu et al., 2009; Yang et al., 2007; Zhang, 1996). In southern China, these adverse developments are augmented by the loss of mangroves diminishing their protective function for coastal marine waters (Li and Lee, 1997). A combination of the aforementioned factors is of relevance in the investigated watersheds of the rivers Wenchang and Wenjiao in north-east Hainan.
Changes in very small watersheds such as the one studied may have significant impacts on local coastal ecosystems such as nearby seagrass beds and coral reefs due to altered mass and elemental fluxes (see e.g. (Herbeck et al., this issue)). Furthermore, small systems such as the Wenchang/Wenjiao Estuary (WWE) are suitable for process studies of spatial and seasonal variability in the distribution and composition of organic matter and its driving factors because of the immediate response of the water body to environmental factors such as heavy rains (Herbeck et al., 2011). These factors are more easily identified in small than in large watersheds, where different and also remote processes, which might dampen the effect of single events, have to be considered.

Within a highly dynamic estuarine environment the distribution and composition of suspended particulate material (SPM) is affected by changing river discharge, the mixing of fluvial and marine particles, and the resuspension of sediments due to tidal currents and wind forcing. The input of organic matter (OM) from allochthonous and autochthonous sources may vary distinctively in space and time leading to a significant variability in the composition and reactivity of estuarine OM (Goñi et al., 2003; Unger et al., 2005a). This, in turn, will influence the cycling of OM and the resulting fluxes from land to sea. Under high discharge condition, e.g. during rainy periods, the contribution from the rivers to estuarine SPM will increase relative to autochthonous material. This fluvial material is to a large extent derived from soils and bedrock (Hedges et al., 1986; Ludwig and Probst, 1996; Meybeck, 1993) and is relatively recalcitrant (Hedges et al., 1997; Ittekkot, 1988). In contrast, under low discharge conditions with low turbidity, in situ production of fresh planktonic OM might dominate riverine and estuarine OM if nutrients are available (Gupta et al., 1997; Ittekkot et al., 1985). This fresh autochthonous OM is prone to intensive respiration which might be enhanced especially in tidally dominated estuaries where residence time is prolonged and mobilization occurs frequently (Middelburg and Herman, 2007).

In our study we combine information on the concentration of SPM and the content of particulate organic carbon (POC) and total nitrogen (TN) with stable carbon isotopes ($\delta^{13}C_{\text{org}}$) and the concentration and composition of amino acids (AA) and hexosamines (HA). While the combined $\delta^{13}C_{\text{org}}$ and the molar ratio of POC to TN (C/N) are useful parameters to differentiate between terrestrial and marine sources (Goni et al., 2006; McCallister et al., 2006; Meyers, 1994), AA are good indicators of organic matter degradation. They are labile relative to bulk OM and undergo significant alteration already during early diagenesis. This is evident from e.g. monomeric composition and the contribution of AA to bulk POC and TN (Cowie and Hedges, 1994; Wakeham et al., 1997). As AA constitute a large fraction of freshly produced aquatic
OM and most of the nitrogen in living organisms they reflect the degradation state of a representative portion of total OM in a collected sample (Dauwe et al., 1999; Jennerjahn and Ittekkot, 1999; Lee et al., 2000; Unger et al., 2005b). As compared to AA, the contribution of HA to fresh OM is lower by an order of magnitude. HA are less reactive than AA because of their association with bacterial cell walls and chitinous material, so that the ratio of AA/HA decreases with degradation (Benner and Kaiser, 2003; Gupta and Kawahata, 2000; Haake et al., 1992; Müller et al., 1986). In riverine and estuarine SPM, the monomeric composition of AA and HA and the ratio of AA/HA may also give clues on the relative dominance of plankton or soil as source for SPM (Ittekkot, 1988; Ittekkot et al., 1983; Jennerjahn et al., 2004; Unger et al., 2005a).

In order to address the seasonal variability of the distribution and composition of SPM and underlying causes, we investigated SPM from the rivers Wenchang and Wenjiao and their estuary during the dry winter season in December 2006, the rainy summer season in August 2007 and August/September 2008 as well as during the transition between dry and wet season in spring March/April 2009. The results are discussed with respect to the sources, transformations and fate of organic matter in an estuarine environment, which has experienced intense human alterations and is characterised by distinct seasonal hydrological variability, as well as by a significant input of nutrients from municipal and aquaculture effluents (Herbeck et al., this issue; Liu et al., 2011).

2. Materials and Methods

2.1 Study area

The WWE is located at the east coast of Hainan Island, South China Sea (Fig. 1a). It is fed by the two small rivers Wenchang and Wenjiao that debouch into the shallow (<5m) Bamen Bay (Fig. 1b). Their length and average discharge rate is 37 km and 9 m$^3$s$^{-1}$, and 56 km and 12 m$^3$s$^{-1}$, respectively (Zeng and Zeng, 1989 cited in Liu et al., 2011). The region comes under the influence of a tropical monsoon climate. Dry winter season spans from November/December-March/April. During this nominal ‘dry season’, however, heavy rain spells can occur occasionally. The rainy summer season spans from May-October (Fig. 2a), during which rain rates are highly variable. Dry periods may alternate with extreme rainfall of up to 300 mm d$^{-1}$ often related to typhoons, which develop mainly between August and October. The WWE is subject to
mixed semidiurnal micro-tides with an average tidal amplitude of <2 m. However, the intrusion of sea water into the bay is highly variable depending on precipitation, the related river discharge as well as by tidal forcing (Krumme et al., submitted). Inflow of sea water affects the entire study area with the exception of the most upstream sites near the cities of Wenchang and Wenjiao. The marine inflow is also strongly reduced during periods with heavy rains (Fig. 1d-l). Mangroves which once covered large areas around Bamen Bay were converted to aquaculture ponds to a large extent. In total, 74% of the mangrove area was lost since the 1960s (Krumme et al., submitted). The remaining mangroves cover 750 ha and are mainly found in the region where the Wenchang River debouches into the bay (Fig. 1b). Additional small stands occur along thin bands between the ponds and adjacent river banks and the bay. Today, approximately 2150 ha of the Bamen bay region are occupied by ponds for fish and shrimp cultivation especially off the inflow of the Wenjiao River (Fig. 1b). They act as a major nutrient source to the WWE (Herbeck et al., this issue; Liu et al., 2011). In the hinterland of the rivers Wenchang and Wenjiao and their tributaries the major land use is the cultivation of rice, coconut and mixed fruit and vegetable cultures. Urban effluents from the cities of Wenchang and Wenjiao (Wenchang: 116,000 inhabitants in 2006) as well as from smaller settlements are drained into the rivers without any treatment. During recent years, in the course of the promotion of Hainan Island as major touristic site a number of tourist facilities, such as large hotels are constructed along the coast. In part, these hotels replace former aquaculture ponds.
Fig. 1: Maps of the study area. a) location of Hainan in the South China Sea and the region of the WWE; b) detailed map of the WWE with distribution of aquaculture, mangrove, sea grass and coral reefs; c-l) location of sampling sites during the individual sampling campaigns in dry season 2006, rainy seasons 2007 and 2008 and spring 2009. Salinities observed during sampling are given as isolines and light grey numbers. Symbols used to indicate sampling sites also denote the individual sample cluster: ■ river cluster, ● bay-offshore cluster, ■ bay-summer cluster, ▲ rain cluster. Respective white symbols in c) denote samples taken on December 12, in f) denote samples taken on August 25.
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2.2 Sample collection

Four sampling campaigns were carried out in the WWE during the dry season in December 2006, during the rainy seasons in August 2007 and July/August 2008 and the transition period between the dry and the rainy season in spring 2009 (March/April) (Fig. 2b). The dry season 2006 sampling was carried out under declining spring tide conditions, rainy season sampling 2007 was carried out under neap tide condition for W1-W11, and spring tide condition for W12-W16, the rainy season August 2008 sampling under neap tide conditions and the spring 2009 sampling under mid-spring tide conditions (March) and neap tide conditions in April. In 2008, we collected samples before (pre-typhoon) and after a typhoon (post-typhoon) which brought heavy rain falls to the study area (Fig. 2b) (Herbeck et al., 2011). Sampling included collection of SPM and sediments and was accompanied by the measurements of physico-chemical parameters along the Wenchang/Wenjiao Rivers and WWE. In addition to SPM and sediments, various soil and plant samples were collected at different locations along the rivers/WWE.

Fig. 2: a) Record of daily precipitation at Haikou for the period 2006-2009 b) daily precipitation for Haikou and Qionghai, the two weather stations closest to the study area, during Nov/Dec 2006, July and August 2007 and 2008, and March/April 2009. Periods during which SPM was collected are marked by a gray-shaded rectangle for each field campaign. Strong rainfalls in August 2008 were associated with typhoon Kammuri. Precipitation data are taken from Climate Center, Utah State University, http://climate.usurf.usu.edu/products/.
Salinity was measured \textit{in situ} with a WTW MultiLine F/Set3 multiparameter probe. Surface water samples for the analysis of SPM were collected with a bucket and were stored in PE tanks until filtration in the lab the same day. Water samples were filtered onto pre-combusted (5 h, 450°C) and pre-weighed Whatman GF/F filters. It was taken care to thoroughly shake the water tanks before taking water for filtration in order to avoid any settling of material within the tanks. The volume filtrated varied mainly between 500-1250 ml depending on SPM concentration, in a few cases 250 or 1500-2000 ml were filtrated. Sediments were taken using a small sediment grab. The device did not allow for sampling of undisturbed surface sediments, but care was taken to use only material from the upper 1-2 cm of the sediment retrieved. Soil and mangrove samples were taken with a spoon from the surface. All these samples were stored in pre-combusted glass vials. Filters, sediments, soil and plant samples were dried at 40°C in an oven in the field lab. All samples were stored under dry conditions until analysis in the lab in Bremen.

\subsection{2.3 Analyses}

All samples were analysed for total carbon and total nitrogen (TN) by high-temperature combustion in a Carlo Erba NA 2100 elemental analyser (Verardo et al., 1990). POC was determined the same way after removal of carbonate by acidification with 1N HCl and subsequent drying at 40°C. Measurements had a precision of 0.06\% for organic carbon (OC) and 0.02\% for TN, based on repeated measurements of a standard (LECO 1012). The stable isotope composition of OC ($\delta^{13}C_{org}$) was determined with a Thermo Finnigan Delta Plus gas isotope ratio mass spectrometer after high temperature combustion in a Flash 1112 EA elemental analyser. Carbonates were removed prior to the analysis by adding 1N HCl and subsequent drying at 40 °C. $\delta^{13}C_{org}$ values are given as \‰-deviation from the carbon isotope composition of the PDB standard and had an analytical precision of 0.14‰. Dried sediments, soil and plant material were analysed after grinding to a fine homogenous powder in a Retsch planetary ball mill PM 100.

Total amounts of hydrolysable amino acids (AA) and hexosamines (HA) were analysed with a Biochrom 30 amino acid analyser after hydrolysation of 0.5-1 filter or 4-600 mg of plant tissue or sediments with 6 N HCl at 110°C for 22 hours. Hydrolysed samples were evaporated, taken up in 2 ml of sodiumcitrate buffer and injected into the analyser for chromatographic separation on a cation exchange column. Detection of monomers was carried out fluorometrically after derivatisation with o-Phtaldialdehyde and mercaptoethanol. HA concentrations were corrected by a factor of 1.4 to
compensate for losses during hydrolysis according to Müller et al. (1986). The detected amino acids were aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acids (Glu), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), ornithine (Orn), lysine (Lys) and arginine (Arg). Additionally, the non-protein amino acids -alanine (b-Ala) and -aminobutyric acid (g-Aba) (non-prot. AA) and two HA, galactosamine (Galam) and glucosamine (Gluam), were analysed. Repeated measurements of a standard solution yielded a relative standard deviation of 0.1-3.1% for the concentrations of individual monomers.

Lignin phenols were analysed according to the method of Hedges & Ertel (1982) after heating the samples with NaOH, CuO and Fe(NH₄)₂(SO₄)₂ at 160 °C for 3h. After separation of solution from solid, the solution was acidified to pH <2 prior to extraction with ethyl acetate. Lignin phenols were analysed using an Agilent 6890 series Gas Chromatography coupled with Flame Ionization Detector (GC/FID). Analytical precision for the total concentration of lignin phenols was <5%. Details are given in Bao et al. (this issue).

2.4 Statistics

Statistics were carried out using software STATISTICA version 9. Cluster analysis (Euclidian distance, Ward’s method) was applied on SPM samples using non-standardised values of salinity, AA concentration (mg g⁻¹), POC (%), non-prot. AA (mol%), C/N and ₁₃Corg (‰).

Factor analysis (FA) was applied on all samples investigated in this study. The resulting first factor represents the one which accounts for most of the sample variation, the second accounts for the second most important variation and so on. The “site score” (distance of the sample on the factor axis) is calculated for each sample and represents the value of this factor for any single sample and can be used as a variable for describing the samples with respect to the factors emerging from the FA.

For our samples we carried out two factor analyses. The respective data sets were normalised by subtracting the mean of all values and dividing each variable by its standard deviation. A first FA was done using AA mg g⁻¹, the contribution of AA and HA to total organic carbon (AA-C% and HA-C%), AA/HA, non-prot. AA Mol%, Glu Mol%, POC%, and C/N. In a first approach we also included ₁₃Corg, but this variable was of no diagnostic value at all probably due to overlapping values of freshwater plankton and terrestrial material. This FA was carried out in order to evaluate the general
composition, i.e. source and quality of OM for the individual samples. A second FA was applied on the AA molar composition of all our samples in order to assess the variability of AA molar composition over the whole range of samples.

3. Results

3.1 Hydrography

Depending on season and tidal cycle, salinity varied significantly over time at similar sampling sites (Fig. 1c-l). During the dry season 2006, low precipitation (Fig. 2b) and high tide led to salinities >29 throughout the bay. At the mouth of the Wenchang River, salinity was 19, and intrusion of salt water was observed far upstream. In contrast, in the rainy seasons 2007 and 2008 (before typhoon), salinities >28 were observed only in coastal waters. Salinities in the rainy season 2007 were 18-26 in the bay and 0-10 in the rivers, and in rainy season 2008 <16 inside the bay and <6-0 in the rivers (pre-typhoon). After typhoon rains during the rainy season 2008, the whole bay had a salinity <1, at the mouth. Off the bay, salinity was significantly lower than during other periods. In spring 2009, strong inflow of seawater under spring tide conditions was reflected in salinities of 29 in the central bay on March 27. Salinities of ≈17-22 were measured inside the bay on March 28 and April 8. Salinity increased from ≈22 in the outlet channel of the Bay to ≈30 at coastal sites.

3.2 Suspended particulate matter

SPM concentrations varied between 5.8 and 58.2 mg L⁻¹ (Fig. 3a). The largest variation was observed at 0 salinity, where both the lowest and highest concentration occurred. Maxima occurred after strong precipitation during the rainy season 2008 and in spring 2009. A large scatter was also observed at salinities >20. Excluding the data from the freshwater sites (0 salinity), we observed a general increase of SPM with increasing salinity which was statistically significant during 2006 dry season ($r^2=0.88$) and the Wenchang sampling in spring 2009 (March)($r^2=0.82$). The POC content of SPM (Fig. 3b) varied between 1.2 -20.9% with an average of 6.7%. At the upstream sites, concentrations >10% POC were found only during dry season 2006, and the rainy seasons 2007 and 2008 (pre-typhoon). After the rains, these values decreased to 7-10% in spring 2009 and to lowest values of 5-6% after the typhoon 2008. Unlike
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SPM, POC\% showed a negative correlation with salinity. It was most significant in dry season 2006 ($r^2 = 0.87$) and spring 2009 ($r^2 = 0.62-0.88$ for three campaigns) and showed a larger scatter during rainy seasons 2007 ($r^2 = 0.27$) and 2008 ($r^2 = 0.62$ before the typhoon and $r^2 = 0.45$ after the typhoon).

AA and HA concentrations in suspended matter were between 7.5-144 mg g$^{-1}$ (Fig. 3c) and 0.5-11.8 mg g$^{-1}$ sample, respectively. Highest values occurred at the upstream sites of both rivers. Average values for rainy season 2008 (pre-typhoon), dry season 2006 and rainy season 2007 were 80.8, 48.0 and 44.4 mg g$^{-1}$ AA+HA whereas concentrations of both compounds were significantly lower with average values of 27.4 and 25.7 mg g$^{-1}$ AA+HA during post-typhoon 2008 and in spring 2009. As for POC, AA and HA concentrations decreased with increasing salinity. AA and HA contributed 14.6-46.2\% and 1.0-3.0\% to total POC (AA-C\% and HA-C\%), and 33.8-89.2\% and 1.3-4.3\% to TN (AA-N\% and HA-N\%). On average, the contribution of both compounds to POC and TN was highest in the pre-typhoon phase of 2008 with AA+HA-C\% of 35.8 and AA+HA-N\% of 69.7\%, respectively. Lowest AA-C\%+HA-C\% of 22.4\% was recorded during the 2008 post-typhoon phase. Monomeric composition of AA was dominated by Asp, Glu, Gly and Ala contributing 11.3 -12.8 \text{ Mol\%} on average. Lys, Val, Thr, Ser and Leu contributed between 5.7 and 8.2 \text{ Mol\%}. The remaining monomers made up less than 5 \text{ Mol\%} each (Tab. 1). $\delta^{13}$C$_{org}$ varied between -31.5 and -21.7\% and was positively correlated with salinity (Fig. 3f).
Tab. 1: Average AA molar composition and standard deviations, as well as the ratio of the two hexosamines glucosamine and galactosamine (Gluam/Galam) for the four SPM clusters, sediments, mangrove sediments, mangrove leaves and water hyacinths. Gluam/Galam ratios could not be calculated for mangroves and water hyacinths due to the absence of Galam.

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Fig. 3: Variation along the salinity gradient of a) SPM concentration, b) POC content of SPM, c) AA concentration of SPM (mg g⁻¹), d) AA concentration in the water (mg L⁻¹), e) concentration of non-prot. AA Mol%, and f) δ¹³Corg ‰. Data are shown by the four clusters. The circle in e) depicts the low non-prot. AA Mol% of the samples collected during summer rainy season.
3.3 Sediments, soils and plant samples

Sediments taken along the estuarine gradient contained 0.1-1.4% organic carbon (Tab. 2). Sedimentary organic carbon (SOC) content did not show any systematic variation along the estuarine continuum, although highest concentrations were found at the upstream Wenchang site (W5), while lowest concentrations were found off the outlet of the bay in coastal waters (W2). Wenjiao River sediments at the most upstream sites contained only 0.2-0.5% SOC. Average C/N value was 20.1, and $\delta^{13}C_{\text{org}}$ varied between -26.9 and 19.5‰. Unlike SOC, $\delta^{13}C_{\text{org}}$ values showed a distinct increase from the upstream areas towards the coast. Sedimentary AA and HA concentrations were 1-2 orders of magnitude lower than for SPM (Tab. 2) and co-varied with SOC content. Sediment characteristics showed similar spatial pattern in both years. Soil samples taken from rice fields and the river bank had SOC contents of 0.2-2.1% and an average C/N value of 13.4 (Tab. 2). $\delta^{13}C_{\text{org}}$ values were between -27.7 and -24.3‰. AA and HA concentrations of soils were in the range of sediments and varied between 0.6-4.8 mg g$^{-1}$ and 0.1-1.2 mg g$^{-1}$, respectively. In contrast to SPM, monomeric composition of AA of sediments and soils was characterised by elevated contributions of Asp, Gly and the non-prot. AA and by reduced Mol% of Leu, Lys, Arg and the aromatic AA (Tab. 1). On average, mangrove leaves and water hyacinths contained 49% and 40% POC and had respective C/N ratios of 51.6 and 15.3 (Tab. 2). Average $\delta^{13}C_{\text{org}}$ was -29.8‰ and -28.8‰, respectively. The concentration of AA+HA was 48.5 mg g$^{-1}$ for mangroves and 126.2 mg g$^{-1}$ for hyacinths.

Monomeric composition of mangrove leaves and water hyacinths varied in the range observed for SPM and sediments and soils. Notably, they contained elevated amounts of non-prot. AA despite their freshness. Water hyacinths contained elevated Asp Mol% (Tab. 1).
Tab. 2: Number of samples (n), average, and standard deviation of biogeochemical characteristics for the four SPM clusters as well as for sediments, soils, mangrove leaves and water hyacinths. DI* is the degradation index derived from our FA2, low values denote high degradation. TERR denotes the site scores derived from the FA1, where low values indicate high contribution from terrestrial sources.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>n</th>
<th>SPM mg l⁻¹</th>
<th>Sal</th>
<th>POC %</th>
<th>N %</th>
<th>C/N</th>
<th>δ¹³Corg %</th>
<th>AA mg g⁻¹</th>
<th>AA mg L⁻¹</th>
<th>HA mg g⁻¹</th>
<th>AA/HA</th>
<th>non-prot mol%</th>
<th>DI*</th>
<th>TERR</th>
</tr>
</thead>
<tbody>
<tr>
<td>rain cluster</td>
<td>17</td>
<td>26.4±13.7</td>
<td>2.7±3.0</td>
<td>6.2±1.6</td>
<td>0.7±0.2</td>
<td>10.0±2.4</td>
<td>-26.1±12.2</td>
<td>31.1±8.7</td>
<td>0.8±0.4</td>
<td>2.7±0.6</td>
<td>11.6±3.2</td>
<td>0.9±0.4</td>
<td>0.4±0.3</td>
<td>0.1±0.36</td>
</tr>
<tr>
<td>river cluster</td>
<td>14</td>
<td>13.8±6.5</td>
<td>4.3±5.6</td>
<td>13.2±3.5</td>
<td>2.0±0.5</td>
<td>8.0±2.2</td>
<td>-27.5±3.2</td>
<td>93±19.2</td>
<td>1.3±0.8</td>
<td>5.8±2.3</td>
<td>16.8±4</td>
<td>0.5±0.1</td>
<td>0.9±0.2</td>
<td>0.9±0.42</td>
</tr>
<tr>
<td>bay-offshore</td>
<td>20</td>
<td>24.8±8.0</td>
<td>26.3±5.7</td>
<td>2.5±1.2</td>
<td>0.4±0.2</td>
<td>7.6±1.5</td>
<td>-22.8±2.6</td>
<td>17.1±6.6</td>
<td>0.4±0.2</td>
<td>1.2±0.5</td>
<td>14.7±5.6</td>
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<td>0.3±0.3</td>
<td>0.7±0.41</td>
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<tr>
<td>bay-summer</td>
<td>17</td>
<td>24.5±9.4</td>
<td>16.8±7.9</td>
<td>6.9±2.3</td>
<td>1.0±0.3</td>
<td>8.1±1.3</td>
<td>-22.8±2.6</td>
<td>44.2±10.9</td>
<td>1.1±0.5</td>
<td>2.1±1.4</td>
<td>31.1±17.5</td>
<td>0.4±0.2</td>
<td>0.8±0.2</td>
<td>1.0±0.42</td>
</tr>
<tr>
<td>bay sediments</td>
<td>20</td>
<td></td>
<td></td>
<td>0.8±0.7</td>
<td>0.1±0.1</td>
<td>20.1±12.6</td>
<td>-23.3±2.2</td>
<td>2.2±2.8</td>
<td>0.3±0.3</td>
<td>6.0±1.5</td>
<td>2.0±0.7</td>
<td>-1.4±0.8</td>
<td>-1.1±0.6</td>
<td>0.6±0.4</td>
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<tr>
<td>soils</td>
<td>8</td>
<td></td>
<td></td>
<td>1.5±1.6</td>
<td>0.1±0.1</td>
<td>13.4±12.7</td>
<td>-25.5±1.4</td>
<td>4.4±6.3</td>
<td>0.6±0.8</td>
<td>5.3±1.6</td>
<td>1.9±0.9</td>
<td>-0.5±0.5</td>
<td>-0.9±0.4</td>
<td>0.7±0.07</td>
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<tr>
<td>mangrove leaves</td>
<td>3</td>
<td>48.9±7.0</td>
<td>1.1±0.1</td>
<td>51.6±8.7</td>
<td>-26.8±0.4</td>
<td>47.8±5.7</td>
<td>0.7±0.3</td>
<td>77.4±30.6</td>
<td>0.9±0.4</td>
<td>0.8±0.07</td>
<td>-1.7±0.1</td>
<td>0.0±0.0</td>
<td>-1.4±1.0</td>
<td></td>
</tr>
<tr>
<td>mangrove sediments</td>
<td>2</td>
<td>1.1±0.3</td>
<td>0.1±0.0</td>
<td>19±2.7</td>
<td>-24.5±1.1</td>
<td>2.8±0.7</td>
<td>0.3±0.2</td>
<td>9.7±1.6</td>
<td>1.3±0.04</td>
<td>0.5±0.1</td>
<td>1.4±1.0</td>
<td>0.0±0.0</td>
<td>-0.3±0.5</td>
<td></td>
</tr>
<tr>
<td>water hyacinth</td>
<td>3</td>
<td>39.5±3.3</td>
<td>3.0±0.3</td>
<td>15.3±0.7</td>
<td>-26.8±0.8</td>
<td>124.9±23.1</td>
<td>1.3±0.2</td>
<td>100.6±27.8</td>
<td>1.5±0.4</td>
<td>0.1±1</td>
<td>-0.3±0.5</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td></td>
</tr>
</tbody>
</table>

*n = 21 for δ¹³Corg *n = 7 for δ¹⁵N Corg
3.4 Statistics

3.4.1 Cluster Analysis

Cluster analysis divided the SPM samples into four distinct groups (Fig. 4). Cluster 1 ("rain cluster") includes all post-typhoon samples 2008 as well as the upstream samples from both rivers collected during spring 2009 (Fig. 1) after strong spring rain falls (Fig. 2b). These samples had POC <10% and AA concentration ≤40 mg g⁻¹. Their δ¹³Corg varied only slightly around -26‰. Additionally, four exceptional samples that were not collected under rainy conditions are included in this cluster: sample 2006-19 was collected in the upstream Wenchang during the dry season, samples 2007-W2 and 2008-W15 were collected in the aquaculture area off Wenjiao, 2008-W6 off the mangrove island in the Wenchang River. These samples had low AA and POC content and elevated SPM concentration relative to near-by samples in common and therefore resemble the rain samples. This was probably caused by dilution from resuspended sediment or lateral material input from the shore. This together with the relatively low salinities led to their allocation with the rain cluster (n=17) although they were not collected during the rainy periods.

Cluster 2 ("summer-bay cluster") mainly consists of samples collected during rainy seasons 2007 and 2008 (pre-typhoon) inside the bay and in the outlet (Fig. 1). Additionally, samples 2006-W13 and 2009 WJ4 and 2009 WC1-1 belong to this cluster. They had intermediate POC and AA and HA contents and a low non-prot. AA Mol % in common and were (apart from the two spring 2009 samples) collected at intermediate salinities (≈10-25). δ¹³Corg was mainly >-24‰ (n=17).

Cluster 3 ("bay-offshore cluster") comprises samples collected during the dry season 2006 inside the bay, during rainy season 2007 and 2008 (pre-typhoon) off the bay, as well as samples collected within the bay and in the offshore region during 2009 (n=17) (Fig. 1). Samples are characterised by lowest POC and AA and HA content and intermediate non-prot. AA Mol%. They were collected at salinities ≈13-35 and typically had a δ¹³Corg >-26‰ which increased with salinity.

Cluster 4 ("river cluster") comprises samples from the upstream sites close to the cities of Wenchang and Wenjiao as well as some samples further downstream the rivers (Fig. 1). These samples were collected in the dry season 2006, the rainy season 2007 and in the pre-typhoon period of 2008 at salinities <10. They are characterised by highest POC and AA concentrations and low non-prot. AA Mol% and show δ¹³Corg mainly <-27‰ (n=14).

From this information, we attribute the following characteristics to the individual clusters: The rain cluster represents a situation when elevated rain falls affect the...
composition and distribution of suspended particulate organic matter, the *summer-bay cluster* is typical for the normal summer rainy season situation without strong episodic rain events. The *bay-offshore cluster* comprises samples from all seasons collected under minor or without influence from land, i.e. samples from offshore sites as well as from the bay during dry winter and spring seasons. Finally, the *river cluster* represents samples collected along the river stretches during any period with the exception of episodic rain events.

![Fig. 4: Grouping of SPM samples by cluster analysis.](image)
3.4.2 Factor analysis

For both factor analyses, two factors were extracted (Tab. 3). The two factors extracted from FA1 accounted for 35.1% and 34.1% of the total variance, respectively, meaning they were equally important for the composition of OM. The first factor was characterised by highest loadings for AA-C% and Glu Mol%, and lowest loadings for non-prot. AA Mol% and the C/N ratio. The second factor showed highest loadings for POC% and AA mg g\textsuperscript{-1} and lowest ones for HA-C%. The first and second factor of FA2 explained 42.3% and 15.5% of the total variance, respectively, which is in the range of results obtained in earlier studies (Dauwe et al., 1999; Ingalls et al., 2003; Unger et al., 2005b). The first factor is characterised by most negative loadings for Asp and Gly, and the non prot.-AA b-ala and g-aba, most positive loadings were observed for Leu, Arg, Phe, Ile and Tyr. Since the site score of this first factor is a well established degradation index (Dauwe and Middelburg, 1998) to evaluate the compositional changes of amino acids during degradation, we refer to it in addition to the non-prot. AA Mol%. For differentiation to the Dauwe DI we denote the site score from our analysis DI\textsuperscript{*}. The loadings for the second factor of FA2 are given in tab. 1 for comparison with literature data but will not be discussed further.

Tab. 3: Factor loadings of factor1 and factor2 for FA1 and FA2 respectively, together with the % of total variance explained by each factor.

<table>
<thead>
<tr>
<th></th>
<th>FA 1 factor 1</th>
<th>FA 1 factor 2</th>
<th>FA 2 factor 1</th>
<th>FA 2 factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA mg g\textsuperscript{-1}</td>
<td>0.493</td>
<td>0.630</td>
<td>GLY</td>
<td>-0.873</td>
</tr>
<tr>
<td>AA-C%</td>
<td>0.922</td>
<td>-0.119</td>
<td>b-ALA</td>
<td>-0.838</td>
</tr>
<tr>
<td>HA-C%</td>
<td>0.172</td>
<td>-0.762</td>
<td>ASP</td>
<td>-0.686</td>
</tr>
<tr>
<td>AA\textsuperscript{HA}</td>
<td>0.152</td>
<td>0.884</td>
<td>g-ABA</td>
<td>-0.650</td>
</tr>
<tr>
<td>non-prot.</td>
<td>-0.818</td>
<td>-0.231</td>
<td>HIS</td>
<td>-0.063</td>
</tr>
<tr>
<td>POC%</td>
<td>-0.192</td>
<td>0.874</td>
<td>SER</td>
<td>-0.012</td>
</tr>
<tr>
<td>C/N</td>
<td>-0.750</td>
<td>0.375</td>
<td>MET</td>
<td>0.364</td>
</tr>
<tr>
<td>GLU</td>
<td>0.625</td>
<td>0.018</td>
<td>GLU</td>
<td>0.384</td>
</tr>
<tr>
<td>VAL</td>
<td>0.580</td>
<td>0.410</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHE</td>
<td>0.756</td>
<td>-0.297</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARG</td>
<td>0.802</td>
<td>0.183</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEU</td>
<td>0.931</td>
<td>-0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% total variation: 35.1% 34.1% 42.3% 15.5%
4. Discussion

4.1 Classification of OM

In order to arrive at a general classification of OM collected in the frame of this study we carried out FA1. The first factor, which accounted for 35.1% of the total variance of the samples, is interpreted as an indicator for the relative proportion of terrestrial OM content in the samples. This conclusion is based on the distinct factor loadings for AA-C% and C/N (Tab. 2). As evident from Fig. 5, AA-C% is negatively correlated with C/N. A C/N ratio between 4 and 10 is considered typical for bacteria and algae, higher C/N ratios of >20 typical for vascular land plants (Hedges et al., 1988; Hedges and Oades, 1997). While the decrease of AA-C% coupled to increasing C/N values observed for SPM can be interpreted as indicator for progressing degradation, the low AA-C values <15% at high C/N values can be attributed to the higher contribution of terrestrial, AA-C poor plant material. In order to further verify our interpretation of the first factor we compared the site scores with the yield of lignin phenols for a subset of samples for which lignin had been measured (Fig. 6). Lignin-derived phenols are unique for vascular plants and represent unequivocal indicators of terrestrial OM in coastal and marine environments (Bao et al., this issue; Bianchi et al., 2002; Goñi et al., 2003; Hernes and Benner, 2006). The lignin contents show a consistent co-variation with the site scores of factor 1 of FA1 ($r^2=0.7$) which supports our interpretation of FA1 factor 1. (Fig. 6) Lignins were most abundant in mangrove leaves and sediments and decreased in SPM in the sequence rain cluster > bay-offshore cluster ≈ river cluster > summer-bay cluster. The low lignin values and high scores for the summer-bay cluster suggest that under moderate discharge conditions during rainy season, the in situ production dominates the OM. In contrast, the higher values for the rain cluster show that high discharge leads to higher terrestrial OM content. In sediments, it appears that terrestrial OM is preferentially preserved, while planktonic OM is recycled. The second factor of FA1 is interpreted as indicator for the content of fresh OM due to high positive loadings for POC% and AA mg g$^{-1}$ and negative loadings for the non-prot. AA Mol%.
Fig. 5: AA-C% plotted against C/N for all analysed samples

Fig. 6: Site scores obtained from the first axis of FA1 (terrestrial vs. aquatic) versus the yield of lignin phenols (lignin mg/100mg OC). BO=bay-offshore cluster, S=summer-bay cluster, R=rain cluster; Ri= river cluster, ML=mangrove leaves, Sed=sediments). A linear regression of the data is shown.

$r^2 = 0.70$
Combining the site scores of FA1 factor1 (terrestrial vs. aquatic) and the independently obtained FA2 factor 1 (DI*; degradation of OM) we derive a comprehensive qualification of all samples with respect to OM composition (Fig. 7). Generally, the freshness of OM increases with increasing contributions of aquatic OM. The only exception from this trend are mangrove leaves which represent fresh and unaltered land-derived OM. Apparently, mangrove leaves did not play a major role in any of the sample groups in the study area, because leaves undergo strong modification before and during transfer into sediments including disintegration, leaching and microbial reworking (Ashton et al., 1999; Tremblay and Benner, 2006; Ziemann et al., 1984) altering their biogeochemical signature. In addition, Bao et al. ((Bao et al., this issue) could show from lignin phenol analysis in a sediment core from the area that with the loss of mangrove coverage during recent decades the direct input of relatively unaltered mangrove OM was to a great extent substituted by the supply of degraded and altered lignin phenols associated with soil-derived SPM. SPM from the river and summer-bay clusters is characterised by fresh aquatic material, which differed only slightly in its composition due to the dominance of fresh in situ-produced OM in both clusters. The relative contribution of OM from freshwater and marine plankton indicated by δ13Corg was not reflected in the FA. The bay-offshore cluster material is more refractory, due to the reduced fresh OM input relative to the summer-bay cluster and a higher contribution from resuspended, degraded sediments. This probably also caused the slightly higher contribution of terrestrial OM in this cluster. A further shift towards terrestrial and degraded material is associated with strong precipitation (rain cluster). This impact becomes even more evident if we examine the data of the post-typhoon samples separately from the complete rain cluster. Soils and sediments are more degraded and have higher contents of terrestrial OM than SPM. Counterintuitive, the FA based classification ranks soils less terrestrial then the aquatic sediments. This could be explained by high microbial reworking of soil OM resulting in relatively low C/N values in soils (Hedges and Oades, 1997) (Tab. 2) and thereby altering the expected typical terrestrial signature. High contents of microbial and algal OM may contribute to the relative freshness of mangrove sediments (Bao et al., this issue).
CHAPTER IV

Fig. 7: Average site scores from the first axis of FA2 (Di*) versus the average site scores from first axis of FA1 (terrestrial vs. aquatic) for the four SPM clusters, post-typhoon SPM samples, sediments, soils, mangrove sediments, and mangrove leaves. Error bars denote the standard deviation for all samples included in each cluster and sample group.

4.2 Variation of suspended particulate matter concentrations

The observed SPM concentrations are at the lower end of published values for small- to mid-scale tropical rivers (Bouillon et al., 2007; Burns et al., 2008; Davies, 2004; Hoover and Mackenzie, 2007; Jennerjahn et al., 2008; Jennerjahn et al., 2004) probably due to the low relief of the drainage basin of the Wenchang and Wenjiao Rivers leading to only limited erosion. Despite the distinct seasonal precipitation pattern, average concentration of SPM did not vary significantly between seasons (dry season 2006: 20.3±9.7 mg L⁻¹; rainy season 2007 and 2008: 22.1±11.8 mg L⁻¹, spring 2009: 25.5±8.8 mg L⁻¹). On the contrary, differences due to short term variation in precipitation as observed during the rainy season 2008 (16.7±5.3 mg L⁻¹ before and 35.4±14 mg L⁻¹ after typhoon rains) were greater than average seasonal changes. We attribute this peculiarity to the small drainage basin size of the two rivers (900 km²) and the distinct short term and small scale variation in precipitation (Fig. 2b), which led to fast fluctuations of SPM with a rapid increase after rains and subsequent decrease of
SPM within days which has been described in detail by (Herbeck et al., 2011). Such a short term variation contrasts the pronounced seasonal pattern of SPM with elevated concentrations during rainy season which has been observed in large drainage basins (Gupta et al., 1997; Ittekkot and Arain, 1986; Ittekkot et al., 1985; Paolini, 1995).

SPM concentration varied along the salinity gradient (Fig. 3a). Excluding the high SPM values of the rain cluster we observed a consistent increase of SPM concentrations with salinity. This is an unusual pattern, if we assume that rivers act as major source of SPM to an estuary. However, the river cluster samples are characterised by lowest SPM concentrations of all samples indicating that the riverine supply is not the dominant source of SPM to the bay except for strong rain events. This suggests an additional input of SPM within the WWE leading to the formation of a maximum turbidity zone, which has been often ascribed to sediment resuspension due to tidal and wind forcing (Chen et al., 2005; Goni et al., 2009; Kessarkar et al., 2009; Ringuet and Mackenzie, 2005). Due to the lack of wind data we cannot assess the role of wind mixing in our study, but considerable tidal variation (Krumme et al., submitted) suggests tidal processes to induce sediment resuspension. Actually, resuspension is evident from elevated SPM concentration in bottom waters (data not shown). It appears to also have an effect on surface SPM especially during the dry season in 2006 and spring 2009 when we observed a strong positive correlation of SPM with salinity (dry 2006: $r^2=0.87$; spring 2009 March Wenchang $r^2=0.82$).

4.3 Sources and composition of organic matter in the rivers and the estuary

4.3.1 Differentiation of organic matter sources

Terrestrial source materials from the study area are mainly characterised by typical signature of C3 plants which have a lower $\delta^{13}C_{org}$ (-22 to -33‰) than C4 plants (-10 to -20‰; (Bender, 1971). Mangroves, that fringe parts of the study area and contribute to the terrestrial OM pool in the area (Bao et al., this issue) by e.g. litter fall, had a lower $\delta^{13}C_{org}$ for leaves (-29.8±0.4‰) than soils and mangrove sediments (Tab.3). The latter can further be differentiated from mangrove leaves by stronger degradation (evident from non-prot. AA Mol% and DI*, Tab. 2), and much lower C/N and AA/HA ratios. The low AA/HA resulted from the preferential degradation of AA, but can additionally be related to the formation of HA rich bacterial biomass. In soils, the enrichment of HA was ascribed to the accumulation of non-living necromass originally derived from microbial sources (Glaser et al., 2004). Significant contribution of bacteria...
to sedimentary OM has also been described (Gupta and Kawahata, 2003; Niggemann and Schubert, 2006) and is supported by the low Gluam/Galam ratio measured in soils and sediments (Tab. 2; Benner and Kaiser, 2003). Freshwater plankton which has a δ\(^{13}\)C\(_{\text{org}}\) between -29 to -32‰ (Martinelli et al., 1999) can be distinguished from terrestrial OM by the typically low C/N values around 6.7 (Redfield et al., 1963).

With a few exceptions, river cluster samples were characterised by δ\(^{13}\)C\(_{\text{org}}\) < -27‰ which in concert with average C/N values of 8.0 (Tab. 2) suggest freshwater plankton rather than terrestrial material as major OM source. This cluster had the highest POC (>10%) and AA concentrations (80-145 mg g\(^{-1}\)) exceeding those from many other rivers worldwide (Chen et al., 2004; Hedges et al., 1994; Jennerjahn et al., 2008; Jennerjahn et al., 2004; Unger et al., 2005a; Zhang et al., 1992). This, together with the observed low non-prot. AA Mol-% which implies little degradation (Ittekkot et al., 1984; Lee and Cronin, 1982), suggests that OM in this cluster is dominated by fresh material from in situ production sustained by high nutrient input from agriculture in the hinterland and municipal sewage from the cities of Wenchang and Wenjiao (Liu et al., 2011). Samples from the summer-bay and bay-offshore cluster had significantly higher δ\(^{13}\)C\(_{\text{org}}\) in common (Tab. 2) reflecting the significance of marine organic matter in the bay and the coast. River cluster OM was generally characterised by lower AA/HA than OM of the summer-bay cluster (Tab. 2). This can be explained by high soil OM content under rain impact. For those river samples which were not affected by rain events, the OM might be influenced by contribution of OM from bacteria that contain elevated amounts of HA in their cell walls (Benner and Kaiser, 2003; Kandler and König, 1978). This is supported by the low Gluam/Galam ratios of 1.9±0.3 hinting also to a bacterial origin of OM (Benner & Kaiser 2003). Bacteria might be associated with organic-rich anthropogenic waste that has been suggested to be a source of AA in the Yangtze River (Wu et al., 2007a). SPM with unusually high δ\(^{13}\)C\(_{\text{org}}\) of -23.8‰ was collected in the vicinity of Wenjiao City during the rainy season 2008. Following the observation by Wu and co-workers (2007a; 2007b) this isotopic signature along with the low C/N value of 6.5 of this sample and the high amount of labile OM can be interpreted as indicator for sewage input which therefore should be considered as potential OM source in the upstream sites of the rivers.

4.3.2 Impact of strong rain events

Strong precipitation led to a distinct increase of SPM concentrations and a reduction of AA concentration to 31.1 mg g\(^{-1}\) and POC of 6.2% in the rain cluster samples (Tab. 2). An inverse trend of SPM and POC concentration and related to
enhanced precipitation and discharge has been observed in other studies, too (Coynel et al., 2005; Gupta et al., 1997). Relative to the river cluster, OM in the rain cluster was characterised by elevated $\delta^{13}$C$_{\text{org}}$ of -26.1‰ that was close to the average of soil samples (-25.5‰; Tab. 2). The lower OM content and AA/HA ratios relative to river cluster samples, together with the high C/N value of 10.0 point to a larger contribution from terrestrial soil sources triggered by the enhanced runoff (Herbeck et al., 2011). Simultaneously, light limitation hampered the in situ production of fresh phytoplankton OM (Herbeck et al., 2011) (Krumme et al., submitted). High non-prot. AA Mol% (Tab. 2) proved that suspended OM collected during rainy periods was degraded. From the degraded nature of the rain cluster samples a lower bioavailability and a higher potential to be transferred into sediments can be inferred (Dauwe et al., 1999). In contrast to refractory soil OM, fresh plankton material that dominated during periods with little or no rain would undergo intense degradation. This might be the reason why the low $\delta^{13}$C$_{\text{org}}$ of river cluster SPM is not transferred into the upstream sediments which had $\delta^{13}$C$_{\text{org}}$ values between -26.3 and -26.9‰. Together with C/N values of 12.4-14.5, the sedimentary $\delta^{13}$C$_{\text{org}}$ points to rain cluster type SPM and soils as major source of sedimentary OM at the river sites.

Detailed inspection of samples included in the rain cluster showed that the magnitude of rain impact on the amount and composition of SPM varied between events (Fig. 8). The strong precipitation associated with the typhoon not only enhanced SPM concentration in the river, but also flushed the whole WWE (Fig. 1i; Herbeck et al., 2011). The much higher salinities in the bay in spring 2009 (Fig. 1, Fig. 8) revealed a lower precipitation impact when compared to the post-typhoon situation 2008. Accordingly, SPM concentration still increased with salinity along the Wenchang transect in spring (March) 2009 (WC1-9) and the associated $\delta^{13}$C$_{\text{org}}$ of $>-22.7$‰ in coastal waters indicated a higher contribution of marine OM relative to the post-typhoon situation (Fig. 8b). In the Wenchang River upstream sites, SPM concentrations were approximately 2-3 times higher than during non-rainy periods (river cluster), but they were still lower than during the post-typhoon situation, indicating only a moderate rain-induced increase of SPM load. POC (Fig. 8c) as well as AA concentrations for the upstream samples in spring 2009 decreased from March to April, and then reached values close to post-typhoon samples. At the same time, non-prot. AA Mol% and the AA/HA ratio decreased, too, reflecting a growing rain impact and associated supply of degraded terrestrial OM to the upstream Wenchang area between the March and April sampling campaigns in 2009. As compared to the immediate impact of the typhoon rains 2008 on the composition of SPM the effect of the initial rains during spring 2009 (Fig. 2b) was delayed. The input of terrestrial OM and its transfer to offshore waters
was reduced probably because soil erosion and the flushing of the estuary had not yet reached the maximum.

Fig. 8: Variation of a) SPM concentration, b) $\delta^{13}$C$_{org}$, and c) POC% of SPM along the salinity gradient for samples taken under rainy conditions (post typhoon 2008, spring 2009: March and April). Dotted line in a) designates the linear regression line for the samples taken in March 2009; dotted line in b) designates the theoretical mixing line for freshwater (salinity 0) and marine SPM (taken at salinity ≥30) with $\delta^{13}$C$_{org}$ -28.15±2.5‰ for freshwater (n=9), and -21.8±0.9‰ marine water (n=6).
4.3.3 Variation of organic matter along the estuary

$\delta^{13}C_{\text{org}}$ of SPM was positively correlated with salinity (Fig. 3f) reflecting the estuarine transition from terrestrial and riverine material to marine sources (Middelburg and Herman, 2007; Middelburg and Nieuwenhuize, 1998; Yu et al., 2010). $\delta^{13}C_{\text{org}}$ of sediments showed the same pattern but varied over a smaller range than SPM and increased gradually from -26.9 to -26.3% at the most upstream sites to -21.2 to -19.5% at coastal sites. The gradient observed for SPM, however, was not evenly distributed along the estuary. Along the pre-typhoon 2008 transect of the Wenchang River towards the bay, for example, we observed a strong gradient in $\delta^{13}C_{\text{org}}$ of >4‰ within the short distance between stations W7 to W8-10 and despite the small change in salinity (from 5.9 to 9.7). This increase was larger than predicted from the theoretical mixing of fluvial and marine endmembers (Fig. 9). We found a comparable pattern also for the rainy season 2007 suggesting that OM from the Wenchang River did not have a strong impact on the composition of OM inside the bay during normal rainy season situation. The separation of river and bay OM was even more pronounced under the dry winter season situation in 2006 when SPM $\delta^{13}C_{\text{org}}$ values were <-27.7‰ in the Wenchang River and of typically marine >-22.7‰ in the bay. Intermediate $\delta^{13}C_{\text{org}}$ values were not observed pointing at two characteristics under low discharge conditions: (i) input of soil OM with $\delta^{13}C_{\text{org}}$ around -25.5‰ was not a major source of OM. This is also corroborated by the low C/N ratios of <8.8 and is consistent with low precipitation rates before and during that sampling period (Fig. 2b). (ii) Despite higher salinities of up to 18 in the river arm during the dry season admixture of marine OM was not evident from the isotopic signature at these intermediate salinities. This hints at a reduced primary production inside the bay relative to summer periods, possibly due to lower nutrient input from aquaculture facilities which are less productive during the cooler winter months (Herbeck et al., this issue). Low production in the bay during winter months is also reflected in the low AA and POC concentrations and the refractory nature of the OM evident from high non-prot. AA Mol% (typical for the bay-offshore cluster).

The separation of river and bay was further corroborated by plankton investigations carried out in spring 2009 which revealed a very specific plankton assemblage in the Wenchang River dominated by chlorophyta and cyanophyta (Maier, 2010). The results of Maier (2010) also implied that the abundant mixotrophic and heterotrophic protist community, which was detected in the bay area, contributed to the consumption of biomass delivered from the river to the bay and hampered its dispersal into the bay.

The concentration of AA in the water (mg L$^{-1}$) (Fig. 3d) did not show a linear decrease along the estuarine gradient. Highest concentrations were observed for the river and
summer-bay cluster (Tab. 2). This can be explained by in situ production of OM, which is favoured by ample nutrients in the river and bay area (Liu et al., 2011) and beneficial light conditions due to low SPM concentrations within the river and in the upper and middle estuary. In contrast, low nutrient concentrations in coastal waters may have been limiting for primary production (Herbeck et al., 2011) thus only allowing for little production of fresh AA in coastal waters. The high concentrations of AA (mg g⁻¹) in the river cluster SPM relative to the summer-bay cluster can be explained by the elevated concentrations of SPM in the summer-bay cluster, which was caused by the resuspension of OM poor sediments and led to the dilution of AA. Similar to AA (mg L⁻¹) (Fig. 3d), the non-prot. AA Mol% (Fig. 3e) did not change systematically along the salinity gradient supporting the high lability of OM for the river cluster and the summer-bay cluster related to the additional production of fresh, organic-rich SPM in the river and inside the bay area (during summer). This implies that the observed reduction of AA (mg g⁻¹) and POC along the estuarine gradient was not caused by degradation but resulted from dilution with OM poor sediment. The input of fresh OM entirely masked the impact of resuspended sediment on OM composition, so that we consider in situ production as the most important POC source during the summer period. This is consistent with the observation of Abril et al. (2002) who reported on high POC content at elevated SPM concentration in the eutrophic Scheldt Estuary due to large contributions of planktonic OM. Within the bay-offshore cluster, however, the lower non-prot. AA Mol% (Fig. 3e) separated the less degraded samples collected during the rainy season (marked by a circle in Fig. 3e) from the more degraded dry season and spring samples. This indicates that during the rainy period, the small amount of OM detected in coastal waters was dominated by fresh OM that had been exported from the bay or generated by low to moderate in situ production. In contrast, offshore samples collected during winter and spring were similarly degraded as the bay samples. This suggests that during winter season, no fresh OM was available in the bay for export to coastal waters, where, in addition, in situ production contributed little to AA content.

Despite the strong flushing, after the typhoon we still observed an increase of AA/HA (8.5 to 12.9), a decrease of non-prot. AA by 0.9 Mol%, and an increase in δ¹³Corg by 1‰ in offshore direction. These gradients suggest that labile marine OM, which prevailed in the bay before the typhoon (summer-bay cluster) was shifted offshore and partly mixed to the degraded SPM delivered to the bay with the flood-river plume.
4.3.4 Source and process-related variation in monomeric composition and applicability of degradation proxies

Monomeric AA composition of different materials is very similar as expected from the ubiquitous occurrence of AA in the living environment (Cowie and Hedges, 1992; Dauwe and Middelburg, 1998; Ittekkot et al., 1983). Nevertheless, we observed systematic variability of monomeric composition between SPM clusters and sediment and terrestrial samples. These differences can mostly be attributed to degradational changes which are known to exert major control on AA composition (Dauwe et al., 1999; Jennerjahn and Ittekkot, 1997; Sheridan et al., 2002; Tab. 1), but did also result from certain source-specific differences. The monomeric composition of mangrove leaves, for example, was characterised by significantly lower Asp and Ala Mol%, but elevated Leu and Phe Mol% as compared to the SPM clusters. For water hyacinths and mangrove leaves, Galam was below the detection limit but both yielded significant g-Aba Mol%. The latter contradicts the expectation that fresh leaves should not contain elevated amounts of non-prot. AA. Actually, b-Ala was present only in traces.

Glu has been found to be generally low in terrestrial material such as vascular plants and soils and SPM from turbid rivers but elevated in fresh estuarine and marine SPM (Cowie and Hedges, 1992; Gaye et al., 2007; Unger et al., 2005a; Unger et al., 2010).
2005b; Wu et al., 2007a). This is consistent with the high Glu Mol% SPM of the summer and bay-offshore cluster which were identified as the clusters with the strongest marine impact, and low Glu Mol% in mangrove leaves and soils (Tab. 1). Interestingly, Glu revealed the same low Mol% in rain and river cluster samples despite the distinct difference in the degradation state of both clusters supporting our assumption that high Glu Mol% have to be attributed to elevated contribution from marine OM and not primarily to the freshness of OM as suggested by e.g. Sheridan and co-workers (2002).

Asp Mol% was lowest in fresh suspended matter and increased in sediments. Highest Asp Mol% of 18-26% were observed along with elevated carbonate contents >50% in coastal sediments as compared to lower Asp Mol% of 9-15% and 0-7% carbonate inside the bay. This supports earlier findings that the preservation and subsequent enrichment of Asp was closely coupled to its association with carbonate matrices (Ittekkot et al., 1984; Jennerjahn and Ittekkot, 1997). However, elevated Asp Mol% has also been found in carbonate-poor but highly degraded material (Lahajnar et al., 2007; Unger et al., 2005a). Degradation appears to be a second process causing the enrichment of Asp as it has also been observed for Gly (Müller et al., 1986; Nguyen and Harvey, 1997; Pantoja and Lee, 2003; Siezen and Mague, 1978). Gly enrichment in the marine environment has been ascribed to the selective preservation in diatom cell walls (Colombo et al., 1998; Gupta and Kawahata, 2000; Hecky et al., 1973). However, we found high Gly contents both in soils and in sediments indicating that its preservation is a general feature inherent in its simple aliphatic structure and its low nutritional value (Cowie and Hedges, 1996; Sigleo and Shultz, 1993) leading to its accumulation in both aquatic and terrestrial environments.

Even small source specific variations of the monomeric composition are of importance because they may lead to erratic interpretation of degradation indicators such as the non-prot. AA Mol% or the Reactivity Index RI (Tyr+Phe Mol%/non-prot. AA Mol%) (Jennerjahn and Ittekkot, 1999). Molar peculiarities were especially evident for fresh green water hyacinth leaves, for which not only RI and non-prot. AA Mol% gave misleading results, but also the DI* indicating strong degradation (Tab. 2). Source related variation of Glu Mol%, and especially the enrichment of Gly from empty diatom shells or soils may compromise the sensitivity of the DI. Misleading results for the DI have been observed for suspended and sinking matter in the Southern Ocean (Ingalls et al., 2003) and sediments along the Chilean coast (Lomstein et al., 2006) as well as for SPM in the Ganges-Brahmaputra estuary (Unger et al., 2005b). It has further been noted that the DI is less sensitive for the early stages of degradation for particulate (Möbius et al., 2011; Unger et al., 2005a) and dissolved AA (Davis et al., 2009).
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The C and N yields of AA have successfully been applied as degradation proxies (Cowie and Hedges, 1994; Lomstein et al., 2006). Recently, Davis et al. (2009) suggested that the AA-C yield is an especially sensitive indicator of early DOM degradation. However, as evident from Fig. 5, AA-C% also varies strongly with the contribution of terrestrial OM. In fact, the correlation of AA-C with any of our degradation proxies is much weaker than with C/N (figures not shown). For the AA-N yields we could not detect any trend with C/N or any of the degradation proxies. In contrast to AA-C (Fig. 5) (Cowie and Hedges, 1994), AA-N% does not vary significantly between terrestrial and aquatic tissues: we obtained average 57.5±4.0 AA-N% for mangroves and water hyacinths, 61.8±11.9 AA-N% for SPM and 51.5±10.2 AA-N% for soils and sediments. In addition, two opposing processes influence the AA-N changes during degradation which when occurring simultaneously in one sample set erase any distinct correlation with degradation proxies. It has been shown that microbial N-immobilization bears the potential to add AA-N to decaying OM (Tremblay and Benner, 2006). The effect of bacterial activity, however, depends on the initial N content of OM. For N-poor terrestrial or degraded marine OM it emerged that exogenous N was added to OM by bacterial re-synthesis of OM, while for N-rich OM organic N was preferentially used or degraded leading to the reduction of AA-N classically interpreted as degradation indicator (Bourgoin and Tremblay, 2010).

Considering our results against this background a combined evaluation of degradation indicators is mandatory to avoid erratic interpretation especially for sample sets receiving a mixture of terrestrial and marine OM and covering a wide range of degradation intensity which is often the case in estuarine and coastal systems.

5. Conclusions

AA and HA, despite being ubiquitous organic compounds in both the terrestrial and aquatic environment proved to be suitable tools to characterise degradation and source-related composition of OM and together with $^{13}$C$_{org}$ and C/N helped to decipher the variable sources, transformation and fate of OM in this small and highly dynamic estuarine system. It is important to note that the multiple OM sources in an estuarine environment require that established degradation proxies are evaluated carefully before application.

Our results revealed that compositional differences of particulate organic matter were primarily driven by the contrasting hydrological and productivity regimes during wet and dry seasons. Fresh material was produced in the rivers and, mainly during
summer, in estuarine bay waters. During strong episodic rain events refractory soil organic matter was introduced into the river and estuarine waters, where it was mixed with autochthonous planktonic OM and sewage material.

From our data it emerged that significant export of river-derived material to the bay and further offshore is restricted to heavy precipitation events, e.g. associated with typhoons. These precipitation events are drivers of the gross SPM fluxes to the coastal sea where seagrass beds and coral reefs may be adversely affected through sedimentation and effects of enhanced OM respiration.

The limited export of fresh OM from the river and the bay to the coastal areas implies that the bay acts as site of OM storage but also as incinerator for OM. A possible mechanism for the latter is the consumption of OM by bacteria, zooplankton and benthic organisms and fish, which in turn are removed from the bay by intensive fisheries/overfishing (Krumme et al., this issue). The storage function of the bay, however, varies with the hydrological regimes and is disrupted by the export associated with episodic strong rain events.

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CHAPTER V

Typhoon-induced precipitation impact on nutrient and suspended matter dynamics of a tropical estuary affected by human activities in Hainan, China

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Typhoon-induced precipitation impact on nutrient and suspended matter dynamics of a tropical estuary affected by human activities in Hainan, China

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Abstract

Typhoons regularly hit the coasts along the northern South China Sea during summer monsoon. However, little is known on the effects of typhoon-related heavy precipitation on estuarine dynamics and coastal ecosystems. We analyzed physico-chemical characteristics, and concentrations and composition of dissolved and suspended matter in the Wenchang/Wenjiao Estuary (WWE) on the tropical island of Hainan, China, prior to and after typhoon Kammuri in August 2008. Before the typhoon, the estuary displayed vertical and horizontal gradients. High nutrient inputs from agriculture and widespread aquaculture were to a large extent converted into biomass inside the estuarine lagoon resulting in low export of nutrients to coastal waters and a mainly autochthonous origin of total suspended matter (TSM). Heavy typhoon-associated precipitation increased river runoff, which moved the location of the estuarine salinity gradient seaward. It resulted in an export of dissolved and particulate matter to coastal waters one day after the typhoon. Dissolved nutrients increased by up to an order of magnitude and TSM increased approximately twofold compared to pre-typhoon values. Lower δ13Corg and δ15N and elevated C/N ratios of TSM together with lower chlorophyll a (chl a) concentrations indicated an increased contribution of terrestrial material originating from typhoon-induced soil erosion. Local uptake of excess nutrients inside the lagoon was inhibited because of reduced water transparency and the lack of phytoplankton, which had been washed out by the initial freshwater pulse. Two weeks after the typhoon, TSM concentration and composition had almost returned to pre-typhoon conditions. However, physico-chemical properties and nutrients were still different from pre-typhoon conditions indicating that the
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An estuarine system had not fully recovered. Unusually high chl a concentrations in the coastal zone indicated a phytoplankton bloom resulting from the typhoon-induced nutrient export. The typhoon-induced flushing of the WWE resulted in hyposalinity, reduced water transparency, siltation, as well as temporary eutrophication of coastal waters. These are physiological stressors, which are known to impair the performance of adjacent seagrass meadows and coral reefs. The predicted increase in typhoon frequency and intensity will lead to a frequently recurring exposure of coastal ecosystems to these threats, particularly in the South China Sea region where aquaculture is widespread and tropical cyclone frequency is at a maximum.

**Key words:** Typhoon Kammuri, precipitation runoff, stable isotopes, eutrophication, Wenchang/Wenjiao River Estuary, South China Sea

1. Introduction

Tropical storms, also known as typhoons, hurricanes and cyclones, are among the most extreme episodic weather events affecting wetlands and adjacent marine and coastal areas in low latitudes. Usually, tropical storms are accompanied by heavy precipitation, which can occur even several hundreds of kilometers away from the storm’s center. Their strength and frequency are predicted to increase in the context of global climate change (Goldenberg et al., 2001; Emanuel, 2005; Webster et al., 2005; Wu et al., 2005; Knutson et al., 2010).

Effects of tropical storms on estuaries and nearshore coastal systems remain poorly studied, partly because of the unpredictability of storm formation and tracks, which make it difficult to arrange for appropriate pre- and post-storm sampling. Additionally, harsh weather conditions often make timely sampling difficult, especially as some effects may already dissipate after a few days (e.g. Valiela et al., 1998). Existing studies have mostly been conducted in large temperate and subtropical estuaries and nearshore coastal areas in highly developed regions (mainly on hurricane events in the USA, e.g. Pamlico Sound, Biscayne Bay), where comprehensive background data exist. These studies reported on changes in salinity, nutrient supply and primary productivity, as well as on modifications of TSM load and composition driven by storm precipitation (Valiela et al., 1998; Pael et al., 2001, 2006a; Peierls et al., 2003; Burkholder et al., 2004; Wetz and Pael, 2008; Piazza and La Peyre, 2009; Zhang et al., 2009). Storm-related perturbations have been found to intensify with the amount of rainfall, as well as with land use intensity and population...
density of the drainage basin (Valiela et al., 1998; Mallin et al., 1999; Paerl et al., 2001, 2006a, 2006b; Mallin and Corbett, 2006). The impact and persistence of such episodic events in estuarine and coastal systems, however, has to be evaluated locally, as they depend on drainage basin size, land use, estuary size and shape, water residence time, tidal effects etc (e.g. Paerl et al., 2006a; Wolanski, 2007).

With an average of 26.9 ±4.3 tropical storms per year, the tropical/subtropical Western Pacific is the most active typhoon basin of the world (Fink and Speth, 1998; Wang et al., 2010). Studies from this region mostly relate to typhoon effects in offshore waters, where phytoplankton blooms were observed as a consequence of wind-driven upwelling and mixing, as well as assumed nutrient input from rivers (Shiah et al., 2000; Lin et al., 2003; Zheng and Tang, 2007; Zhao et al., 2009; Sun et al., 2010). Knowledge from the pacific region on the effects of typhoons on river, estuarine and nearshore coastal dynamics is mainly limited to Taiwan (e.g. Kao and Liu, 1996; Chen et al., 2001; Milliman and Kao, 2005; Milliman et al., 2007; Goldsmith et al., 2008; Hilton et al., 2008), while almost nothing is known on typhoon effects on coastal tropical habitats, such as mangroves, seagrass meadows and coral reefs (Victor et al., 2006; Wolanski et al., 2003). These tropical regions often experience particularly intense anthropogenic stress from high population density and ongoing land use change, which is projected to further increase in the future (Seitzinger et al., 2002). As in many other parts of tropical SE Asia, mangroves in China have been replaced to a large extent by paddy fields and aquaculture ponds (Li and Lee, 1997). These and other activities contribute to eutrophication, one of the most problematic anthropogenic impacts on estuarine and coastal waters worldwide (Turner and Rabalais, 1994; Vitousek et al., 1997; Justic et al., 2002; Diaz and Rosenberg, 2008). Resulting adverse effects, such as reduced water transparency, formation of hypoxia, altered nutrient ratios and cycling, as well as toxic algal blooms, contribute to the degradation of coastal habitats (Vitousek et al., 1997; Rabalais and Gilbert, 2008). Increased sediment input caused by soil erosion from deforested watersheds threatens estuarine and coastal water quality and habitats. This is further accelerated by the loss of mangrove forests (e.g. Wattayakorn et al., 1990; Alongi, 2002; Victor et al., 2004, 2006; Thampanya et al., 2006; Wolanski, 2007).

Here, we report on the effects of a typhoon on the biogeochemistry of the tropical Wenchang/Wenjiao Estuary (WWE) and its adjacent coastal zone at the east coast of Hainan, northern South China Sea. The region is the major landfall corridor of typhoons in Hainan (Zeng and Zeng, 1989) and has experienced intense land use changes in its watershed, most notably the conversion of mangroves into aquaculture ponds. We compare sources and processes affecting nutrients and suspended matter.
along the estuary during two weeks before and two weeks after typhoon-induced heavy precipitation and evaluate the potential impact on nearby seagrass meadows and fringing coral reefs.

2. Materials and methods

2.1. Study area

Hainan is the largest Chinese island in the South China Sea, located in the marginal tropics (Fig. 1a). The Wenchang/Wenjiao Estuary (WWE) is situated at Hainan’s east coast (19°36’ N, 110°49’ E) and comprises an area of approximately 40 km² (Fig. 1b, c). The lowland rivers Wenchang and Wenjiao enter the shallow (mean depth: 3 m), kidney-shaped lagoon Bamen Bay, which is connected to the sea via a narrow natural channel (maximum depth: 10 m; Fig. 1c). The estuary is subject to mixed semidiurnal microtides. The tidal range at spring and neap tides is around 1.5 m and 0.5, respectively (Fig. 2). Hence, we can expect stronger tidal mixing during spring tides. Neap tides are semidiurnal, whereas spring tides are mixed semidiurnal (Fig. 2). In the lagoon the currents are clearly bi-directional, upstream at flood and downstream at ebb tides. At spring tides, flood current speeds reached a maximum of 0.3 m s⁻¹, while ebb tide current speeds could exceed 0.6 m s⁻¹, so that the system is ebb-dominated during this period (Ye, 1988; Krumme et al., under review). There are no reliable data on water discharge of the estuary available. Krumme et al. (under review) estimated a water residence time of 5.6 days using the empirical formula of Uncles et al. (2002).
The WWE can be divided into three zones: the upper estuary, including the lower reaches of the rivers Wenchang and Wenjiao, the middle estuary, covering the lagoon and the channel, and the outer estuary, which is located in the nearshore coastal zone (Fig. 1c). The watersheds of the rivers Wenchang and Wenjiao are characterized by mixed agriculture dominated by rice, coconut palm and fruit cultures (mainly C3 plants). The Wenchang River passes the county capital Wenchang (55,800 inhabitants in 2009; Fig. 1) and enters the lagoon in the north-west. Since the Wenjiao River at the north-east of the lagoon is dammed, the Wenchang brings the major freshwater input, as well as untreated urban effluents and sediments into the system. 73% of the fringing riverine mangrove has been lost since the 1960s (Krumme et al., MFR under review) and has been replaced by aquaculture ponds with semi-intensive and intensive shrimp and fish culture, which now cover approximately 15 km² (~35%) of the lagoon (Fig. 1c). Despite some mangrove remnants that fringe the pond complexes on their seaward edge, the former mangrove area has lost its ecological function as filter and sediment trap. Instead, the area has become a major source of untreated effluents, released into the estuary from aquaculture ponds. On top of that,
feces and excess feed are discharged from hundreds of floating net cages containing live fishes and invertebrates that are mainly lined up opposite of Qinglan harbor. In the outer estuarine zone, fringing coral reefs occur in a few hundred meters offshore and seagrass meadows are found in their back-reef areas (Fig. 1c).

The region is characterized by a tropical monsoon climate with a dry season from November to April and a wet season from May to October (Fig 2a). The annual average air temperature is 22-26 °C, the coldest monthly average air temperature is 15.3 °C (Wang, 2002). Between 1959 and 2000, Hainan Island was impacted by an annual mean of 7.9 typhoons and directly hit by 2.6 typhoons (Liu, 1984 cited in Huang, 2003). The north-eastern Wenchang-Haikou region has the highest frequency of typhoon landfall incidents on the island (Zeng and Zeng, 1989), where typhoon-induced rainfall accounts for 35-60% (>700mm) of the total annual precipitation of 1500-2000 mm (Huang, 2003; Wang et al., 2008). Typhoon activity on the island lasts from June to November with the peak frequency between July and September, when extreme rainfall alternates with dry periods of several weeks (Fig 2a; Zeng and Zeng, 1989; Wang et al., 2010).
Fig. 2: (a) Daily precipitation at the weather station Haikou from 2000 to 2008; (b) zoom-in of the sampling time in July/August 2008: daily precipitation at the weather stations in Haikou and Qionghai (upper panel) and time series of tidal changes in water level (lower panel) in the WWE during the pre- and post-typhoon Kammuri phase (for location of station names see Fig. 1c). Data of precipitation from Climate Center, Utah State University, http://climate.usurf.usu.edu/products/data.php?tab=gsod (locations of weather stations see Fig. 1b); water level data from tidal calendar predictions for Qinglan (real water levels were approximately 1-2 m higher during the typhoon).
2.2. Typhoon Kammuri

Kammuri developed as a tropical depression in the northern South China Sea north of the Philippine island of Luzon on August 4, 2008 (Fig. 1a). During its passage towards mainland China, Kammuri developed into a severe tropical storm as categorized by the Hong Kong Observatory (HKO) and the Japan Meteorological Agency (JMA) in the late evening of August 5. Kammuri made landfall along the South coast of China in the Western Guangdong Province (close to Hong Kong) at about 12 pm UTC on August 6. It passed the study area in a distance of approximately 200km in the night to August 7 before it moved into the Gulf of Tonkin. It made landfall for a second time in the Guangxi Province, China, before it dissipated on August 8. The maximum radius of gale wind was 440 km with maximum sustained wind speeds of 95 km h\(^{-1}\) (KITAMOTO Asanobu, 2010). Although, according to the Saffir-Simpson Hurricane Scale and the storm scale of Japan Meterological Agency, Kammuri was classified as a severe tropical storm, the denomination ‘typhoon Kammuri’ was commonly used. Therefore, we will also use the term ‘typhoon’.

No precipitation data are available for the Wenchang region, but our observations showed that Kammuri caused a first peak of heavy rain on August 6, followed by a second peak on August 9. Precipitation records from weather stations in Haikou and Qionghai (Fig. 1b) reveal that heavy precipitation of 40-145 mm per day were a widespread phenomenon during the passage of Kammuri (Fig. 2b). As typhoon Kammuri passed the study area in quite a distance, heavy precipitation was expected to be the major pressure on the system, while wind-driven effects were of minor importance (own observations).

2.3. Sampling design

Sampling in the WWE took place in July/August 2008 with two sampling campaigns before and two after the passage of typhoon Kammuri (Fig. 2b). Sampling focused on the western part of the lagoon and the lower reaches of the Wenchang River (Fig. 1c). The Wenjiao River is dammed, which results in negligible river discharge. Sampling at neap tides (13 days before and 1 day after the typhoon) included collection of surface water, suspended matter and physico-chemical measurements by boat and was carried out along the WWE at 10 stations before and 6 stations after the typhoon (Fig. 1c). Additionally, bottom water was collected at some stations in the middle and outer estuary, where water depth was ≥3 m. Both neap tide cruises were conducted at similar tide levels between low and high tide (55-90 cm). Sampling at spring tides (4-9 days before and 7-12 days after the typhoon) was conducted at three fixed stations along the WWE (Fig. 1, 2): stations A (upper estuary),
B (middle estuary) and C (outer estuary). Each station was sampled over a 24 h period. Physico-chemical parameters were measured in situ and samples for the analysis of dissolved and suspended matter of surface water were taken every three hours from an anchored boat, yielding n = 8 per parameter per tidal cycle. In addition to spring and neap tide sampling campaigns, water, suspended matter, sediment, soil and plant samples were collected before the typhoon at different locations along the estuary and the watershed.

2.4. Data collection and sample preparation

Salinity (±0.1), water temperature (±0.1 °C) and pH (±0.1) were measured in situ with a WTW MultiLine F/Set3 multi-parameter probe and dissolved oxygen (DO; ±0.02 mg l⁻¹) was measured with a LDO™ HQ40d portable dissolved oxygen meter. Vertical Secchi depth readings were taken during daylight hours (06:00-18:00). Water samples were collected with a bucket and a Niskin bottle for surface and bottom water, respectively. Water samples for nutrient analyses were filtered immediately after sampling through single use Sartorius Minisart ® membrane filters (0.45 μm pore size) into PE bottles, which were rinsed three times with the filtered sampling water beforehand. Samples were preserved with a mercury chloride solution (50 μl of a 20 g l⁻¹ HgCl₂-solution added to 100 ml sample) and stored cool and dark until analysis.

Water samples for the analysis of total suspended matter (TSM) were stored in PE tanks until filtration in the laboratory the same day. Samples were filtered under constant pressure onto pre-combusted (5 h, 450°C) and pre-weighed Whatman GF/F filters and dried at 40°C. For chlorophyll a (chl a) analysis, water was filtered onto GF/F filters, which were stored frozen until analysis within the following days. Samples of surface sediments and soils were collected directly with a spoon, where possible, or with a grab sampler and stored cool in plastic bags or glass vials. Each plant sample consisted of 2-10 fresh leaves of a plant, which were rinsed and stored in plastic bags. All sediment, soil and plant samples were dried in a drying oven at 40°C within the same day after collection.

2.5. Analyses

Dissolved nutrients were analyzed using a continuous flow analyzing system (Skalar SAN™ System). Nitrate+Nitrite (NOₓ⁻), nitrite (NO₂⁻), phosphate (PO₄³⁻) and silicate (Si(OH)₄) were detected spectrophotometrically and ammonium (NH₄⁺) fluorometrically as colored/fluorescence dye (Grasshoff et al., 1999). Nitrate (NO₃⁻) is NOₓ⁻ - NO₂⁻. Dissolved inorganic nitrogen (DIN) is the sum of NO₃⁻, NO₂⁻ and NH₄⁺. Daily equidistant 10 point calibrations for the necessary concentration ranges were
established. Detection limits of the procedure were 0.03 μM, 0.01 μM, 0.02 μM, and 0.06 μM for NO\textsubscript{x}, NO\textsubscript{2}, NH\textsubscript{4}, PO\textsubscript{4}\textsuperscript{3-}, and Si(OH)\textsubscript{4}, respectively; determination limits were 0.08 μM, 0.04 μM, 0.06 μM, 0.07 μM and 0.19 μM, respectively, according to DIN 32645 (German Institute of Standardization). The coefficient of variation of the procedure was <3.4%. For chlorophyll \textit{a} (chl \textit{a}) determination, pigments were extracted from the filters in 10 ml 90% acetone at 4°C in the dark for approximately 24 hours and vials were shaken once a while. Extracts were centrifuged at 60 rpm for three minutes. The supernatant was measured with a Lovibond PC Spectro 1.0 photometer (Tintometer GmbH) and chl \textit{a} concentrations were calculated according to the method of Lorenzen (1967). Concentrations of total suspended matter (TSM) were determined by weighing the dried filter, subtracting the original weight of the empty filter and dividing it by the respective volume of water filtered. Values given are the average of 3-5 filters. TSM on GF/F filters was analyzed for total carbon (C\textsubscript{tot}) and total nitrogen (TN) by high-temperature combustion in a Carlo Erba NA 2100 elemental analyzer (Verardo et al., 1990). Organic carbon (C\textsubscript{org}) was determined the same way after removal of carbonate by acidification with 1N HCl and subsequent drying at 40 °C. Measurements had a precision of 0.06% for C\textsubscript{org} and 0.02% for TN, based on repeated measurements of a standard (LECO 1012). The nitrogen isotope composition (δ\textsuperscript{15}N) was determined with a Thermo Finnigan Delta Plus gas isotope ratio mass spectrometer after high-temperature combustion in a Flash 1112 EA elemental analyzer. The carbon isotope composition (δ\textsuperscript{13}C\textsubscript{org}) was determined similarly after removal of carbonates by adding 1N HCl and subsequent drying at 40 °C. δ\textsuperscript{13}C\textsubscript{org} and δ\textsuperscript{15}N are given as ‰-deviation from the carbon isotope composition of the PDB standard and the nitrogen isotope composition of atmospheric air, respectively. The precisions of the method, measured by an internal standard, were 0.20‰ (δ\textsuperscript{15}N) and 0.14‰ (δ\textsuperscript{13}C\textsubscript{org}). Dried sediment, soil and plant material was analyzed for C\textsubscript{org}, TN, δ\textsuperscript{13}C\textsubscript{org} and δ\textsuperscript{15}N contents following the same procedure as described for filters after homogenizing and grinding to a fine powder in a planet mill. For each spring tide station, means and standard deviations of the 8 measurements were calculated and water parameters were tested for differences between pre- and post-typhoon conditions using the t-test for independent samples by groups (p<0.05; STATISTICA 9).
3. Results

3.1 Physico-chemical characteristics

Before the typhoon, the salinity gradient was mainly located within the middle estuary with brackish water reaching up to station 2/A in the Wenchang River (upper estuary; Fig. 3a; Tab. 1). Only at station 1 during neap tide, surface water salinity was 0. Bottom water salinities were always higher than surface salinities, indicating water column stratification along the estuary. During spring tide, surface water salinities were higher than during neap tide and differences between surface and bottom water salinities were smaller (Fig. 3a). Surface water temperature, pH, DO and Secchi depth showed a clear trend along the estuarine gradient and hardly varied for neap and spring tide (Tab. 1). High standard deviations of most parameters at stations A-C reflected large tide-induced changes during 24 hr sampling at spring tide, especially in the upper estuary. Here, DO values, which were relatively high in the middle and outer estuary (>6.5), decreased to up to 2.7 mg L⁻¹ during the night.

One day after landfall of typhoon Kammuri (August 8, see Fig. 2b) during neap tide, surface water salinity had dropped to values <3 throughout the upper and middle estuary, while in the outer estuary, surface salinity was 7.5 compared to 20 before the typhoon (Fig 3b). Estuarine stratification still persisted but bottom water salinities were lower than under pre-typhoon conditions (Fig. 3) indicating that tidal mixing was minimal and the entire estuary was dominated by freshwater. Surface water temperature, pH, DO and Secchi depth were considerably lower at all stations immediately after the typhoon and the variability in water parameters from the river to the coast was less pronounced (Tab. 1).

Spring tide measurements at stations A-C showed that one to two weeks after the typhoon (August 14-19, see Fig. 2b), salinity, water temperature, pH, DO and Secchi depth had started to rise again compared to immediately after the typhoon at most of the stations (Fig. 3b; Tab. 1). However, salinity was still significantly lower compared to pre-typhoon spring tide conditions and also average temperature and Secchi depths were below pre-typhoon values. Average pH and DO in the middle and outer estuary were elevated compared to pre-typhoon values during spring tide. A very low variability of salinity at station A as compared to before the typhoon indicates that the upper estuary was still dominated by freshwater during most of the spring tidal cycle, while large standard deviations at stations B and C reflect tidal mixing in the lower parts of the estuary (Fig. 3; Tab. 1).
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Fig. 3: Surface and bottom water salinities along the WWE under (a) neap and spring tide pre-typhoon and (b) neap and spring tide post-typhoon conditions. Spring tide samples with mean and standard deviation (n = 8).

Tab. 1: Physico-chemical characteristics of the surface water in the upper, middle and outer regions of the WWE under pre- and post-typhoon conditions during neap and spring tide. Spring tide samples with mean and standard deviation (n=8).

<table>
<thead>
<tr>
<th>Pre/post typhoon</th>
<th>upper estuary</th>
<th>middle estuary</th>
<th>outer estuary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>post</td>
<td>pre</td>
</tr>
<tr>
<td>Neap tide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Station</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.0*</td>
<td>0.1*</td>
<td>15.5</td>
</tr>
<tr>
<td>Temperature [°C]</td>
<td>33.7</td>
<td>28.1*</td>
<td>31.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>7.8*</td>
<td>8.5</td>
</tr>
<tr>
<td>DO [mg L⁻¹]</td>
<td>6.7</td>
<td>5.7*</td>
<td>8.5</td>
</tr>
<tr>
<td>Secchi depth [m]</td>
<td>0.7</td>
<td>0.3*</td>
<td>1.0</td>
</tr>
<tr>
<td>Spring tide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Station</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Salinity</td>
<td>9.2 (±3.7)</td>
<td>0.2 (±0.4)</td>
<td>22.4 (±3.8)*</td>
</tr>
<tr>
<td>Temperature [°C]</td>
<td>31.0 (±0.9)</td>
<td>30.7 (±0.8)</td>
<td>31.4 (±0.8)</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 (±0.4)*</td>
<td>5.9 (±0.7)</td>
<td>8.1 (±0.1)*</td>
</tr>
<tr>
<td>DO [mg L⁻¹]</td>
<td>4.5 (±2.0)</td>
<td>5.5 (±0.4)</td>
<td>7.5 (±0.8)*</td>
</tr>
<tr>
<td>Secchi depth [m]</td>
<td>1.0 (±0.2)</td>
<td>0.7 (±0.2)</td>
<td>1.1 (±0.2)</td>
</tr>
</tbody>
</table>

* values are significantly different (t-test for independent samples by group; p<0.05)
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3.2 Dissolved inorganic nutrients

Before typhoon Kammuri, all dissolved nutrients decreased along the salinity gradient during neap and spring tide resulting in low concentrations in offshore direction to the outlet (Fig 4). Nitrate and silicate decreased sharply with increasing salinity, while there was an increase in nitrite at salinities around 5 and in ammonium at salinities between 10 and 17. In the upper estuary, nitrate dominated the DIN pool, while in the middle (and outer) estuary strong nitrate removal along the estuary accompanied with partial ammonium increases resulted in ammonium dominance in the DIN pool (Fig. 5). Ammonium concentrations above the mixing line at station A indicate temporarily high N input into the estuary causing ammonium dominance in the DIN pool during spring tide (Figs. 4, 5).

One day after the typhoon, all nutrients decreased very little along the estuary and passed the outlet in much higher concentrations than before the typhoon (Fig 4). Ammonium, nitrite and silicate were lower in the upper estuary compared to pre-typhoon conditions. In contrast, nitrate and phosphate concentrations in the upper estuary were greater than before the typhoon and only decreased slightly with increasing salinity. This resulted in an estuary-wide shift from ammonium to nitrate dominance in the DIN pool (Fig. 5), which was slightly attenuated toward the outer estuary because of higher ammonium concentrations with salinity (Fig. 4).

7-12 days after the typhoon, silicate and nitrite in the upper estuary were higher compared to directly after the typhoon (Fig. 4). Ammonium concentrations in the upper estuary were still similar to directly after the typhoon. In the middle and outer estuary, ammonium concentrations were low indicating effective removal in the lagoon. The few exceptions suggest local ammonium input into the lagoon. Phosphate concentrations in the upper estuary still exceeded pre-typhoon concentrations, but were lower compared to directly after the typhoon and decreased with increasing salinity. Nitrate levels in the upper estuary were still greater than before the typhoon and decreased less from station A to C compared to before the typhoon, resulting in comparatively high concentrations throughout the estuary, export of nitrate into the coast and prevailing nitrate dominance in the DIN pool in the upper and middle estuary (Fig. 5).
Fig. 4: Surface water concentrations of dissolved inorganic nutrients along the WWE under neap and spring tide pre-typhoon (left column) and neap and spring tide post-typhoon conditions (right column). The end-member mixing line indicates nutrient concentrations expected from conservative mixing of fresh and marine water masses with nutrient concentrations given in Tab. 2. Vertical dotted and dashed lines denote salinity at the outlet during neap and spring tide, respectively. Pre-typhoon phosphate concentrations during spring tide could not be determined due to sample loss.
3.3 Suspended particulate matter concentration and composition

Under pre-typhoon conditions (neap and spring tide), TSM concentrations ranged between 8 and 43 mg l\(^{-1}\) with a TSM maximum in the middle estuary (Fig. 6a). Chl a concentrations were especially high in the middle estuary during neap tide (11-27 μg l\(^{-1}\)), while during spring tide concentrations were considerably lower (0-15 μg l\(^{-1}\)) throughout the estuary with a distinct offshore decrease (Fig. 6b). Pre-typhoon organic matter (OM) contents of the TSM at neap and spring tide were high in the upper estuary and decreased towards the coast: At neap tide, C\(_{org}\) and TN contents decreased from 20.9% to 5.4% and from 1.7% to 0.9%, respectively. Average spring tide C\(_{org}\) and TN contents were lower, decreasing from 7.6% to 3.5% and from 1.3% to 0.4% from station A to C, respectively. The C/N ratio varied from 14.0 in the upper to 5.4-9.9 in the middle and outer estuary, while average spring tide C/N ratios were more homogenous along the estuary (6.3-7.9). δ\(^{13}\)C\(_{org}\) and δ\(^{15}\)N values increased abruptly
downstream of station 1 and revealed the same pattern during neap and spring tide (Fig. 6c, d).

One day after Kammuri (at neap tide), TSM concentrations had increased two-to three-fold at all stations to values between 21 and 58 mg l$^{-1}$ (Fig. 6a), while chl $a$ concentrations had dropped to values between 5 and 10 μg l$^{-1}$ (Fig. 6b). C$_{org}$ and TN contents were lower as compared to pre-typhoon contents at all stations (range: 4.8-6.2% and 0.4-0.8%, respectively). C/N ratios were higher in the middle and outer estuary (between 8 and 16), whereas in the upper estuary C/N ratios decreased from 14 to 12.4. $\delta^{13}$C$_{org}$ and $\delta^{15}$N of TSM were higher in the upper estuary and lower in the middle and outer estuary as compared to before the typhoon, so that they were virtually uniform (approximately -26‰ and 4‰, respectively) throughout the estuary (Fig. 6 c, d).

One to two weeks after the typhoon (at spring tide), TSM concentrations had decreased to below pre-typhoon spring tide levels in the upper and middle estuary, while in the outer estuary, TSM concentrations were greater than before the typhoon (Fig. 6a). Chl $a$ concentrations remained low in the upper estuary, while they were elevated 3- and 15-fold on average compared to pre-typhoon spring tide concentrations in the middle and outer estuary, respectively (Fig. 6b). In the middle and outer estuary, contents of C$_{org}$ (11.0±3.0 and 6.7±1.6) and TN (1.9±0.4 and 1.1±0.3) were significantly higher compared to pre-typhoon spring tide values. Average C/N ratios were in the same range as before the typhoon in the middle (7.4±0.8) and outer estuary (6.9±0.6) and significantly higher compared to before Kammuri in the upper estuary (8.9±0.6). $\delta^{13}$C$_{org}$ and $\delta^{15}$N of TSM had returned to pre-typhoon values (Fig. 6c, d) except station A, where TSM was still significantly enriched in $^{15}$N as compared to before the typhoon (p<0.05).
Fig. 6: Concentration of (a) total suspended matter (TSM), (b) chlorophyll a (chl a), (c) $\delta^{13}$Corg-TSM and (d) $\delta^{15}$N-TSM along the WWE under neap and spring tide pre-typhoon (left column) and neap and spring tide post-typhoon conditions (right column). Spring tide samples with mean and standard deviation (n=8).
4. Discussion

4.1 Sources and distribution of dissolved and particulate matter before the typhoon

Under 'normal' summer monsoon conditions (pre-typhoon), the distribution of dissolved and particulate matter in the WWE was dominated by the interaction of freshwater discharge and tide-mediated variations in vertical and horizontal mixing with marine water (Fig. 3). High nutrient and particulate organic matter concentrations as well as low $\delta^{13}$C$_{org}$ and $\delta^{15}$N of TSM (Fig. 7a) distinguished sources and biogeochemical composition of dissolved and particulate components in the freshwater-dominated upper estuary from the area downstream. High chl $a$ concentrations, C$_{org}$ and TN contents of TSM, and a very low $\delta^{13}$C$_{org}$ value of up to -32‰ indicative of freshwater phytoplankton (Martinelli et al., 1999) point towards autochthonous riverine production as primary organic matter source at station 1. A C/N ratio of 14 in combination with the occurrence of plant detritus in this sample suggest TSM at this station to mainly consist of a mixture of freshwater phytoplankton and vascular plants (e.g. limnic macrophytes, such as water hyacinths, and/or mangroves; Tab. 2). During neap tide, the upper estuary had a stronger fluvial/terrestrial influence (low salinity, high organic matter, high C/N ratio), while greater tidal mixing during spring tide caused a mixture of riverine TSM with TSM originating from the middle estuary (see below). C isotopes cannot tell us the origin of TSM in a definite matter but the higher mean $\delta^{13}$C$_{org}$ in combination with a lower C/N ratio close to the Redfield ratio at station A compared to station 2 are a strong indicator for a greater portion of marine phytoplankton (typical $\delta^{13}$C$_{org}$ of -18‰ to -22‰ in tropical regions; e.g. Fischer, 1991) in the TSM of the upper estuary during spring tide. The comparatively low $\delta^{15}$N of TSM at station 1 was most likely linked to sources and processes of dissolved nitrogen in the upper catchment, as N-Input from in situ N$_2$-fixation by phytoplankton as source of isotopically light N can be excluded since cyanobacterial biomass was consistently low in water samples from station 1 (D. Maier, pers. comm.). Possible processes resulting in such low $\delta^{15}$N of TSM are strong fractionation by primary producers during N-uptake due to very high DIN concentrations (Cifuentes et al., 1988; Montoya et al., 1991; Montoya and McCarthy, 1995) and/or uptake of light nitrogen originating e.g. from Haber-process produced chemical fertilizers applied in the agriculture. Although, the N sources and processes cannot easily be separated using N isotopes alone, our data suggest that high nitrate concentrations may arise from input of agricultural fertilizers from the watershed, which are usually leached from soils in the form of nitrate (Mian et al., 2009 and references therein) and typically have a $\delta^{15}$N of -2 to 2‰ (e.g. Lee et al.,
Untreated municipal sewage from Wenchang City is another potential source of dissolved nutrients and particulate organic matter, but this should be enriched in $\delta^{15}N$ (Costanzo et al., 2001; Lee et al., 2008), which is not reflected in the observed low $\delta^{15}N$ values of TSM. Porewater inputs from the mangrove-fringed tidal flats (Tab. 2) may also contribute to the nutrient inventory of the upper estuary, as it has been shown from other regions (e.g. Dittmar and Lara, 2001; Akamatsu et al., 2009). High $C_{org}$ and TN contents in TSM and sediments and temporarily low DO concentrations suggest high remineralization rates of OM in the upper estuary. Remineralized N in porewater may potentially have low $\delta^{15}N$ values because mangroves typically have a significant amount of $N_2$ fixation associated with them (Holguin et al., 2001). Nitrite concentrations in the surface water above the mixing line at salinities around 5 are indicative for nitrification processes, which could also be responsible for the allocation of isotopically light nitrate. Denitrification can be neglected as an important process because DO concentrations measured were high throughout the estuarine transect. Nitrification in addition to nutrient uptake by phytoplankton and mangroves may also result in a greater removal of ammonium compared to nitrate, phosphate and silicate along the upper estuary (Fig. 4). High inputs of ammonium at station A may be related to elevated porewater export from larger inundated intertidal areas during spring tide (Dittmar and Lara, 2001) and/or local inputs of effluents from aquaculture ponds (Tab. 2) observed during sampling. Due to the higher contribution of nitrate to the DIN pool in the upper estuary compared to the middle and outer estuary (Fig. 5) and the very low $\delta^{15}N$ of the TSM at station 1, it is conceivable that a combination of fertilizers leached from agriculture soils and nitrified ammonium from organic matter regeneration in the sediment (some of it potentially coming from $N_2$-fixers in mangroves) were the main DIN sources in the upper estuary. Further studies are needed to clarify the relative relevance of each process.

In the middle and outer estuary, high $C_{org}$ and TN values and a C/N ratio in the range of the Redfield ratio in combination with $\delta^{13}C_{org}$ values between -22 and -24‰ indicate TSM to be mainly of planktonic origin dominated by marine phytoplankton (Tab. 2; Fischer et al. 1991). The higher abundance of ciliates and other zooplankton at station 4 compared to all other sites (D. Maier, pers. comm.) suggests that planktonic TSM delivered by the Wenchang River (upper estuary) is mainly consumed before being transported into the lagoon and may have caused the distinct shift in $\delta^{13}C_{org}$ from the upper to the middle estuary. The strong increase of $\delta^{15}N$ of TSM from the upper to the middle estuary indicates a shift in the DIN source. The high $\delta^{15}N$ may result from uptake of $^{15}N$-enriched nitrate and ammonium and/or intermixture with $^{15}N$-enriched TSM, both released into the lagoon with shrimp and fish pond effluents (Fig. 7a, Tab. 243.
2). Generally, elevated $\delta^{15}$N values in particulate matter have been related to aquaculture impact in various systems (Jones et al., 2001; Costanzo et al., 2004; Lin and Fong, 2008).

In general, mixing with nutrient poor marine waters is responsible for the seaward decrease in nutrient concentrations. Additionally, non-conservative behavior of all nutrients with salinity (Fig 4) designates strong uptake by primary producers, which is corroborated by high chl $a$ concentrations and the predominance of planktonic organic matter in TSM. The local increase in ammonium at the lagoon stations indicates drainage of untreated effluents from abundant aquaculture ponds (Tab. 2) as an important source of nutrients in the middle estuary. High porewater ammonium concentrations in sediments of the middle estuary (Tab. 2) and a sufficiently long residence time of $\approx 5.6$ days indicate that remineralization of sedimentary organic matter may also be a relevant process for N supply to the water column in the middle estuary. Ammonium addition and simultaneous removal of nitrate in the middle estuary result in a shift in the DIN pool from nitrate dominance in the upper to ammonium dominance in the middle and outer estuary (Fig. 5).

Neap- and spring tide concentrations $\leq 1$ $\mu$M ammonium, nitrate, nitrite and phosphate in the outer estuary indicate that export of dissolved inorganic nitrogen and phosphate to the coast does not allow for accumulation in coastal waters and appears therefore negligible under ‘normal’ summer monsoon conditions. Silicate, which in contrast to all other nutrients determined is not related to anthropogenic emission, was the only dissolved nutrient exported into coastal waters. This implies that the WWE acts as a sink for dissolved N and P derived from land, and released from aquaculture ponds or from internal nutrient regeneration. This filter function is facilitated by the morphology of the middle estuary (medium-sized lagoon with a narrow inlet channel) that hampers the immediate outflow and mixing with coastal marine waters and thus fosters the uptake of nutrients by brackish-water phytoplankton, its consumption by organisms of higher trophic levels, as well as deposition in sediments within the lagoon. This filtering capacity of the estuary is most likely an important prerequisite allowing the development of coral reefs and seagrass meadows in close vicinity to the lagoon despite continuous riverine nutrient and sediment input, and may buffer the adverse effects of additional inputs from aquaculture sources.
Fig. 7: (a) Pre-typhoon and (b) post-typhoon $\delta^{13}\text{C}_{\text{org}}$ vs. $\delta^{15}\text{N}$ of estuarine TSM at neap tide stations (1-10) and spring tide stations (A-C) and of selected potential end-members as listed in Tab. 2. Spring tide and end-member samples with mean and standard deviation. Numbers refer to station numbers in Fig. 1c.
Tab. 2: Concentrations (mean±SD) of potential sources (end-members) of particulate matter and inorganic nutrients in the WWE in July/August 2008. Samples were mostly taken before the passage of typhoon Kammuri.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Particulate matter</th>
<th>Inorganic nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ¹³Corg [‰]</td>
<td>δ¹⁵N [‰]</td>
</tr>
<tr>
<td>Typhoon rain (August 6/7, 2008)</td>
<td>-27.2 (±3.2)</td>
<td>20.4 (±12.3)</td>
</tr>
<tr>
<td>Freshwater (salinity = 0)</td>
<td>-21.0 (±0.6)</td>
<td>6.0 (±2.3)</td>
</tr>
<tr>
<td>Marine water (salinity = 33.5)</td>
<td>-24.0 (±3.4)</td>
<td>8.8 (±3.5)</td>
</tr>
<tr>
<td>Aquaculture ponds (water)</td>
<td>-25.8 (±0.9)</td>
<td>6.1 (±0.3)</td>
</tr>
<tr>
<td>Sediments of upper estuary (0-5 cm)</td>
<td>-22.8 (±2.2)</td>
<td>8.5 (±2.1)</td>
</tr>
<tr>
<td>Sediments of middle estuary (0-5 cm)</td>
<td>-18.0 (±0.9)</td>
<td>6.9 (±1.3)</td>
</tr>
<tr>
<td>Sediments of outer estuary (0-5 cm)</td>
<td>-25.4 (±2.1)</td>
<td>15.0 (±2.7)</td>
</tr>
<tr>
<td>Soils (from agriculture fields)</td>
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<td>40.0 (±18.8)</td>
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<td>C₃-plants:</td>
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<tr>
<td>mangrove leaf</td>
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<td>2.9 (±2.9)</td>
</tr>
<tr>
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<td>sugar cane leaf</td>
<td>-11.8 (±0.1)</td>
<td>6.4 (±2.7)</td>
</tr>
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</table>

4.2 Sources and distribution of dissolved and particulate matter during and after the typhoon

Typhoon-associated rains enhanced river discharge and turned the estuary into a river-dominated system, as documented by the reduction of salinity during and after the typhoon (Fig. 3; Tab. 1).

One day after the typhoon, seawater intrusion into the lagoon was essentially restricted to the bottom layer and the estuarine salinity gradient was shifted toward the coastal sea (Fig. 3). This indicates that rainwater inputs caused an almost complete flushing of the lagoon; intrusion of marine waters triggered by typhoon-induced wind and wave forcing through the inlet channel was not evident. The relatively uniform distribution of physico-chemical properties, nutrients and TSM concentration and composition along the transect sampled indicates that due to extreme runoff, dissolved and particulate matter from the upper catchment dominated in the entire estuary and was eventually exported into coastal waters. A high similarity in δ¹³Corg and δ¹⁵N values of TSM and C₃ plants, upstream sediments and soils from the watershed designates terrestrial matter as a major organic matter source of TSM after the typhoon (Fig. 7b). C/N ratios of TSM between 8 and 16 specify soils as the most likely source of suspended matter (Tab. 2). This implies that the torrential rainfall increased erosion of the predominantly agricultural soils in the upper catchment leading to a high input of allochthonous particles into the lagoon and coastal zone. Post-hurricane δ¹⁵N values around 4‰ were also observed in the Pamlico Sound System and also ascribed to such processes (Paerl et al., 2006a). Typhoon-derived soil erosion also had a
dominant impact on δ¹⁵N-TSM in the eutrophic Danshuei Estuary in northern Taiwan (Chen et al., 2001; Liu et al., 2007), causing δ¹⁵N values to rise to >2‰, where under normal conditions high fractionation during planktonic uptake led to a very low δ¹⁵N signal (<0‰). Wind-driven sediment resuspension along the shallow margins of the lagoon may also play a role, however dissimilarity in the δ¹³Corg and δ¹⁵N of TSM and lagoon sediments (Tab. 2) proofs that this process is likely of minor importance. Reduced chl a concentrations and lower C org and TN contents 1 day after the typhoon reflect that the rich phytoplankton population of the lagoon was pushed seaward with the first flush after the precipitation event. Homogenous and elevated nutrient levels along the sampled transect suggest that nutrient uptake by phytoplankton was restricted. Reduced concentrations of silicate after the typhoon indicate dilution of brackish waters with rain water containing low levels of silicate (Tab. 2). Concentrations of ammonium along the transect were in the range of ammonium in rain water, but nitrate concentrations in the water column were 3-11 times higher than in rain water. This indicates that in addition to direct atmospheric inputs of reactive nitrogen by rain (Tab. 2), which can be a significant ‘new’ N source in estuaries after heavy rains (Paerl, 1985; Paerl and Fogel, 1994), especially along the Chinese coast (e.g. Duce et al., 2008), nitrate was leached from agricultural fields in high quantities by a massive freshwater input, leading to a strong enhancement in nitrate concentrations after the typhoon. Peierls et al. (2003) also related an increase of nitrate in Pamlico Sound in the wake of a hurricane to freshwater input. The increase in phosphate is probably also linked to leaching from agricultural soils, though, phosphate concentrations may have declined through adsorption onto the typhoon-derived larger amount of particulates in the water column. In contrast, typhoon-related resuspension of estuarine sediments and associated release of nitrate and ammonium from sediment porewater into the water column was probably negligible compared to the afore-mentioned external nutrient inputs: a theoretical release of porewater from a 1 m² wide and 5 cm deep sediment layer having an assumed porosity of 0.6 and porewater concentrations as listed in Tab. 2 into a overlying water column of 3.5 m depth would only have augmented water nutrient concentrations by <1% relative to pre-typhoon concentrations. Therefore, nitrate from agriculture soils and rain inputs are considered the major source of dissolved nitrogen replacing aquaculture-derived ammonium before the typhoon and causing a shift in the DIN pool towards nitrate dominance in the middle and outer estuary after the typhoon (Fig. 5). High nutrient concentrations measured at station 9 directly after the typhoon indicate that nutrients were not completely consumed inside the estuarine lagoon, as usual. Nutrient uptake inside the lagoon by phytoplankton was restricted due to the short residence time during typhoon-
associated flushing and light limitation resulting from high loads of allochthonous TSM in the water column, as reflected by low Secchi depths (Tab. 1). Hence, the typhoon-induced flushing of the estuarine lagoon led to an export of nutrients into the outer estuary and fertilization of the coastal zone, so that under typhoon conditions the WWE acted as a nutrient source toward the coastal sea.

Two weeks after Kammuri’s passage, the biogeochemical conditions in the estuarine system had started to return to pre-typhoon conditions. However, a comparison of physico-chemical properties and dissolved nutrients 1-2 weeks after the typhoon with pre-typhoon values (both at spring tide) illustrates the persistent impact of the flushing. Relatively low surface and bottom water salinities despite spring tide conditions designate that typhoon-induced runoff was still draining from the watershed causing diminished impact of tidal forcing and a seaward shift of the estuarine gradient (Fig. 3, 4). Reduction of silicate, ammonium and phosphate with salinity to levels below the end-member mixing line indicate nutrient removal by phytoplankton and other primary producers along the estuarine transect, as observed before the typhoon. However, elevated nitrate and phosphate concentrations compared to pre-typhoon spring tide conditions indicate that the supply of nutrients from the upper catchment into the estuary continued. High nitrite levels suggest nitrification, which may have resulted from recycling of organic matter transported into the estuary with precipitation runoff. Also after the typhoon, denitrification appears to be unlikely because of relatively high DO concentrations (Tab. 1). Nitrate inputs from land accompanied by nitrification processes accounted for the persisting dominance of nitrate in the DIN pool one to two weeks after the typhoon (Fig. 5). Higher silicate levels in the upper estuary compared to directly after the typhoon indicate increased silicate inputs from land. In contrast, TSM concentrations were even lower than before the typhoon in the upper and middle estuary and the isotope composition distinguishes particulate organic matter from the upper estuary from that of the middle and lower estuary, as it was under pre-typhoon conditions (Fig. 7). This suggests a first flush effect: Only the initial freshwater pulse delivered considerable amounts of eroded soils and sediments into the estuary. Within a few days after the typhoon’s passage, these allochthonous particles were to a large extent exported offshore favored by the strong ebb dominance in the lagoon during spring tide and/or removed from the water column of the lagoon by rapidly settling particles (e.g. Victor et al., 2006). Resultant relief from light limitation, as indicated by the increased Secchi depth, together with the ample nutrient availability, stimulated phytoplankton growth in the middle and in particular in the outer estuary. This was evident from increased concentrations of chl a and DO, a $\delta^{13}C_{org}$ signal typical
for estuarine/marine phytoplankton and low ammonium and phosphate levels at these sites.

### 4.3 Relevance of typhoon precipitation for the estuarine system and nearshore coastal zone

Our results show that the nutrient input to coastal waters is very low at 'normal' low discharge, despite significant land use changes along the estuary and in the watershed (Fig 8a). Under these conditions, the estuary acts as a sink for dissolved nitrogen and phosphorus due to high nutrient uptake by primary producers inside the lagoon. Strong alterations in the amount and composition of dissolved nutrients and total suspended matter in the WWE after typhoon Kammuri showed that heavy precipitation had significant effects on the estuarine biogeochemistry persisting over several weeks. It caused increased soil erosion in the catchment and flooding of the estuarine lagoon with nutrient- and sediment-laden freshwater (Fig 8b). Thus, the estuarine filter function was interrupted for several days until most of the precipitation runoff had passed through the estuary. Nutrients derived from agriculture, aquaculture and urban effluents and organic matter regeneration, which are normally retained inside the estuarine lagoon, are then exported into coastal areas together with additional atmospheric nutrient inputs through rain (Fig. 8b, c). During and after major precipitation events, the estuary represents a temporal source of nutrients and organic matter toward the coastal ocean lasting for at least two weeks. The fertilization of the coastal ocean stimulated phytoplankton growth within a few days after the initial freshwater pulse. Phytoplankton blooms induced by increased N loadings have been observed in a range of estuaries and coastal seas worldwide several days after major storms (e.g. Valiela et al., 1998; Paerl et al., 2001, 2006a; Peierls et al., 2003; Burkholder et al., 2004; Wetz and Paerl, 2008; Zhang et al., 2009, Zhao et al., 2009). Consequences of excessive phytoplankton growth are well known and include proliferation of toxic algal species and hypoxic conditions (e.g. Paerl, 1988; Anderson et al., 2002; Livingston, 2007).

This study showed that even comparatively moderate and distant typhoons, as observed here, can induce a rapid export of freshwater, excess nutrients and TSM into nearshore regions, resulting in hyposalinity, reduced water transparency, siltation, as well as temporary eutrophication. Major storms can also cause other extreme conditions besides strong precipitation, such as seiching and other wind-driven effects. These were not observed during this study because of the relatively distant passage of Kammuri. During greater typhoon exposure (e.g. a direct typhoon landing), the estuary and coastal zone may be impacted by large storm surges resulting in sediment and
nutrient resuspension or hypersalinity, as e.g observed in Breton Sound (Louisiana) after Hurricane Katrina (Piazza and Peyre, 2009). Though, it is likely that in the WWE the relatively narrow inlet channel and adjacent fringing reefs serving as wave breakers to some extent limit the marine influence in the lagoon during typhoons.

Long-term precipitation data (Fig. 2a) reveal that pulsed rainfall events of comparable and even larger intensity frequently occur in the region. They are not necessarily related to typhoons but may also be linked to monsoonal thunderstorms. These pulsed natural disturbances are an inherent part of the overall system dynamics and most probably an important control of nutrient and material fluxes in the WWE. Because of the random occurrence of tropical storms and logistic constraints to investigate their effects, the latter are rarely accounted for in estuarine carbon and nutrient budgets. Due to a lack of some physical factors (e.g. local precipitation, river flow, etc.) we were not able to calculate nutrient and TSM fluxes. However, our biogeochemical data clearly reflected estuarine-wide changes in the water column in the wake of such an event and emphasized the quantitative significance of tropical storms for carbon and nutrient budgets.

As our data only show partial recovery of the estuarine system within 7-12 days after the typhoon, we conclude that full recovery from a typhoon of the Kammuri type to ‘normal’ water column conditions will take longer than 2 weeks. The similarity between salinity, nutrient and chl a concentrations 1-2 weeks after the typhoon (during spring tide) and neap tide concentrations before the typhoon emphasizes that it is important to consider both tidal phases (spring and neap tide) to distinguish typhoon effects from tidal variations and to clearly evaluate the recovery time of a system. This, however, is seldomly accounted for in other studies. The duration of recovery of an estuarine system is generally related to precipitation rates and frequency of rain events, as well as morphologic (e.g. size of watershed and estuary), hydrologic (e.g. flushing and residence times) and other regional factors. Due to the relatively short water residence time of ~5.6 days and the small catchment size, it is conceivable that the recovery time of the WWE is at the lower end of the range reported in other studies varying between days (e.g. Valiela et al., 1998), months (e.g. Zhang et al., 2009) and years (e.g. Paerl et al., 2001; Burkholder et al., 2004).

Hainan’s seagrasses and coral reefs are under stress from overfishing, habitat destruction and pollution (Hutchings and Wu, 1987; Fiege et al., 1994; Huang et al., 2006). The episodic flushing of freshwater, excess nutrients and organic matter into coastal waters we observed can additionally impair the performance of adjacent seagrass meadows and coral reefs, which are adapted to oligotrophic conditions. Besides direct physiological stress from siltation, low light availability and hyposalinity,
the nutrient pulses can stimulate excessive growth of benthic algae, which may ultimately result in a phase shift in coral reefs and seagrass meadows toward algal dominance (e.g. Valiela et al., 1997; McCook, 1999). It is likely that during recent decades the detrimental effects of estuarine flushing have been significantly reinforced by the anthropogenically enhanced nutrient and organic matter input from expanding agriculture and aquaculture and the loss of wetland areas, which under natural conditions act as a buffer retaining nutrients and sediments (Valiela and Cole, 2002; Walsh, 2004; Victor et al., 2004; Wolanski, 2007). China lost about 50% of its coastal wetlands between 1950 and 2000 (An et al., 2007). Besides that, the loss of wetlands and flood plains fostered by e.g. the construction of aquaculture ponds accelerates runoff velocity and thereby aggravates typhoon effects. Projected increase in typhoon intensity, duration and possibly frequency (e.g. Knutson et al., 2010) in combination with enhanced nutrient and sediment inputs into the estuary related to human activities in the catchment will expose the estuarine and coastal habitats more often and for longer time periods to eutrophication and hyposalinity stress, thereby extending their recovery time and eventually weakening their resilience.
Fig. 8: Simplified sketch summarizing the major biogeochemical processes in the WWE (a) before, (b) directly after and (c) 1-2 weeks after the typhoon. (a) Before the typhoon, the salinity gradient ranges over the whole estuary, the net discharge is relatively low and dissolved N and P released into the estuary from agriculture, aquaculture and benthic recycling are taken up by phytoplankton leading to a low export of N and P to the outer estuary. (b) Directly after the typhoon, heavy precipitation causes a dominance of freshwater along the estuarine system, an increased net discharge and high amounts of allochthonous particles (sediment and soil) derived from erosion in the upper catchment. Added nutrients from rain and leaching of agricultural fields are exported to the outer estuary. They can not be taken up by phytoplankton because it was flushed out. (c) 1-2 weeks after the typhoon, freshwater prominence and net discharge were lower enabling settlement of the allochthonous particles and subsequently the recurrence of phytoplankton. They consume introduced nutrients along the estuary, which results in a lower but still evident export of N to coastal waters.
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CHAPTER VI

Tracing nitrogen dispersal from aquaculture pond effluents using nitrogen stable isotope ratios (δ^{15}N) in seagrass, epiphytes and phytoplankton bioassays

by Lucia Herbeck, Daniela Unger, Angela Scharfbillig and Tim Jennerjahn

In Preparation
Tracing nitrogen dispersal from aquaculture pond effluents using nitrogen stable isotope ratios ($\delta^{15}$N) in seagrass, epiphytes and phytoplankton bioassays

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Abstract

Nutrient pollution from land-based aquaculture represents a significant anthropogenic threat to coastal waters. The aim of this study was to trace the dispersal of effluents from fish and shrimp ponds and to determine their biological impact on coastal back-reef areas in NE Hainan, tropical China. Besides nutrient concentrations, we determined the stable isotopic composition of ammonium ($\delta^{15}$N-NH$_4^+$) and nitrate ($\delta^{15}$N-NO$_3^-$) in aquaculture ponds. Furthermore, we analyzed nutrients and chlorophyll a (chl a) as well as the nitrogen content (TN) and stable isotopic composition of total suspended matter ($\delta^{15}$N-TSM), seagrass leaves and epiphytes along transects perpendicular to the shore in three back-reef areas exposed to different nitrogen loads from pond aquaculture. Additional samples were taken from a phytoplankton bioassay experiment, during which we incubated offshore surface water at stations along these transects. At the back-reef areas of Chang qi gang and Qingge, where 8.7 and 2.4 km$^2$ of the coastal hinterland are covered by shrimp and fish ponds, respectively, concentrations of NH$_4^+$ and NO$_3^-$ as well as TN in TSM, seagrass and epiphytes were highest close to the shore and decreased in offshore direction indicating substantial nitrogen input from land. High chl a (~10 $\mu$g L$^{-1}$) and significantly elevated chl a levels in the bioassay after incubation suggest stimulation of primary production by an increased nitrogen availability. For the first time, it could be confirmed that pond effluents are heavily enriched in $^{15}$N ($\sim$17‰ $\delta^{15}$N-NH$_4^+$, $\sim$7‰ $\delta^{15}$N-NO$_3^-$ and $\sim$10‰ $\delta^{15}$N-TSM). Consequently, high $\delta^{15}$N values in TSM (5-12‰), seagrass leaves (5-9‰) and epiphytes (7-11‰) proved nitrogen in the coastal waters to be derived from aquaculture ponds. Moreover, a significant $\delta^{15}$N increase of phytoplankton in the bioassay up to 2500 m from the shore identified aquaculture effluents as the dominant
nitrogen source in the entire back-reef areas. In contrast, low nutrient concentrations and tissue TN close to shore at the control site Ye Lin suggest little N inputs from the comparatively small pond area (0.04 km²). However, the $\delta^{15}$N increase of phytoplankton in the bioassay revealed that aquaculture effluents reach this site as well. Analysis of $\delta^{15}$N in the bioassay was therefore indentified as the most sensitive tool to trace the nutrient dispersal from aquaculture ponds. Our results indicate that effluents from large-scale pond aquaculture cause eutrophication in back-reef areas exceeding a distance of 2500 m from the shore, which may lead to degradation of valuable coastal habitats.

Key words: aquaculture, shrimp and fish pond effluents, nitrogen stable isotopes ($\delta^{15}$N), seagrass, bioassays, China

1. Introduction

Aquaculture has undergone rapid development within the past 30 years. Land-based pond aquaculture is a common practice for production of shrimp and fish, especially in SE Asia. One of the key concerns about pond aquaculture is the direct release of nutrient-rich effluents into natural water bodies (Dierberg and Kiattisimkul, 1996; Páez-Osuna, F., 2001). Those effluents are especially enriched in nitrogen from animal faeces, added feed and fertilizers (Burford and Williams, 2001) and can lead to eutrophication of adjacent coastal waters. This is of major concern as nutrient enrichment has been identified as one of the primary reasons for worldwide degradation of seagrass meadows and coral reefs (McGlathery, 2001; Bellwood et al., 2004; Lapointe et al., 2004; Waycott et al., 2009).

Inorganic nutrient concentrations have often been used to assess the impacts of nutrient release to coastal and marine waters. Though, it is difficult to trace nutrient input and dispersal in coastal systems by this method due to rapid dilution and biological uptake. Nutrient release from aquaculture farms may be pulsed and of large spatio-temporal variability (Herbeck et al., subm.). Furthermore, changes in hydrodynamic pattern may affect distribution and uptake processes generating highly variable nutrient regimes in coastal and marine waters and making it difficult to relate observed nutrient pattern at one time to general input from aquaculture sources.

In order to overcome those limitations, biomarkers have recently been developed as a tool for tracing nutrient pollution along coastal and marine systems. Many algal species have been shown to absorb and store excess nutrients and to
respond with growth (Fong et al., 2001). That is why the tissue composition of primary producers can reflect the conditions of the surrounding water body. Due to their longer turnover time, these plant tissues yield an integrated signal of both persistent and pulsed nutrient inputs over time (Costanzo et al., 2001).

Stable nitrogen isotopes have been used to trace the sources of nutrients in the water column of different environments (e.g. Cifuentes et al., 1996; Udy and Dennison, 1997; McClelland & Valiela, 1998; Thimdee et al., 2002; Pinón-Gimate et al., 2009). Their applicability is based on the fact that individual sources of nitrogen to coastal ecosystems have distinguishable specific δ¹⁵N signals associated to fractionation during different microbial processes, during which the lighter ¹⁴N is preferably converted (Heaton, 1986; Peterson and Fry, 1987).

Marine water typically has a δ¹⁵N-NO₃⁻ value ranging from 5-7‰ (Miyake and Wada, 1967; Wada et al., 1975) with deep water NO₃⁻ having mostly invariable values around 5‰ (Liu and Kaplan, 1989; Sigman et al., 2000). In contrast, denitrification leads to a ¹⁵N enrichment of the NO₃⁻ causing values up to 19‰ in water column oxygen minimum zones of the Pacific and the Arabian Sea (Cline and Kaplan, 1975; Liu et al., 1987; Liu and Kaplan, 1989; Brandes et al., 1998; Voss et al. 2001). Fertilizer-rich agricultural run-off has typically a very low δ¹⁵N value close to 0‰, because NH₄⁺ and NO₃⁻ fertilizers are usually produced by industrial fixation of atmospheric nitrogen (e.g. Lee et al., 2008). To the contrary, animal or sewage wastes usually have elevated δ¹⁵N values of >10‰, due to strong fractionation during volatilization of ammonia (NH₃) leaving NH₄⁺ enriched in ¹⁵N, which is subsequently converted to ¹⁵N-enriched NO₃⁻ (Heaton, 1986; Valiela et al., 2000). Dissolved nitrogen in aquaculture effluents has also been suggested to be enriched in ¹⁵N (Costanzo et al., 2001), despite direct evidence of δ¹⁵N values from dissolved nitrogen so far.

According to this background, TN and δ¹⁵N in seagrasses, algae and mangroves have been used to investigate the effects of effluents from land-based shrimp and fish farms (Jones, et al. 2001; Costanzo et al., 2004; Vizzini and Mazzola, 2004) and offshore fish farms (Sarà et al. 2006; Pérez et al., 2008) on estuarine and marine waters. Those studies report on elevated TN and δ¹⁵N in plant tissues close to farms indicating significant nitrogen enrichment in the respective areas.

Macroalgae and phytoplankton bioassays have found to be effective tools to trace effluents along horizontal gradients (Costanzo et al., 2001; Dalsgaard and Krause-Jensen, 2006; Deutsch and Voss, 2006; Pitta et al., 2009; García-Sanz, et al., 2010, 2011). In this experimental approach, macroalgae or phytoplankton are deployed at variable distances to the respective nitrogen source and incubated for a certain time period. This way, restrictions due to naturally irregular macrophyte distribution can be
overcome and results benefit from the standardized set-up. Using this method, Dalsgaard and Krause-Jensen (2006) as well as Pitta et al. (2009) found high primary productivity near offshore fish cages in the Mediterranean, which rapidly decreased with distance from the farms. García-Sanz, et al. (2010, 2011) found enhanced TN and $\delta^{15}$N in macroalgae in >1 km distance from offshore fish farms in the Mediterranean and Atlantic using bioassays. One single study also applied macroalgal bioassays to detect nutrient enrichment from a shrimp farm in French Polynesia (Lin and Fong, 2008) and reported on elevated $\delta^{15}$N in Acanthophora spicifera tissue extending out to 800 m away from the shrimp farm.

Despite its effectiveness, the application of macroalgal bioassays is limited due to a potential lack of suitable macroalgae. In contrast, phytoplankton bioassays may be applied everywhere due to worldwide occurrence of phytoplankton. However, to our knowledge this work is the first to use phytoplankton bioassays for evaluating the impact of land-based pond aquaculture on coastal waters and testing the suitability of TN and $\delta^{15}$N obtained by phytoplankton bioassays for tracing pond effluents. Furthermore, bioindicators have not been used to quantify effects of large-scale shrimp and fish pond complexes, as they are found e.g. in SE Asia. China is the worldwide leading producer of aquaculture goods accounting for 62% of global production in terms of quantity and 51% of global value (FAO, 2010). Especially in the southern parts of the country, hundreds of hectares along the shoreline have been converted to shrimp and fish ponds, often in direct adjacency to valuable coastal habitats and there is still little known about the consequences of those extremely dense shrimp and fish pond agglomerations.

Aim of this study was to assess the dispersal and impact of anthropogenic dissolved nitrogen released from shrimp and fish ponds along coastal back-reef areas in NE Hainan, tropical China, by means of biogeochemical composition and different bioindicators. We measured concentrations of dissolved inorganic nitrogen and chl a along with TN and $\delta^{15}$N in phytoplankton, seagrass leaves and epiphytes, as well as a phytoplankton bioassay to quantify the spatial extent and magnitude of nitrogen enrichment along three back-reef areas exposed to various nitrogen loads derived from pond aquaculture. We also determined $\delta^{15}$N-NO$_3^-$ and $\delta^{15}$N-NH$_4^+$ in shrimp and fish ponds to verify the causal relationship between elevated $^{15}$N in coastal waters and the hypothesized aquaculture nitrogen source. This are, to our knowledge, the first values of $\delta^{15}$N-NO$_3^-$ and $\delta^{15}$N-NH$_4^+$ in aquaculture effluents reported in the literature so far.
2. Material and Methods

2.1 Study area and sampling design

The study area is located at the NE coast of the island Hainan, South China, in the marginal tropics (Fig. 1a). Mangroves used to cover sizable parts of the study area, but were converted to aquaculture ponds to large extents. Coral reefs fringe parts of the coast in 0.5 to 4 km distance from the shore and seagrass meadows dominated by *Thalassia hemprichii* exist in their back-reef areas (Fig. 1b). The maximum depth in the back-reef areas is 1.5-3 m (during spring high tide). The area is subject to mixed semidiurnal microtides with a tidal range of about 0.5 and 1.5 m at neap and spring tide, respectively, causing large parts of the back-reef areas being exposed to air during spring low tide. The region is characterized by a tropical monsoon climate with a dry season from November to April and a rainy season from May to October. The total annual precipitation is 1500 to 2000 mm with typhoon-induced rainfall accounting for 35-60% (>700mm), especially from July to September (Huang, 2003; Wang et al., 2008). Average air temperatures range between 14.6-20.8 °C in January and 25.2-33.1 °C in July.

![Figure 1](image-url)

**Fig. 1:** Location of the study area situated at the NE coast of Hainan, China (a) and overview on the study area (b) with location of the three study sites Ye Lin (c), Chang qi gang (d) and Qingge (e) including position of the sampling stations (black dots) along a distance gradient from the shore and extension of coastal habitats and aquaculture ponds.
During the past 30 years, the area has become an important site for brackish water pond aquaculture for intensive production of shrimp and fish. An area of 39.6 km² along the approximately 45 km shoreline of the study area is used for brackish water pond aquaculture (Herbeck et al., subm.). The main shrimp species cultured are *Litopenaeus vannamei* (white shrimp), *Penaeus chinensis* (chinese shrimp) and *Penaeus monodon* (black tiger shrimp) and the main fish species cultured are *Epinephelus awoar* (banded grouper) and *Epinephelus lanceolatus* (gentiana grouper). Three to four crops of shrimp and one crop of fish can be cultured per year. Aquaculture effluents are released directly into the environment without prior treatment. They are either drained into natural creeks or drainage channels or pumped directly into the estuaries or coast with tubes. Operation characteristics of brackish pond aquaculture in NE Hainan are described in detail in Herbeck et al. (subm.).

This study was carried out during March and April 2009 at the transition between dry and rainy season. We chose three back-reef areas that varied in extent of pond aquaculture production in their hinterland and received different loads of nitrogen: Ye Lin (19°31.3 N, 110°52.0' E; Fig. 1c) with low pond exposure, Qingge (19°19.7' N, 110°41.3' E; Fig. 1d) with medium pond exposure and Chang qi gang (19°27.2’ N, 110°47.8’ E; Fig. 1e) with high pond exposure. Nitrogen loadings and other characteristics of the study sites are summarized in Tab. 1. The site Ye Lin was close to the outlet of the Wenchang/Wenjiao Estuary (WWE) and temporarily came under its influence (Fig. 1b).

At each site, sampling stations were established along a perpendicular transect reaching from the shore line to the reef crests. Those stations were situated in a distance of 50, 100, 250, 500, 1000 and 2500 m from the shoreline, depending on the size of the respective back-reef areas (Fig. 1c-e).

Tab. 1: Size of pond and back-reef areas and nitrogen loadings from pond aquaculture at the three investigated study sites (Herbeck et al., subm.).

<table>
<thead>
<tr>
<th></th>
<th>Ye Lin</th>
<th>Qingge</th>
<th>Chan qi gang</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of back-reef area [km²]</td>
<td>1.5</td>
<td>8.4</td>
<td>23.2</td>
</tr>
<tr>
<td>Area covered by ponds [km²]</td>
<td>0.04</td>
<td>2.4</td>
<td>8.7</td>
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<tr>
<td>Average export of aquaculture effluents [10⁶ m³ yr⁻¹]</td>
<td>0.4</td>
<td>23.7</td>
<td>87.9</td>
</tr>
<tr>
<td>Average nitrogen export from aquaculture ponds [t yr⁻¹]:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved inorganic nitrogen (DIN)</td>
<td>0.4</td>
<td>21.9</td>
<td>81.3</td>
</tr>
<tr>
<td>Dissolved organic nitrogen (DON)</td>
<td>0.3</td>
<td>16.2</td>
<td>60.2</td>
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<td>Particulate nitrogen (PN)</td>
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<tr>
<td>Total nitrogen (TN)</td>
<td>1.9</td>
<td>105.8</td>
<td>392.7</td>
</tr>
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</table>
2.2 Sampling and sample analysis

2.2.1 Aquaculture ponds and drainage channels

Water from shrimp ponds, fish ponds and drainage channels was collected by submersion of a bottle from the pond/channel edge. Water for the analysis of dissolved inorganic nitrogen \([\text{DIN} = \text{nitrate (NO}_3^-) + \text{nitrite (NO}_2^-) + \text{ammonium (NH}_4^+)]\) was filtered immediately after sampling through single use Sartorius Minisart® membrane filters (0.45 µm pore size). DIN samples were stored in PE bottles, which were rinsed three times with the filtered sampling water beforehand, preserved with a mercury chloride solution (50 µL of a 20 g L\(^{-1}\) HgCl\(_2\)-solution added to 100 ml sample) and stored cool until analysis. Water samples for the analysis of total suspended matter (TSM), its nitrogen content (TN-TSM) and its nitrogen isotopic composition (\(\delta_{15}N\)-TSM), as well as for stable nitrogen isotopes of ammonium and nitrate (\(\delta_{15}N\)-NH\(_4^+\) and \(\delta_{15}N\)-NO\(_3^-\)) were stored in PE tanks in the dark until filtration within the same day.

Under constant pressure, a specific volume of water was filtered onto pre-combusted (5 h, 450 °C) and pre-weighed Whatman GF/F filters, which were dried at 40 °C. The filtered water was acidified to pH 1.5 with concentrated HCl and stored frozen in pre-acidified 1 L-PE bottles until analysis.

DIN constituents were analyzed using a continuous flow injection analyzing system (Skalar SAN++ System), by which NO\(_x\) (NO\(_3^-\) + NO\(_2^-\)) were detected spectrophotometrically and NH\(_4^+\) fluorometrically as a colored complex (Grasshoff et al., 1999). Respective determination limits were 0.08 µM, 0.03 µM and 0.06 µM and the coefficient of variation of the procedure was <3.4%.

The TSM concentration was calculated by dividing the weight difference between dried filters and the original weight of the empty filter by the respective volume of water filtered. TSM was analyzed for total nitrogen (TN) by high-temperature combustion in a Carlo Erba NA 2100 elemental analyzer (Verardo et al., 1990). Measurements had a precision of 0.02%, based on repeated measurements of a standard (LECO 1012). \(\delta_{15}N\)-TSM was determined with a Thermo Finnigan Delta Plus gas isotope ratio mass spectrometer (MS) after high temperature combustion in a Flash 1112 EA elemental analyzer (EA). \(\delta_{15}N\) is given as ‰-deviation of atmospheric air. The precisions of the method, determined by an internal standard, were 0.20‰.

For the analysis of \(\delta_{15}N\)-NH\(_4^+\) and \(\delta_{15}N\)-NO\(_3^-\), NH\(_4^+\) and NO\(_3^-\) were extracted from the water onto acidified Whatman GF/D glass fibre filters using a modification of the “ammonium diffusion method” of Holmes et al. (1998) and the “nitrate extraction method” of Sigman et al. (1997) and subsequently measured with a coupled EA-MS system as described for \(\delta_{15}N\)-TSM above. In short, NH\(_4^+\) in water samples was
converted to NH₃ at a pH ~11, which was trapped on an acidified filter packed between two teflon membranes. Subsequently, this step was repeated after reduction of NO₃⁻ in the remaining water samples to NH₄⁺ using Devada’s alloy. The filters were dried and packed into silver caps for later combustion. Double or triplicate measurements were carried out for each sample.

2.2.2 Dissolved and suspended matter in coastal waters

Surface water samples were taken repeatedly from each coastal station (n=4-12) by boat. DIN constituents, TSM and TN-TSM, as well as δ¹⁵N-TSM, were determined as described above. In addition to that, chl a of TSM was determined: water was filtered onto a GF/F filter, which was then frozen until analysis. Pigments were extracted from the filters in 10 ml 90% acetone at 4 °C in the dark for approximately 24 hours, and extracts were subsequently centrifuged at 60 rpm for three minutes. Chl a in the supernatant was determined with a TURNER 10-AU field fluorometer after Arar and Collins (1997).

2.2.3 Seagrass and epiphytes

Approximately 10 shoots of T. hemprichii, the only seagrass species present at all sites, were collected randomly at 5 different positions at each station. Additionally, shoots of T. hemprichii were collected at five time intervals over the study period at the 100 m station in Ye Lin and the 500 m station in Qingge, in order to determine potential temporal variability in TN and δ¹⁵N signals. Plants were rinsed, and leaf epiphytes were removed, dried at 40 °C and stored in glass vials. The first 2 cm of the second youngest leaf of each T. hemprichii shoot of the pooled samples were sorted, and dried at 40 °C. Subsequently, dried seagrass leaf and epiphyte tissues were ground to a fine powder and analyzed for TN and δ¹⁵N as described above.

2.2.4 Experiment – phytoplankton bioassay

A phytoplankton bioassay was installed at each station based on the method described in Dalsgaard and Krause-Jensen (2006). The set-up of the bioassay was partly modified in order to adapt it to conditions in a shallow reef lagoon with strong tidal effects. Dialysis bags were made from Spectra/por 1 dialysis membrane (flat width 12 cm) made of regenerated cellulose with a molecular weight cut-off of 6-8 kDa. Dialysis tubes were cut into 30 cm long pieces, which were sealed at one end using cable ties. All dialysis bags at a study site were filled with water collected approximately 500 m offshore of the reef crest of the respective study sites. The filling water of four of the seven bags, which were deployed at each station, was previously filtered through a
50 μM sieve in order to remove larger grazers, while in three of the seven bags unfiltered water was used. After filling, the dialysis bags were closed with a cable tie. Each dialysis bag had a volume of approximately 700 ml and a diameter of about 7.6 cm. The bags were placed into an envelope made from see-through nylon fishing net with a mesh size of 2 cm. Cable ties were used to attach the wrapped bags to a set-up deployed at each station, which varied depending on the depth of the respective station. At stations with a minimal ebb tide depth of <1 m (= all but the station furthest offshore at each site), two metal poles (90 cm long) were hammered about 40 cm into the sediment and two 1.5 m long ropes were spanned between the poles, to which bags were tied (Fig. 2a). This set-up helped avoiding dragging of fragile dialysis bags over coral rubble at low tide, which could have caused breakage of dialysis membranes. At stations with a minimal ebb tide depth of >1 m (= station furthest offshore at each site), bags were tied to a buoyant plastic tube, which was anchored with a rope approximately 50 cm beneath the surface (Fig. 2b). Buoys were attached to each set-up to mark their positions. After an incubation time of four days, the water content of the bags was filled into 1 L PE-bottles and stored cool and dark until processing the same day.

Water samples were filtered under constant pressure onto pre-combusted and pre-weighed Whatman GF/F filters. Half of each filter was dried at 40 °C and analyzed for TSM, TN-TSM, and δ^{15}N-TSM and the other half was analyzed for chl a, as described above.

![Fig. 2: Set-up of phytoplankton bioassays](image)
2.3 Data analysis

The statistic tool of SIGMAPLOT 11.0 was used to perform the statistical analyses. The data were tested for normal distribution before choosing parametric or non-parametric statistical methods. ANOVAs or ANOVAs on ranks followed by the Tukey or Mann-Whitney test were performed to analyze variance and determine significant differences (p<0.05) between stations along the distance gradient and between sites. The student t-test or Mann-Whitney test was used to determine significant differences between the two time intervals of the bioassay experiment. The Pearson Product Moment Correlation analysis or Spearman Rank Order Correlation analysis was performed to test for significant correlations.

3. Results

3.1 Nitrogen and its isotopic composition in aquaculture effluents

All nitrogen constituents were high in shrimp ponds, fish ponds and in drainage channels (Tab. 2). NH₄⁺ accounted for the main part of the DIN (63% on average), whereas the portion of NO₃⁻ and NO₂⁻ was minor. In shrimp ponds, TN-TSM was significantly lower compared to fish ponds and drainage channels, and DIN was higher than TN-TSM. In fish ponds and drainage channels, nitrogen was higher in the particulate than in the dissolved fraction.

δ¹⁵N-NH₄⁺ values were between 15.5 and 18.1‰, while average values of δ¹⁵N-NO₃⁻ from 6.5-7.1‰ were significantly lower than values of δ¹⁵N-NH₄⁺. Average values of δ¹⁵N-TSM varied between 8.8 and 11.4‰.

All nitrogen constituents and their δ¹⁵N values varied considerably between the different ponds and drainage channels analyzed, as indicated by a high range and standard deviation.
Tab. 2: Concentration (mean±SD) and range of dissolved and particulate nitrogen constituents and their isotopic composition in fish ponds, shrimp ponds and drainage channels during March/April 2009.

<table>
<thead>
<tr>
<th></th>
<th>DIN</th>
<th>NH$_4^+$</th>
<th>NO$_3^-$</th>
<th>NO$_2^-$</th>
<th>TN-TSM</th>
<th>δ$^{15}$N-NH$_4^+$</th>
<th>δ$^{15}$N-NO$_3^-$</th>
<th>δ$^{15}$N-TSM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[μM]</td>
<td>[μM]</td>
<td>[μM]</td>
<td>[μM]</td>
<td>[mg L$^{-1}$]</td>
<td>[%]</td>
<td>[%]</td>
<td>[%]</td>
</tr>
<tr>
<td><strong>Shrimp ponds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>88.8</td>
<td>56.1</td>
<td>26.4</td>
<td>0.5</td>
<td>0.7</td>
<td>15.4</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>19.9-332</td>
<td>7.7-179</td>
<td>4.3-117</td>
<td>0.1-1.0</td>
<td>0.1-1.5</td>
<td>14.9-16.0</td>
<td>2.8-20.8</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>121.4</td>
<td>65.7</td>
<td>44.6</td>
<td>0.4</td>
<td>0.7</td>
<td>0.8</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fish ponds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>76.4</td>
<td>48.3</td>
<td>25.5</td>
<td>2.7</td>
<td>1.8</td>
<td>16.2</td>
<td>7.1</td>
<td>11.4</td>
</tr>
<tr>
<td>Range</td>
<td>6.4-200</td>
<td>3.0-122</td>
<td>0.0-145</td>
<td>0.2-6.7</td>
<td>0.3-6.6</td>
<td>2.6-24.2</td>
<td>2.9-12.1</td>
<td>5.9-18.6</td>
</tr>
<tr>
<td>SD</td>
<td>60.0</td>
<td>41.6</td>
<td>35.1</td>
<td>2</td>
<td>1.9</td>
<td>9.1</td>
<td>3.5</td>
<td>4.3</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>13</td>
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<tr>
<td><strong>Drainage channels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>100.0</td>
<td>63.4</td>
<td>37.3</td>
<td>2.8</td>
<td>2.6</td>
<td>18.1</td>
<td>6.5</td>
<td>9.1</td>
</tr>
<tr>
<td>Range</td>
<td>10.1-284</td>
<td>3.4-189</td>
<td>0.4-163</td>
<td>0.2-11.2</td>
<td>0.01-17.3</td>
<td>12.8-24.8</td>
<td>3.2-10.4</td>
<td>5.4-15.1</td>
</tr>
<tr>
<td>SD</td>
<td>80.3</td>
<td>56.3</td>
<td>41</td>
<td>3.1</td>
<td>4.5</td>
<td>4.0</td>
<td>2.4</td>
<td>3.1</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>17</td>
<td>18</td>
<td>18</td>
<td>13</td>
<td>9</td>
<td>9</td>
<td>14</td>
</tr>
</tbody>
</table>

3.2. Distribution of dissolved and particulate nitrogen and chl a in coastal waters

There was a strong temporal variability in all parameters measured in the surface water, as indicated by a high standard deviation at most study sites (Fig. 3). NH$_4^+$ concentrations were usually higher than NO$_3^-$ concentrations (Fig. 3a, b). Highest average concentrations of NH$_4^+$ (8.6 μM) and NO$_3^-$ (7.2 μM) were measured in Chang qi gang, followed by Qingge. At both sites, NH$_4^+$ and NO$_3^-$ concentrations decreased with increasing distance from the shore (significant correlation; Tab. 3), whereas at Ye Lin, average concentrations increased slightly with distance and only decreased again at the station furthest offshore.

Concentrations of chl a and TN-TSM (Fig. 3c, d) were on similar levels at all three study sites and showed the same trends over the distance gradient as observed for NH$_4^+$ and NO$_3^-$. Average values of δ$^{15}$N-TSM were between 4.8 and 9.9‰ and were highest at the nearshore stations in Qingge, where values up to 13.0‰ were measured (Fig. 3e). At Qingge, the δ$^{15}$N became lighter with increasing distance, while the opposite trend was observed in Ye Lin and Chang qi gang. There was a positive correlation of chl a with NH$_4^+$ in Ye Lin and with NO$_3^-$ in Qingge and Chang qi gang (Tab. 3). TN-TSM correlates significantly with NO$_3^-$ and NH$_4^+$ in Chang qi gang, but no significant correlations were detected for δ$^{15}$N-TSM.
Fig. 3: Concentration (mean±SD) of NH$_4^+$ (a), NO$_3^-$ (b), chl a (c), TN-TSM (d) and $\delta^{15}$N-TSM (e) over a logarithmic distance gradient at the three study sites. Samples (n≥4) were taken at different days and tidal levels during the study period. The dashed line represents the position of the reef crest.
Tab. 3: Correlation of individual water column parameters at Ye Lin (YL), Qingge (Qg) and Chang qi gang (Cqg) as indicated by the correlation coefficient (r²) and level of significance determined by the Spearman Rank Order Correlation Analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Distance [m]</th>
<th>NH₄⁺ [µM]</th>
<th>NO₃⁻ [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YL</td>
<td>Qg</td>
<td>Cqg</td>
</tr>
<tr>
<td>NH₄⁺ [µM]</td>
<td>r²</td>
<td>0.02</td>
<td>-0.40</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>NO₃⁻ [µM]</td>
<td>r²</td>
<td>0.11</td>
<td>-0.69</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>chl a [µg L⁻¹]</td>
<td>r²</td>
<td>0.55</td>
<td>-0.60</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>TN-TSM [µg L⁻¹]</td>
<td>r²</td>
<td>0.59</td>
<td>-0.73</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>**</td>
<td>***</td>
</tr>
</tbody>
</table>

ns p≥0.05 (not significant)  ** p<0.01 (highly significant)  * p<0.05 (significant)  *** p<0.001 (extremely significant)

3.3 Nitrogen content and N isotopic composition in seagrass and epiphytes

The average nitrogen content in the seagrass T. hemprichii (2.6-3.5%) was significantly higher than in its epiphytes (0.5-1.4%), while the δ¹⁵N composition in seagrass leaf and epiphyte tissue (~7.7%) was similar (Fig. 4). TN and δ¹⁵N in seagrass and epiphytes mostly showed the same trends over distance at the respective sites.

TN contents in seagrass samples were on similar levels around 3% with significantly lower values at 250 m in Ye Lin and 500 m in Chang qi gang compared to the other stations at the respective distance. Seagrass TN content in 100 m at Ye Lin was significantly higher than in Qingge and Chang qi gang at the same distance from the shore, and significantly lower in Chang qi gang at 500 m distance. The TN content in epiphytes ranged from 0.38-1.54% and was significantly lower at 250 m in Ye Lin and 500 m in Qingge compared to the other stations at the respective sites. TN content in epiphytes tended to be lower at Chang qi gang compared to the other sites. There was no significant relationship between TN contents in seagrass and epiphytes with distance or average NO₃⁻ and NH₄⁺ concentrations.

In Qingge and Chang qi gang, the δ¹⁵N in seagrass and epiphyte samples in 100 m distance from the shore was significantly higher than in 1000 m from the shore. The opposite trend was found in Ye Lin, where seagrass samples at 50 m from the shore had a significantly lower δ¹⁵N than stations further offshore. There were no
significant differences between $\delta^{15}N$ in seagrass tissue between the sites except at 1000 m from the shore, where values were significantly lower in Chang qi gang than in Qingge. $\delta^{15}N$ in epiphytes in Chang qi gang were at most stations significantly higher than those in Ye Lin and Qingge and also higher than $\delta^{15}N$ in seagrass leaves. $\delta^{15}N$ in seagrass correlated significantly with NO$_3^-$ concentrations in Qingge and Chang qi gang, while $\delta^{15}N$ in epiphytes correlated with NH$_4^+$ in Ye Lin and with NO$_3^-$ in Qingge.

![Diagram](image)

**Fig. 4:** TN and $\delta^{15}N$ in tissue of *T. hemprichii* seagrass leaves and epiphytes over a distance gradient at the three study sites in 2009. Significant differences (ANOVA & Tukey; p<0.05) between stations along a distance gradient at the respective sites are indicated with ° for seagrass leaves and * for epiphytes.

There were no significant differences in TN and $\delta^{15}N$ contents in seagrass leaf tissue at Ye Lin and Qingge over time (Fig. 5). TN content in epiphyte tissue in Ye Lin and Qingge increased over the sampling time with a significantly lower value at the first and significantly higher values at the last two sampling events in Ye Lin and a significantly lower TN content at the first sampling time in Qingge. In Ye Lin, there was a significant correlation of TN in epiphyte tissue with NO$_3^-$ ($r^2=0.96$; p<0.05) and NH$_4^+$ ($r^2=0.94$; p<0.05). The $\delta^{15}N$ in epiphyte tissue in Ye Lin was significantly lower at the
first sampling event compared to the second, while it increased over the sampling time in Qingge with a significantly lower value at the first and significantly higher value at the last sampling event.

Fig. 5: Concentration of water DIN and NH$_4^+$ as well as $\delta^{15}$N and TN in tissue of $T$. hemprichii seagrass leaves and epiphytes over the sampling time in Ye Lin at 100 m distance from the shore and in Qingge at 500 m from the shore. The difference in concentration between DIN and NH$_4^+$ accounts for NO$_3^-$ and NO$_2^-$.

3.4 Experiment – phytoplankton bioassay

In Qingge and Chang qi gang, there was a significant increase in chl a concentration for both the filtered and unfiltered treatments at most stations (Fig. 6). The chl a increase was most pronounced at the stations close to the shore. In Qingge, it decreased linearly with distance and was negative at 1000 m distance from the shore for the unfiltered treatment. In Chang qi gang, the chl a increase was minimal at 500 m distance from the shore and was higher again further offshore. In Ye Lin, initial chl a concentrations were significantly higher than at the other stations. The only significant chl a increase over the incubation time in bags with unfiltered water in Ye Lin was at the 500 m station, while there was a significant chl a decrease in the bags with filtered...
water at most stations of Ye Lin. Chl a increase in the bags with unfiltered water was at most stations higher than in those with filtered water. However, in Ye Lin and Qingge, initial chl a concentrations in bags with unfiltered water were also 2.2 times higher in Qingge and 1.3 times higher in Ye Lin than in bags with filtered water, whereas concentrations were similar in Chang qi gang.

![Fig. 6](image.png)

**Fig. 6:** Chl a concentration (mean±SD) in the dialysis bags at the time of deployment (initial) and at retrieval after 4 days at the three stations with (a) unfiltered (n=3) and (b) 50 μm filtered water (n=4). Significant differences (p<0.05) in chl a concentrations between the two time intervals are indicated with * as tested with the Students t-test.

There were no significant differences in the TN and δ15N of particulate matter between the bags with filtered and with unfiltered water. Therefore, TN and δ15N of both treatments were combined (Fig. 7). TN contents were at all stations significantly elevated after the incubation period, except in Ye Lin at 250 m (Fig. 7a). In Qingge and Chang qi gang, the TN increase was most pronounced close to the shore, where TN contents were on average 4 and 5 times higher, respectively, than the initial values, and the increment decreased in offshore direction. In Ye Lin, TN contents were on similar levels over the distance gradient. The δ15N of particulate matter in the bags was also significantly elevated at all stations of Qingge and Chang qi gang and at the 50 and 100 m stations in Ye Lin (Fig. 7b). The strongest δ15N increase was in Qingge at 50 m distance from the shore, where values increased from 6.1 to 13.2‰. In Chang qi
gang, the $\delta^{15}$N was increased from 6.0 to values $>8.0\%$ even at the station in 2500 m distance from the shore.

![Graphs showing TN and $\delta^{15}$N in the TSM in the dialysis bags (mean±SD) at the time of deployment (initial) and at retrieval after 4 days at the three stations (n=7). Data for bags with filtered and with unfiltered water were combined, because there were no significant differences in TN and $\delta^{15}$N between the treatments. Significant differences (p<0.05) in TN and $\delta^{15}$N between the two time intervals are indicated with * as tested with the Students t-test.]

**Fig. 7:** TN (a) and $\delta^{15}$N (b) of the TSM in the dialysis bags (mean±SD) at the time of deployment (initial) and at retrieval after 4 days at the three stations (n=7). Data for bags with filtered and with unfiltered water were combined, because there were no significant differences in TN and $\delta^{15}$N between the treatments. Significant differences (p<0.05) in TN and $\delta^{15}$N between the two time intervals are indicated with * as tested with the Students t-test.

4. Discussion

4.1 Nitrogen and N isotopic composition of aquaculture effluents

Elevated average DIN concentrations in aquaculture ponds and drainage channels compared to coastal waters reflect high nitrogen inputs. Remineralisation of organic matter dominated by ammonification and excretion of NH$_3$ by the cultured animals results in predominance of NH$_4^+$ in the DIN pool. This is concordant with other studies reporting on high dissolved nitrogen concentrations in shrimp ponds (Briggs and Funge-Smith, 1994; Jackson et al., 2003; Wahab et al., 2003; Islam et al., 2004) and adjacent creeks (Burford et al., 2003; Biao et al., 2004; Costanzo et al., 2004). Nutrient and organic matter concentrations in shrimp and fish ponds specific to brackish water pond culture in NE Hainan are discussed in detail in Herbeck et al., (subm.).
The assumption of previous studies that aquaculture effluents are enriched in $^{15}$N (e.g. Jones et al., 2001; Costanzo et al. 2004; Lin and Fong, 2008) can now be confirmed by our high $\delta^{15}\text{N}$ signatures of NH$_4^+$ ($\sim$17‰), which to our knowledge are the first published data on aquaculture NH$_4^+$ in shrimp ponds, fish ponds and drainage channels (Tab. 2). There are two reasons for the high $\delta^{15}\text{N}$ in aquaculture pond effluents: First, the nitrogen in ponds originates from animals of a relatively high trophic level added with fish and shrimp feed based on fish meal and small fishes. Typically, the $\delta^{15}\text{N}$ increases by $\sim$3‰ with each trophic level (Minawage and Wada, 1984). Therefore, remineralization of the $^{15}\text{N}$ enriched surplus feeds and excretions from farmed animals results in relatively high $\delta^{15}\text{N}$-NH$_4^+$ in ponds, despite fractionation during organic matter decomposition. In addition, fractionation during volatilization and nitrification of NH$_3$ causes further $^{15}\text{N}$ enrichment in the remaining NH$_4^+$ pool (Heaton, 1986; Cifuentes et al., 1989). Nitrification results in the comparatively light NO$_3^-$, which is consistent with average $\delta^{15}\text{N}$ of NO$_3^-$ of 6.7‰ in the ponds of the study area. NH$_4^+$ uptake by primary producers causes further $^{15}\text{N}$ enrichment of the remaining NH$_4^+$, while the planktonic OM is comparatively lower (e.g. Montoya, 2008). This process is of considerable importance as TSM in aquaculture ponds has been shown to consist mostly of phytoplankton (Jackson et al. 2003, Herbeck et al., subm.). Uptake of the isotopically lighter NO$_3^-$ appears to contribute less to the TSM signal, as the $\delta^{15}\text{N}$ of the TSM was still significantly higher than that of NO$_3^-$, so that a preference of plankton for NH$_4^+$ can be expected. Vizzini and Mazzola (2004) reported on similar $\delta^{15}\text{N}$-TSM values of $\sim$8‰ in fish farm effluents in Italy.

A few exceptional low $\delta^{15}\text{N}$ values of $<$5‰, especially of NO$_3^-$ in fish ponds, suggest contributions of artificial fertilizers. Those are added to ponds mainly at the beginning of crop production in order to stimulate phytoplankton growth serving as feed for the young animals raised. In summary, $\delta^{15}\text{N}$-NH$_4^+$ values $>$14‰ and $\delta^{15}\text{N}$-TSM values $>$7‰ in 75% of the ponds and drainage channels indicate that aquaculture effluents are generally enriched in $^{15}\text{N}$. This contradicts assumptions of previous studies, which found comparatively lower $\delta^{15}\text{N}$-TSM values in shrimp farms of $\sim$ 6‰ and stated those to be generally less enriched in $^{15}\text{N}$ than e.g. sewage having a $\delta^{15}\text{N}$-TSM value of $\sim$10‰ (Jones et al. 2001; Costanzo et al. 2004). Obviously, there is a significant regional variety of $\delta^{15}\text{N}$ in shrimp aquaculture N and the previously reported values rather mark the lower end of possible $\delta^{15}\text{N}$ values. This variability could be related to operation characteristics of the farms by measures inhibiting or favouring certain conversion processes. High pH values, for example, may support volatilization of NH$_3$ causing $^{15}\text{N}$ enrichment of NH$_4^+$. It is also possible that volatilization is higher in...
NE Hainan due to a generally higher overall NH$_4^+$ availability sustained by the compared to other regions much larger number of ponds per area.

4.2 Concentrations of dissolved nitrogen as tracer for effluent dispersal

High concentrations of NH$_4^+$ and NO$_3^-$ close to shore compared to the offshore sites in Chang qi gang and Qingge, indicate land-derived nitrogen enrichment at the two coastal sites that host large areas of shrimp and fish ponds in their hinterland. The higher NH$_4^+$ and NO$_3^-$ concentrations in coastal waters of Chang qi gang relative to Qingge further point to a correspondence of coastal DIN concentrations to annual nitrogen exports from aquaculture ponds, which is about four times higher in Chang qi gang than in Qingge (Tab. 1). In Chang qi gang and Qingge, nitrogen concentrations decrease in offshore direction to concentrations similar to those found close to shore at the control site Ye Lin. This results on the one hand as a function of dilution with nutrient-poor oceanic waters indicated by a salinity increase from ~27 close to shore to ~33 near the reef crest (Herbeck et al., subm.), and on the other hand by nitrogen uptake of primary producers and microbes. Increasing NH$_4^+$ and NO$_3^-$ concentrations with distance from the shore at Ye Lin, however, indicate nitrogen input to the offshore region. This is likely due to transient exposure to the river plume of the WWE. Nutrient export from the WWE was low under dry conditions, due to efficient conversion of nutrients inside the estuarine lagoon of the WWE (Herbeck et al., 2011). During strong rain events, however, high run-off caused direct freshwater and nutrient export from the estuary to coastal waters. Depending on tidal currents, the river plume was occasionally deflected in northward direction influencing the outer coastal stations at Ye Lin as confirmed by a salinity range between 12 and 33. This is also confirmed by the specific DIN composition at the offshore stations in Ye Lin. While a higher NH$_4^+$ contribution to the DIN in Chang qi gang and Qingge points to NH$_4^+$-rich aquaculture effluents as nitrogen source (Tab. 2), NO$_3^-$ dominance at the offshore stations at Ye Lin rather point to impacts of NO$_3^-$-rich estuarine water being exported to the coast after rain events (Herbeck et al., 2011) as a result of locally heavy rain showers during the sampling time. Therefore, elevated NH$_4^+$ and NO$_3^-$ concentrations at those stations are likely not related to effluents released from aquaculture ponds in the Ye Lin region, but to a mixture of effluents from sewage, aquaculture and agriculture drained into the WWE (Herbeck et al., 2011; Liu et al., 2011; Unger et al., subm.).

Although concentrations of NH$_4^+$ and NO$_3^-$ showed spatial trends in nitrogen dispersal, those were usually not statistically significant. This was mainly because of a high short-term variability in concentrations due to tidal mixing, variable uptake and variable nutrient supply from the aquaculture ponds, which masked spatial gradients.
4.3 Chl a and nitrogen and δ¹⁵N contents of suspended matter as indicator for biotic response to anthropogenic nitrogen

High chl a concentrations at stations with high NH₄⁺ and NO₃⁻ concentrations indicate stimulation of planktonic primary production. In Chang qi gang and Qingge, concentrations of chl a declined with decreasing nutrient availability in offshore direction to concentrations close to 1 μg L⁻¹, the maximum found in other reef areas (Furnas et al., 1990; Liston et al. 1992; Van Duyl et al., 2002; Otero and Carbery, 2005). In contrast, increasing chl a concentrations in offshore direction at Ye Lin indicate elevated stimulation of primary production due to dissolved nitrogen from the river plume or contribution of phytoplankton exported from the WWE.

TN and δ¹⁵N of TSM mirrored the spatial trends observed for NO₃⁻, NH₄⁺ and chl a. A higher TN-TSM reflects a high in-situ production as well as storage of excess nitrogen by phytoplankton, both pointing to a high nitrogen availability close to shore in Chang qi gang and Qingge and at the offshore stations at Ye Lin. Irrespective the effect of fractionation during planktonic nitrogen uptake, TSM close to shore in Qingge and Chang qi gang is characterized by high δ¹⁵N values of up to 13‰ strongly suggesting assimilated dissolved nitrogen to originate primarily from aquaculture effluents (Tab. 2). In contrast, δ¹⁵N values <6‰ near the shore in Ye Lin do not indicate any distinct influence of aquaculture effluents at this site. While decreasing δ¹⁵N-TSM in offshore direction at Chang qi gang and Ye Lin suggest decreasing impact of effluents and the admixture of ¹⁵N-depleted marine water, increasing δ¹⁵N of TSM at the most offshore station at Ye Lin points to exposure to dissolved nitrogen from the river plume of the WWE. This is consistent with δ¹⁵N values of the TSM in the river plume (Herbeck et al. 2011) as well as higher NO₃⁻ concentrations at this station.

Average δ¹⁵N of TSM at the 50 m station in Chang qi gang was exceptionally low (4.8‰) while TSM at all other stations of this location was more enriched in ¹⁵N (6.7-7.6‰). In fact, TSM in two shrimp ponds in direct adjacency to this station also had exceptionally low δ¹⁵N signals of 2.8‰ and 4.3‰, probably due to the application of lighter nitrogen fertilizers. It is therefore conceivable that the 50 m station received effluents from those recently fertilized ponds draining effluents directly into the sea, while all other stations at Chang qi gang were rather influenced by aquaculture effluents exported from the tidal creek, which received effluents of fish and shrimp ponds being more enriched in ¹⁵N.

Although, the temporal variability was less than that of DIN and chl a concentrations, results of TN and δ¹⁵N in TSM only revealed few statistically significant trends along individual transects. Most likely, this was due to a fast response of small-cell planktonic organisms to specific nutrients, which are therefore incapable in
integrating a signal over time. Besides that, the TN and δ^{15}N analysis not only reflects the pure phytoplankton but the bulk of TSM, which may contain various amounts of allochthonous matter that offset the informative value of those parameters for in-situ processes. Variability in chl a values may also be associated with variable light conditions and grazing affecting phytoplankton abundance.

4.4 Bioindicators as tracers for effluent dispersal

4.4.1 Seagrass and epiphytes: tissue N and δ^{15}N contents

TN contents in *T. hemprichii* in Hainan were within the same range than values between 2.5 and 3.2% found in previous studies (Yamamuro et al., 2003). Clear spatial gradients in TN contents in seagrass leaf and epiphyte tissue along the distance transect were not evident in Qingge and Chng qi gang. At Ye Lin we observed decreasing values towards offshore sites opposing the trends of water column parameters. This proves TN in seagrass and epiphyte tissue to be a poor indicator for describing small-scale spatial distribution of nitrogen enrichment.

In contrast, δ^{15}N in seagrass leaves and epiphytes displayed clear spatial trends similar to that of water parameters. Seagrass and epiphytes were mostly similarly enriched in ^15N as was suspended matter/phytoplankton. Overall average δ^{15}N of *T. hemprichii* (5.3-8.6‰) was much higher than measurements from the same species in rather unimpacted areas of Thailand, Japan and Australia (Yamanuro et al. 2003), as well as Palau (Yamanuro et al., 1995) and Indonesia (Evrard et al., 2005) reporting on values between 1.8 and 5‰. Similarly, average δ^{15}N of epiphytes (7.1-10.4‰) were substantially higher than values of epiphytes between 2.1 and 3‰ reported from unpolluted areas (Jennings et al., 1997; Lepoint et al., 2000) and of 6.5‰ from an area impacted by fish farms (Vizzini and Mazzola, 2004). This reflects the strong influence of aquaculture effluents in our study area relative to many other regions worldwide. Likewise, studies in NE Australia found δ^{15}N values of ~10‰ in seagrass tissue close to sewage impacted sites, while signals ranged between 2 to 5‰ at pristine sites (Udy and Dennison, 1997; Jones et al., 2001). This proves δ^{15}N in seagrass and epiphyte tissue as suitable indicator to detect small-scale differences in nitrogen exposure of polluting sources.

Seagrass tissues did, however, not appear to be sensitive to short term changes in nitrogen availability, since the TN and δ^{15}N signal in seagrass tissue did not change over time despite increasing concentrations of DIN in course of the study period as a result of rain events (Fig. 5). This is likely due to a relatively long seagrass
turn-over time with leaf growth values of *T. hemprichii* of approximately 1.8 cm per shoot and day (Terrados et al. 1999). Furthermore, seagrasses may display complex strategies to meet their nitrogen requirements and often rely on a variable mixture of nutrients from the water column and nutrients from the pore water pool (Touchette and Burkholder, 2000). This makes them less suitable as bioindicators for fast changes compared to many algae that take up nitrogen exclusively from the water column and have a much faster turnover. In fact, TN and δ¹⁵N in epiphyte tissue did show an immediate response to increasing DIN concentrations over the study period (Fig. 5). Elevated δ¹⁵N in epiphyte tissue at the end of the study period points to exposure to elevated nitrogen primarily originating from aquaculture effluents or uptake of ¹⁴N-depleted nitrogen due to strong previous fractionation of the high DIN concentrations provided with the rain.

Anyhow, the suitability of epiphytes as bioindicators appears limited since the bulk material on top of seagrasses may vary in their composition between the different study sites to compare, which may affect the TN and/or δ¹⁵N content. On the one hand, epiphytes may be composed of different algae species that may have species-specific uptake and fractionation capacities. On the other hand, the bulk of epiphytes may contain different amounts of sediments trapped in the filaments of epiphytic algae, which do not reflect water column conditions at a site but may originate from other (e.g. terrestrial) sources. From visual observations, we assume high contributions of sediment in the epiphyte loads, especially in Qingge and Chang qi gang. An intermixture with sediments, which are usually poor in nitrogen and of different isotopic composition, could therefore bias the TN and δ¹⁵N signals of the “true epiphytes”.

### 4.4.2 Phytoplankton bioassay: Chl a increase and tissue N and δ¹⁵N contents

The phytoplankton bioassay showed significant enhancement of phytoplankton biomass as well as of nitrogen contents and δ¹⁵N signatures of the incubated phytoplankton at the majority of stations (Fig. 6, 7).

Likewise trends displayed by water, seagrass and epiphyte parameters, chl a increments over the incubation time in the phytoplankton bioassays with unfiltered water reflect highest nutrient availability close to shore decreasing in offshore direction in Chang qi gang and Qingge, but reveal an opposite trend at Ye Lin. While a clear linear decrease in chl a increments was observed in Qingge, this did not account for Chang qi gang. It is likely that phytoplankton growth in the dialysis bags at Chang qi gang was temporarily light limited due to rain-related flush-out of particles from the adjacent tidal creek during the incubation time. This is further indicated by high TSM concentrations during the experiment between 25-55 mg L⁻¹ (unpublished data).
Generally, the chl a increase (up to 31 $\mu$g L$^{-1}$) was higher than in most other published phytoplankton bioassays reporting on increases from 0.2 to $<4$ $\mu$g L$^{-1}$ over a 4 day incubation (Dalsgaard and Krause-Jensen, 2006). This indicates substantially higher nutrient availability and related growth conditions at our study area.

In the treatment with the filtered phytoplankton, growth increments were also high in Qingge and Chang qi gang, while chl a concentrations even decreased in Ye Lin implying negative phytoplankton growth and degradation of the phytoplankton initially present in the dialysis bags at the end of the incubation time. Higher chl a increase in bags with unfiltered than with filtered water was opposite to results of a phytoplankton bioassay in the mainly oligotrophic Mediterranean Sea (Pitta et al., 2009). There, grazing inhibited any significant increase of primary production due to nitrogen derived from fish farm wastes in bags with unfiltered water. On the contrary, top down control on phytoplankton by grazers seemed not to be of relevance in the shallow coastal system of Hainan. In fact, a lower initial biomass in the bags with the filtered water than in those with unfiltered water indicates that filtration rather removed large phytoplankton than zooplankton. In contrast to the Mediterranean, where the phytoplankton community consisted mainly of small cell-size primary producers (Pitta et al., 2009), the phytoplankton in the study is mainly composed of species larger than 50 $\mu$M (Maier, 2010), which are not easily grazed by zooplankton. Therefore, it is likely that in coastal back-reef areas, zooplankton is of minor importance in controlling phytoplankton biomass.

The significant increase of TN and $\delta^{15}$N in the phytoplankton bioassays at all stations in Qingge and Chang qi gang suggests that the whole back-reef areas exceeding distances of 2500 m from the shore are affected by aquaculture effluents, though with decreasing intensity in offshore direction. The enormous increase in $\delta^{15}$N in the bioassay to values close to those measured in aquaculture ponds and drainage channels clearly confirms that nitrogen enrichment in the back-reef areas is derived from the aquaculture ponds. Elevated $\delta^{15}$N after incubation in the bioassays close to shore in Ye Lin reflect exposure to aquaculture waste, despite the relatively low abundance of ponds at this site. This indicates that $\delta^{15}$N and TN in bioassays are very sensitive tracers even detecting low amounts of nitrogen input, which were not detected by any other tracers. Unchanged $\delta^{15}$N in the bioassays after incubation at the offshore stations at Ye Lin indicate a nitrogen source other than pond effluents to cause the enhanced nitrogen availability reflected by the elevated nitrogen content in the bioassays at these stations. As discussed before, this is likely related to waters exported from the WWE influenced by various nitrogen sources such as agriculture, urban wastes and aquaculture ponds and floating net cages (Herbeck et al., 2011).
Compared to other studies using bioassays, the growth increments of TN and \( \delta^{15}N \) over the incubation were substantially higher in our phytoplankton bioassays. Growth increments of the %TN content from 0.3-1.7% mostly exceeded the maximum growth increments of the %TN of 1.1% determined in macroalgae assays next to offshore fish farms in the Mediterranean (García-Sanz et al., 2011) and that of 0.7% next to a relatively small shrimp farm in Polynesia (Lin and Fong, 2008) after 3-4 day incubation. Also the relative increases in \( \delta^{15}N \) of up 7.1‰ were exceptionally high compared to those studies reporting maximal relative increases of 1.3‰ (García-Sanz et al., 2011) and 0.8‰ (Lin and Fong, 2008). In our study, TN and \( \delta^{15}N \) in the phytoplankton bioassay reflected the exposure to aquaculture effluents at stations at distances of >2500 m from the shore contrasting the results from other studies, where effects were detectable only up to 500 m distance from land-based shrimp farm (Lin and Fong, 2008) and between 100-2000 m from fish farms (García-Sanz et al., 2011). The strong increase in TN and \( \delta^{15}N \) at sites close to shore and the detectability of effects at larger distances are most likely related to the much larger aquaculture pond area and the related large amount of effluents in Hainan compared to e.g. the study in Polynesia, where the shrimp farm only covered 2 ha, representing only half the size of the pond area in our least affected site in Ye Lin. This indicates that TN and \( \delta^{15}N \) in bioassays with primary producers not only serve as suitable qualitative indicators of nutrient enrichment from aquaculture effluents, but that these parameters may also reflect the impact related to the size of the farm area. Generally, the modified set-up of the phytoplankton bioassay proved appropriate for shallow back-reef areas under microtidal regime. Thus, the phytoplankton bioassay serves as a suitable method, especially in areas that lack the occurrence of macroalgae, which could else be used for a bioassay.

5. Summary and Conclusion

Different approaches were applied to study the spatial extent of nitrogen dispersal released from three different coastal pond areas varying in size into back-reef areas. While shrimp pond effluents have previously only been presumed to be enriched in \( ^{15}N \), this is the first study that proved ammonium and nitrate in aquaculture ponds to have high \( \delta^{15}N \) values. This confirms the suitability of \( \delta^{15}N \) as a tracer for pond effluents in coastal systems. For the first time, we used a phytoplankton bioassay to successfully trace nutrients released from aquaculture ponds. While a high short-term variability in standard water quality parameters such as DIN and chl a as well as TN
and $\delta^{15}\text{N}$ in TSM masked significant spatial gradients, the bioassay gave the clearest results. TN and $\delta^{15}\text{N}$ increase over time in the bioassay proved to be the best indicators, because compared to chl a increase, those parameters are less affected by light conditions. Therefore, TN and $\delta^{15}\text{N}$ increases in a phytoplankton bioassay can be regarded as a very precise tool to trace the spatial extension of pond effluent derived nutrient enrichment in coastal waters. TN and $\delta^{15}\text{N}$ tissue contents of seagrasses and epiphytes also indicated general trends, but were less specific in determining spatial gradients in exposure to pond effluents. However, this approach is an easy as well as less time and resources consuming way to investigate if an area is exposed to pond effluents, and serves as excellent indicator for large-scale comparison e.g. between different countries.

All approaches indicated significant impact of aquaculture effluents close to shore at the sites Chang qi gang and Qingge, whereas the impact close to shore at the control site Ye Lin was low. Increasing nitrogen concentrations further offshore at this site, however, indicated temporal exposure to the river plume of the Wenchang/Wenjiao Estuary transporting a mixture of nitrogen-rich effluents from urban sewage, aquaculture and agriculture. Despite decreasing concentrations of dissolved nitrogen in offshore direction at Qingge and Chang qi gang, elevated $\delta^{15}\text{N}$ in seagrasses, epiphytes and TSM as well as $\delta^{15}\text{N}$ increases in phytoplankton in the bioassay after incubation revealed persisting exposure to effluents. This effect was detected up to a distance of 1000 m at Qingge and 2500 m at Chang qi gang. This indicates that enhanced phytoplankton growth as revealed by high in-situ chl a concentrations and enormous chl a increase in a phytoplankton bioassay have to be related to nutrient rich pond effluents affecting the entire back-reef areas. This is likely reinforced by the limited tidal mixing in those partly enclosed coastal areas. Thus, we concluded that large-scale pond aquaculture endangers present seagrass meadows and coral colonies in NE Hainan. Our results emphasize the necessity to develop technical solutions to reduce nutrient inputs from aquaculture ponds into coastal areas.

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Presentations and further co-author publications

First author presentations on international conferences:


Co-author presentations on international conferences:


Further co-author publications:


Sources and diagenesis of organic matter in a sediment core in mangrove tidal flat during the past 100 years--- a multi-biomarker approach.

Journal: Continental Shelf Research  
Current status: Submitted  
Contributions: L. Herbeck contributed the $\delta^{15}$N data, wrote the part of the study area section that is related to aquaculture and contributed to the manuscript text.


Biogeochemical behavior of organic carbon in a small tropical river/estuary, Hainan.

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Hiermit erkläre ich, dass ich die Arbeit mit dem Titel:

“Ecological impact of land-derived anthropogenic nutrients and organic matter on tropical estuarine and coastal systems of Hainan, China”

selbständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

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