

Pond aquaculture effluents traced along back-reef waters by standard water quality parameters, $\delta^{15}\text{N}$ in suspended matter and phytoplankton bioassays

Lucia S. Herbeck^{1,*}, Daniela Unger^{1,2}

¹Leibniz Center for Tropical Marine Ecology, 28359 Bremen, Germany

²Present address: DESY Deutsches Elektronen-Synchrotron, Forschungszentrum der Helmholtz-Gemeinschaft, 22607 Hamburg, Germany

ABSTRACT: Despite the enormous growth in aquaculture in recent years, little is known of the impact of effluents from large-scale pond agglomerations on tropical coastal ecosystems. The aim of the present study was to evaluate the dispersal and ecological impact of effluents from fish and shrimp ponds along 3 seagrass-covered back-reef areas on Hainan Island, tropical China, with pond areas in their hinterland differing in extent (Yelin: 0.04 km²; Qingge: 2.4 km²; Changqi: 8.7 km²). Concentrations of dissolved and particulate nitrogen, chlorophyll *a* (chl *a*) and stable nitrogen isotopes of ammonium ($\delta^{15}\text{N-NH}_4^+$), nitrate ($\delta^{15}\text{N-NO}_3^-$) and suspended matter ($\delta^{15}\text{N-TSM}$) were used to trace pond effluents along transects perpendicular to the shore. Additionally, $\delta^{15}\text{N-TSM}$ and chl *a* samples were taken from a phytoplankton bioassay experiment, during which offshore surface water was incubated in dialysis bags at stations along these transects. High nutrient concentrations, particularly ammonium, in combination with high chl *a* ($\sim 10 \mu\text{g l}^{-1}$) and elevated chl *a* levels in the bioassays after incubation indicate eutrophication of the effluent-exposed back-reef areas Qingge and Changqi, with decreasing intensity in the offshore direction. We report the first $\delta^{15}\text{N-NH}_4^+$ of pond effluents which were as high as 17‰. Consequently, elevated $\delta^{15}\text{N}$ values in TSM (5 to 12‰) and a $\delta^{15}\text{N}$ increase from <7 up to 14‰ over time in the phytoplankton bioassays specified pond effluents as the predominant nutrient source. The effluents affect the entire back-reef areas over a distance of at least 2.5 km from shore. Our results show that analysis of $\delta^{15}\text{N}$ in phytoplankton bioassays is a powerful bioindicator for tracing pond-derived nutrient dispersal and eutrophication effects.

KEY WORDS: Aquaculture · Shrimp- and fish-pond effluents · Nitrogen stable isotopes · $\delta^{15}\text{N}$ · Phytoplankton bioassay · Back-reef area · Eutrophication · China

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INTRODUCTION

Production of shrimp and fish in pond aquaculture has undergone rapid development within the past 30 yr, 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

& Kiattisimkul 1996, Páez-Osuna 2001). Those effluents are especially enriched in nitrogen from animal feces, added feed and fertilizers (Burford & Williams 2001). Nutrient enrichment can lead to eutrophication of adjacent coastal waters and is one of the primary reasons for the worldwide degradation of seagrass meadows and coral reefs (McGlathery 2001,

*Email: lucia.herbeck@zmt-bremen.de

Bellwood et al. 2004, Lapointe et al. 2004, Waycott et al. 2009). China is the leading producer of aquaculture goods, accounting for 62% of global production in terms of quantity and 51% of global economic value (FAO 2010). Production is especially high in the southern parts of the country, where hundreds of hectares along the shoreline have been converted to ponds for the intensive culture of shrimp and fish. These ponds are often directly adjacent to seagrass meadows and coral reefs, although little is known of their effects on these coastal systems. Inorganic nutrient concentrations are often used for assessing the impacts of nutrient release into coastal and marine waters, although it is difficult to trace nutrient input and dispersal in coastal systems due to rapid dilution and biological uptake. Changes in hydrodynamic pattern may also affect distribution and uptake processes. Moreover, nutrient release from aquaculture farms may be pulsed and have large spatio-temporal variability (Herbeck et al. 2012). This results in highly variable nutrient regimes, making it difficult to relate an observed nutrient pattern at any one time to general input from aquaculture sources.

To overcome those limitations, bioindicators have recently been developed as a tool for tracing nutrient pollution in coastal and marine systems. Many algal species absorb and store excess nutrients and respond with increased growth rates to high nitrogen and phosphorus concentrations in the water (Fong et al. 2001). Thus, the growth rate and elemental composition of primary producers can reflect the conditions of the surrounding water body. Due to the longer turnover time of algal biomass compared to the turnover of nutrients in the water, algae yield an integrated signal of both persistent and pulsed nutrient inputs over time (Costanzo et al. 2001).

Stable nitrogen isotopes ($\delta^{15}\text{N}$) can be used to trace the sources of dissolved nutrients in different aquatic environments (e.g. Cifuentes et al. 1996, Udy & Denison 1997, McClelland & Valiela 1998, Thimdee et al. 2002, Piñón-Gimate et al. 2009). Individual sources of nitrogen to coastal ecosystems have distinguishable specific $\delta^{15}\text{N}$ signals associated with fractionation during different microbial processes, whereby the lighter ^{14}N is preferentially converted (Heaton 1986, Peterson & Fry 1987). The $\delta^{15}\text{N}$ value of nitrate ($\delta^{15}\text{N}\text{-NO}_3^-$) in marine water typically ranges from 5 to 7‰ (Miyake & Wada 1967, Wada et al. 1975), with deep water NO_3^- having mostly invariable values around 5‰ (Liu & Kaplan 1989, Sigman et al. 2000). Fertilizer-rich agricultural run-off typically has a very low $\delta^{15}\text{N}$ value close to 0‰ because ammonium (NH_4^+) and nitrate (NO_3^-) fertilizers are

usually produced by industrial fixation of atmospheric nitrogen (e.g. Lee et al. 2008). However, strong fractionation during volatilization of ammonia (NH_3) leads to a ^{15}N enrichment of the remaining NH_4^+ , which is subsequently converted to ^{15}N -enriched NO_3^- (Heaton 1986, Valiela et al. 2000). Especially strong volatilization processes from animal or sewage wastes usually lead to elevated $\delta^{15}\text{N}$ values of >10‰. Accordingly, it is suggested that dissolved nitrogen in aquaculture effluents is enriched in ^{15}N (Costanzo et al. 2001), despite missing evidence of $\delta^{15}\text{N}$ values from dissolved nitrogen to date.

Within this context, total nitrogen (TN) and $\delta^{15}\text{N}$ levels 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 & Mazzola 2004) and offshore fish farms (Sarà et al. 2006, Pérez et al. 2008) on estuarine and marine waters. Those studies report elevated TN and $\delta^{15}\text{N}$ values in plant tissues close to farms, indicating significant nitrogen enrichment in the respective areas.

Macroalgal and phytoplankton bioassays are effective tools for tracing effluents along horizontal gradients (Costanzo et al. 2001, Dalsgaard & Krause-Jensen 2006, Deutsch & Voss 2006, Pitta et al. 2009, García-Sanz et al. 2010, 2011). Macroalgae or phytoplankton are deployed at variable distances to the respective nitrogen source and incubated for a certain period of time. This approach provides a standardized experimental set-up and overcomes restrictions due to naturally irregular macrophyte distribution. Using this method, Dalsgaard & 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 $\delta^{15}\text{N}$ in macroalgae in >1 km distance from offshore fish farms in the Mediterranean and Atlantic. A single study also applied macroalgal bioassays to detect nutrient enrichment from a small shrimp farm in French Polynesia (Lin & Fong 2008) and reported elevated $\delta^{15}\text{N}$ values in *Acanthophora spicifera* tissue extending out to 800 m away from the shrimp farm.

Despite its effectiveness, the application of a macroalgal bioassay may be limited by the lack of suitable macroalgae. Instead, phytoplankton bioassays may be more advantageous as they can be applied everywhere due to the ubiquitous occurrence of phytoplankton. However, the suitability of $\delta^{15}\text{N}$ in phytoplankton bioassays for tracing land-based effluents in adjacent natural water bodies has

not been previously evaluated. The interpretation of $\delta^{15}\text{N}$ signals from phytoplankton bioassays, which usually contain several different phytoplankton species, may be more complicated than for a single species approach because N fractionation during assimilation is species dependent (e.g. Wada & Hattori 1978, Montoya & McCarthy 1995).

The aim of the present study was to make the first assessment of the influence of anthropogenic dissolved nitrogen released from large-scale shrimp and fish pond complexes on back-reef coastal waters. Our specific objectives were to quantify the extent and magnitude of nitrogen enrichment along offshore gradients in 3 back-reef areas in NE Hainan, tropical China, that are exposed to various nitrogen inputs from aquaculture ponds, using (1) standard water quality parameters (dissolved inorganic nitrogen [DIN], chlorophyll *a* [chl *a*] and total suspended matter [TSM]), (2) measurements of TN and $\delta^{15}\text{N}$ in suspended matter and (3) a phytoplankton bioassay (to determine changes in chl *a*, TN and $\delta^{15}\text{N}$ over time). The present work is the first to use phytoplankton bioassays to evaluate the impact of land-based

pond aquaculture on coastal waters and test the suitability of using TN and $\delta^{15}\text{N}$ obtained by phytoplankton bioassays to trace pond effluents. Furthermore, we determined for the first time $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{15}\text{N}\text{-NH}_4^+$ values from shrimp and fish pond effluents in order to verify the causal relationship between elevated ^{15}N in coastal waters and the hypothesized aquaculture nitrogen source.

MATERIALS AND METHODS

Study area

The study area is located on the NE coast of the island Hainan, South China, in the marginal tropics (Fig. 1a). Much of the study area was formerly covered in mangrove forests, but a large extent has been converted to aquaculture ponds. Coral reefs fringe parts of the coast between 0.5 and 4 km from the shore, and seagrass meadows dominated by *Thalassia hemprichii* exist in the back-reef areas (Fig. 1b). The maximum depth in the back-reef areas is 1.5 to

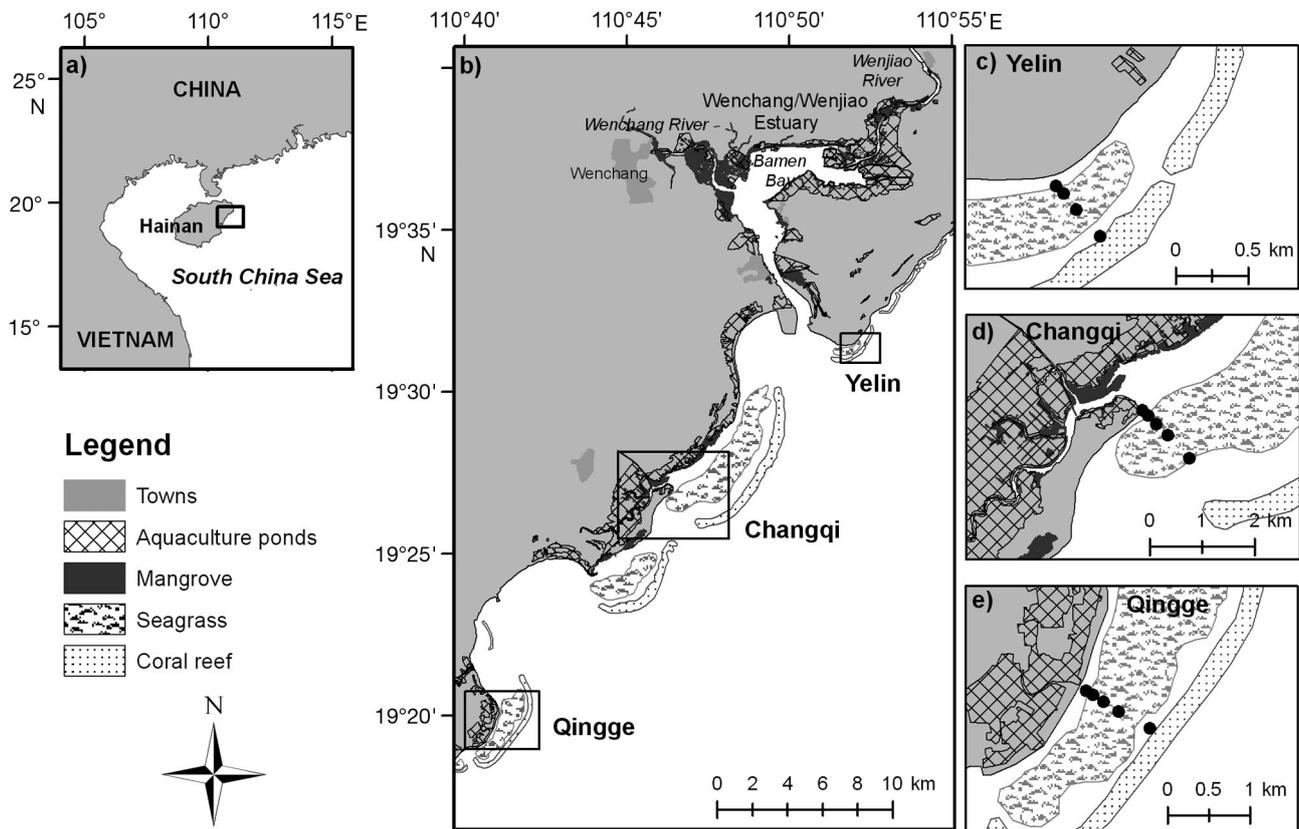


Fig. 1. (a) Location of the study area situated at the NE coast of Hainan, China and (b) overview of the study area with location of the 3 study sites (c) Yelin, (d) Changqi and (e) Qingge including position of the sampling stations (black dots) along a distance gradient from the shore and distribution of coastal habitats and aquaculture ponds

3 m (during spring high tide). The area is subject to mixed semidiurnal microtides with a tidal range of ~0.5 to 1.5 m at neap and spring tide, respectively, causing large parts of the back-reef areas to be 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 (Huang 2003, Wang et al. 2008). Mean air temperatures range between 14.6 and 20.8°C in January and 25.2 and 33.1°C in July.

During the past 30 yr, the area has become an important site for the production of shrimp and fish in semi-intensive and intensive brackish water pond aquaculture. Ponds cover an area of ~39.6 km² along ~45 km of shoreline in the study area (Herbeck et al. 2012). 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* (giant grouper). A total of 3 to 4 crops of shrimp and 1 crop of fish can be cultured per year. Aquaculture effluents are released directly into the environment without prior treatment. They are either drained directly from the ponds into estuarine or coastal waters or reach there via natural creeks or drainage channels. The operation of the brackish pond aquaculture in NE Hainan is described in detail by Herbeck et al. (2012).

The present study was carried out during March and April 2009 at the transition between the dry and rainy seasons. We chose 3 back-reef areas that varied in extent of pond aquaculture production in their hinterland and received different loads of nitrogen: (1) Yelin—low pond exposure, (2) Qingge—medium pond exposure and (3) Changqi—high pond exposure (Fig. 1b–e). Nitrogen loadings and other characteristics of the study sites are summarized in Table 1. The site Yelin was close to the outlet of the Wenchang/Wenjiao Estuary (WWE) and was temporarily affected by its outflow (Fig. 1b).

Water sampling

Water from shrimp ponds (n = 6), fish ponds (n = 20) and drainage channels (n = 18) randomly selected over the study area was collected by submersing a bottle from the pond/channel edge. In the back-reef areas of each site, sampling stations were

established along a perpendicular transect reaching from the shoreline to the reef crests. Individual stations were situated at 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). Buoys were deployed to mark the positions of the stations. Surface water samples were collected from each coastal station every time the sites were visited (n = 4 to 12).

Water for the analysis of DIN (= NO₃⁻ + NO₂⁻ + NH₄⁺) was filtered immediately after sampling through single-use Sartorius Minisart® membrane filters (0.45 µm pore size). The filtrate was stored in polyethylene (PE) bottles (pre-rinsed 3 times with the filtered water), preserved with a mercury chloride solution (50 µl of a 20 g l⁻¹ HgCl₂ solution added to 100 ml sample) and stored refrigerated until analysis at our laboratory in Germany. For the determination of total suspended matter (TSM), nitrogen content (TN-TSM) and nitrogen isotopic composition (δ¹⁵N-TSM) as well as chlorophyll *a* (chl *a*) and stable nitrogen isotopes of ammonium and nitrate (δ¹⁵N-NH₄⁺ and δ¹⁵N-NO₃⁻), field water samples were stored in PE tanks in the dark until vacuum filtration in the field laboratory within the same day.

Phytoplankton bioassay

Phytoplankton bioassays were set up at each station in the back-reef areas based on the method of Dalsgaard & Krause-Jensen (2006). Dialysis tubing (flat width 12 cm, Spectra/por 1 dialysis membrane, regenerated cellulose with a molecular weight cut-off of 6 to 8 kDa) was cut into 30 cm long pieces, which were sealed at 1 end using cable ties to make dialysis bags. All dialysis bags at each site were filled with the same water collected ~500 m offshore of the reef crest of the respective site. The filling water of 4 of the 7 bags deployed at each station was filtered through a 50 µm sieve to carry out the

Table 1. Size of pond and back-reef areas, average export of aquaculture effluents and nitrogen loadings (DIN: dissolved inorganic nitrogen, DON: dissolved organic nitrogen, PN: particulate nitrogen, TN: total nitrogen) from pond aquaculture at the 3 investigated study sites (Herbeck et al. 2012)

| Site | Back-reef area (km ²) | Pond area (km ²) | Effluent export (10 ⁶ m ³ yr ⁻¹) | N export (t yr ⁻¹) | | | |
|---------|-----------------------------------|------------------------------|--|--------------------------------|------|-------|-------|
| | | | | DIN | DON | PN | TN |
| Yelin | 1.5 | 0.04 | 0.4 | 0.4 | 0.3 | 1.2 | 1.9 |
| Qingge | 8.4 | 2.4 | 23.7 | 21.9 | 16.2 | 67.7 | 105.8 |
| Changqi | 23.2 | 8.7 | 87.9 | 81.3 | 60.2 | 251.1 | 392.7 |

bioassay experiment with and without grazer exclusion as also done by Pitta et al. (2009). Samples of the filtered and the untreated filling water were additionally collected in plastic containers. After filling, the dialysis bags were closed with cable ties. Each dialysis bag had a volume of ~700 ml and a diameter of ~7.6 cm. The bags were placed into an envelope made from transparent nylon fishing net with a mesh size of 2 cm. Slight modifications to the method of Dalsgaard & Krause-Jensen (2006) were made for the deployment of the dialysis bags to adapt the experiment to conditions in a shallow reef lagoon with tidal effects. At stations with a minimal ebb tide depth of <1 m (i.e. all but the station furthest offshore at each site), 2 metal poles (90 cm long) were hammered ~40 cm into the sediment, and two 1.5 m long ropes were spanned between the poles, to which the bags were tied with cable ties (Fig. 2a). This set-up aimed to prevent the fragile dialysis bags from being dragged over the coral rubble at low tide, which could have caused breakage of the dialysis membranes. During the period of the bioassay experiment (13 to 20 April 2009), tide-related variations in water level were ~90 cm in

daylight periods, with a water depth of ~60 cm in the mornings and late afternoons and ~150 cm around midday. At stations with a minimal ebb tide depth of >1 m (i.e. the station furthest offshore at each site), bags were tied to a buoyant plastic tube, which was anchored ~50 cm beneath the surface by a rope (our Fig. 2b; Dalsgaard & Krause-Jensen 2006). After an incubation period of 4 d, the water content of the bags was transferred into PE bottles. The samples of initial filling water and water from the incubated dialysis bags were stored cool and dark until further processing and analysis for chl *a* concentrations, TN content and $\delta^{15}\text{N}$.

Sample processing and analytical procedure

In the field laboratory, a defined aliquot volume of each water sample was filtered under mild pressure onto pre-combusted (5 h, 450°C) and pre-weighed Whatman GF/F filters. The filters for the analysis of TSM, TN-TSM and $\delta^{15}\text{N}$ -TSM were dried at 40°C, while those for chl *a* analysis were frozen. One liter of the filtrate from the aquaculture pond and drai-

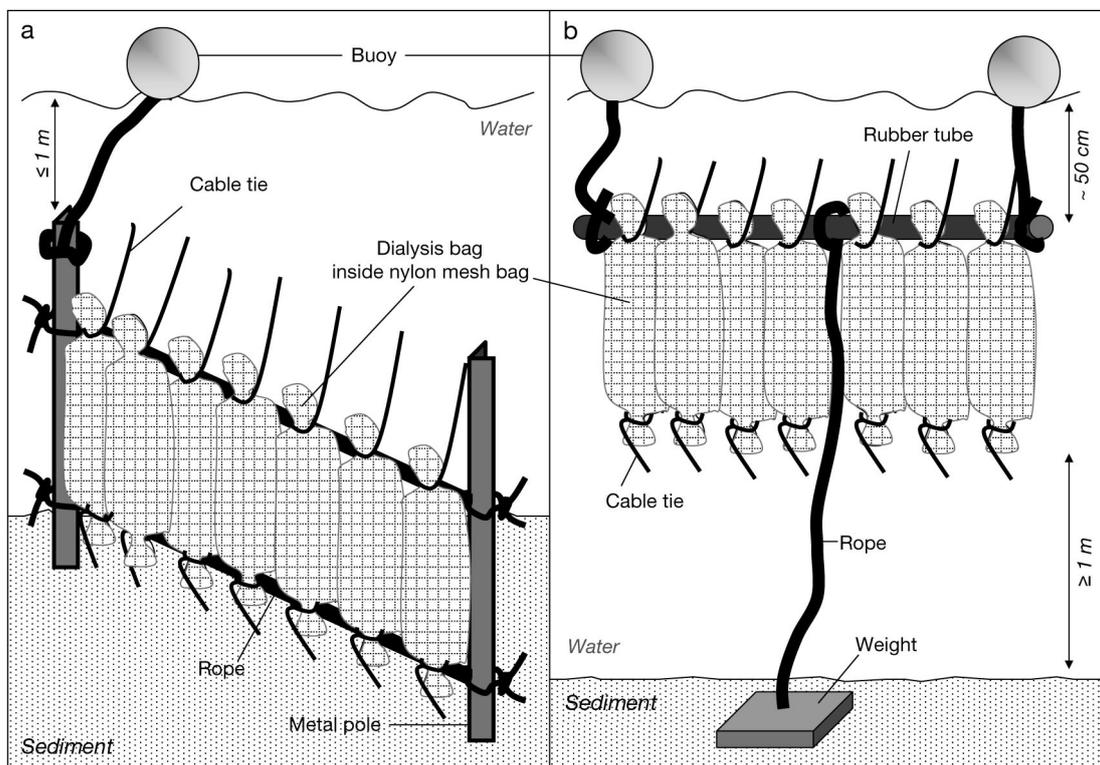


Fig. 2. Set-up of phytoplankton bioassays (a) attached to the sea bottom if minimum depth <1 m or (b) buoyant at a minimum depth >1 m. See 'Materials and methods — phytoplankton bioassay' for details

nage channel samples was acidified to pH 1.5 with concentrated HCl and stored frozen in PE bottles pre-washed with a 10% HCl solution for later analysis of $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$.

Chl *a* concentrations were determined by 24 h extraction of the pigments from the frozen filters in 10 ml of 90% acetone at 4°C under dark conditions with constant shaking. The extracts were subsequently centrifuged at 60 rpm for 3 min, and the chl *a* in the supernatant was determined with a TURNER 10-AU field fluorometer following the method of Arar & Collins (1997). The TSM concentration was calculated by dividing the difference between the weight of the dried filters and the original weight of the empty filter by the respective volume of water filtered. TSM was analyzed for TN by high-temperature combustion (1100°C) in a Carlo Erba NA 2100 elemental analyzer (Verardo et al. 1990). Measurements had a precision of 0.02%, based on repeated measurements of an internal standard (LECO 1012). $\delta^{15}\text{N-TSM}$ was determined with a Thermo Finnigan Delta Plus gas isotope ratio mass spectrometer (MS) after high temperature combustion (1000°C) in a Flash 1112 EA elemental analyzer (EA). $\delta^{15}\text{N}$ is given as the ‰ deviation from atmospheric air. IAEA N1 and N2 were used as international reference standards. The precision of the method, determined by repeated measurements of internal standards (Pepton and OAS), was 0.2‰. DIN constituents were analyzed using a continuous flow injection analyzing system (Skalar SAN⁺⁺⁺System), by which NO_x^- ($\text{NO}_3^- + \text{NO}_2^-$) were detected spectrophotometrically and NH_4^+ fluorometrically as a colored complex (Grasshoff et al. 1999). Respective determination limits were 0.08, 0.03 and 0.06 μM , and the coefficient of variation of the procedure was <3.4%. For the analysis of $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$, NH_4^+ and NO_3^- were extracted from the water onto acidified Whatman GF/D glass fiber filters using modifications 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 $\delta^{15}\text{N-TSM}$ above. For analysis, samples needed to have NH_4^+ and NO_3^- concentrations $\geq 8 \mu\text{M}$, which mostly applied only to those from aquaculture ponds and drainage channels. In short, NH_4^+ in $\leq 500 \text{ ml}$ water was converted to NH_3 at pH ~ 11 , which was trapped on an acidified filter packed between 2 Teflon membranes. Subsequently, this step was repeated after reduction of NO_3^- to NH_4^+ in the remaining water samples, using Devarda's alloy. The filters were dried and packed into silver (Ag) caps for

later combustion. Double or triplicate measurements were carried out for each sample. Laboratory internal nitrate and ammonium standards and blanks were processed the same way as samples. Overall, the methods had an analytical precision of $\leq 1\%$.

Statistical analysis

Statistical analyses were performed using SIGMA-PLOT 12.0. The data were tested for normal distribution before choosing parametric or non-parametric statistical methods. Two-way analysis of variance (ANOVA or ANOVA on ranks) followed by Tukey's post hoc test was performed to analyze variance and determine significant differences ($p < 0.05$) between stations along the distance gradient and between sites. Student's *t*-test or the Mann-Whitney *U*-test was used to determine significant differences between initial and end concentrations of the bioassay experiment. Spearman rank order correlation analysis was performed to test for significant correlations.

RESULTS

Nitrogen and its isotopic composition in aquaculture effluents

All dissolved and particulate nitrogen components showed high mean concentrations and $\delta^{15}\text{N}$ values in shrimp ponds, fish ponds and drainage channels (Table 2). However, large ranges and standard deviations observed in most parameters indicated considerable variability between the different ponds and drainage channels analyzed. Mean NH_4^+ concentrations in shrimp ponds, fish ponds and drainage channels were higher than NO_3^- concentrations, indicating that NH_4^+ typically accounted for the major part of the DIN (63% on average), while NO_3^- typically accounted for one third, and NO_2^- was present in low concentrations. The range of NH_4^+ and NO_3^- concentrations was, however, similar. In shrimp ponds, mean TN-TSM concentrations were lower than in fish ponds and drainage channels and were lower than mean DIN concentrations. In fish ponds, nitrogen was higher in the particulate than in the dissolved fraction, whereas in drainage channels, dissolved and particulate nitrogen levels were about equal. Fish ponds and drainage channels had significantly lower mean $\delta^{15}\text{N-NO}_3^-$ values (7.1 and 6.5‰) than $\delta^{15}\text{N-NH}_4^+$ values (16.2 and 18.1‰), whereas mean $\delta^{15}\text{N-TSM}$ values were at an intermediate level (9.1 and 11.4‰).

Table 2. Concentration (mean, standard deviation [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. TN: total nitrogen; TSM: total suspended matter; nd: no data

| | DIN (μM) | NH_4^+ (μM) | NO_3^- (μM) | NO_2^- (μM) | TN-TSM (μM) | $\delta^{15}\text{N-NH}_4^+$ (‰) | $\delta^{15}\text{N-NO}_3^-$ (‰) | $\delta^{15}\text{N-TSM}$ (‰) |
|--------------------------|--------------------------|--------------------------------------|--------------------------------------|--------------------------------------|-----------------------------|-------------------------------------|-------------------------------------|----------------------------------|
| Shrimp ponds | | | | | | | | |
| Mean | 88.8 | 56.1 | 26.4 | 0.5 | 53.0 | 15.4 | nd | 8.8 |
| SD | 121.4 | 65.7 | 44.6 | 0.4 | 51.1 | 0.8 | | 6.4 |
| Range | 19.9–332 | 7.7–179 | 4.3–117 | 0.1–1.0 | 5.0–106 | 14.9–16.0 | | 2.8–20.8 |
| n | 6 | 6 | 6 | 6 | 4 | 2 | | 6 |
| Fish ponds | | | | | | | | |
| Mean | 76.4 | 48.3 | 25.5 | 2.7 | 131.4 | 16.2 | 7.1 | 11.4 |
| SD | 60.0 | 41.6 | 35.1 | 2 | 134.5 | 9.1 | 3.5 | 4.3 |
| Range | 6.4–200 | 3.0–122 | 0.0–145 | 0.2–6.7 | 18–471 | 2.6–24.2 | 2.9–12.1 | 5.9–18.6 |
| n | 20 | 20 | 20 | 20 | 14 | 7 | 7 | 13 |
| Drainage channels | | | | | | | | |
| Mean | 100.0 | 63.4 | 37.3 | 2.8 | 98.7 | 18.1 | 6.5 | 9.1 |
| SD | 80.3 | 56.3 | 41 | 3.1 | 69.8 | 4.0 | 2.4 | 3.1 |
| Range | 10.1–284 | 3.4–189 | 0.4–163 | 0.2–11.2 | 0.7–213 | 12.8–24.8 | 3.2–10.4 | 5.4–15.1 |
| n | 18 | 17 | 18 | 18 | 12 | 9 | 9 | 14 |

Distribution of dissolved and particulate nitrogen, TSM and chl *a* in coastal waters

There was a strong temporal variability in all parameters measured in the surface water, as indicated by the large data ranges at most stations (Fig. 3). NH_4^+ and NO_3^- concentrations were usually on similar levels in Yelin, whereas DIN was dominated by NH_4^+ in Qingge (~75%) and in Changqi (~60%) (Fig. 3a,b, Table 3). Highest median concentrations of NH_4^+ (9.2 μM) and NO_3^- (7.3 μM) were found in Changqi close to shore, followed by nearshore concentrations in Qingge. At both sites, NH_4^+ and NO_3^- concentrations decreased significantly with increasing distance from the shore (Table 4). While at Changqi this decrease was almost linear, concentrations at Qingge decreased strongly within the first 250 m from the shore and then remained constant. Comparatively higher NO_3^- concentrations were, however, measured at the station furthest offshore at both sites. At Yelin, NH_4^+ concentrations remained fairly constant along the transect (~2.5 μM on average), while NO_3^- concentrations even increased slightly between 50 and 500 m distance from the shore and only decreased to almost 0 μM at the station furthest offshore. Overall, NH_4^+ and NO_3^- concentrations were significantly higher at Changqi than at the other 2 sites.

TSM, chl *a* and TN concentrations were positively correlated to each other except at Yelin, where a negative relationship of TSM with TN and chl *a* was observed (Fig. 3c–e, Tables 3 & 4). At Qingge and

Changqi, median concentrations were highest close to shore and decreased toward offshore stations. Nearshore stations, however, displayed a large concentration range especially at Qingge. At Yelin, the opposite trend was observed, with highest TN and chl *a* concentrations measured at the station furthest offshore. At Changqi, concentrations of all 3 parameters increased significantly with increasing NH_4^+ and NO_3^- concentrations.

Median $\delta^{15}\text{N-TSM}$ values were highest at the 50 m station at Qingge (9.8‰; Fig. 3f, Table 3). At this site, $\delta^{15}\text{N-TSM}$ decreased significantly with increasing distance and decreasing NH_4^+ , NO_3^- and TN-TSM concentrations (Table 4). Median $\delta^{15}\text{N-TSM}$ ranged from 5.6 to 6.7‰ at Yelin and from 6.4 to 8.2‰ at Changqi and were not correlated to distance from shore (Fig. 3f, Table 4). Overall, $\delta^{15}\text{N-TSM}$ values were significantly lower at Yelin than at the other 2 sites.

Phytoplankton bioassay

At the majority of stations at Qingge and Changqi, there was a significant increase in chl *a* concentrations after incubation in the dialysis bags, for both the filtered and unfiltered treatments. This chl *a* increase was most pronounced at the nearshore stations (Fig. 4). In the unfiltered treatment at Qingge, the chl *a* values after incubation decreased linearly with distance and were even lower than the initial concentration at 1000 m distance from the shore. In the

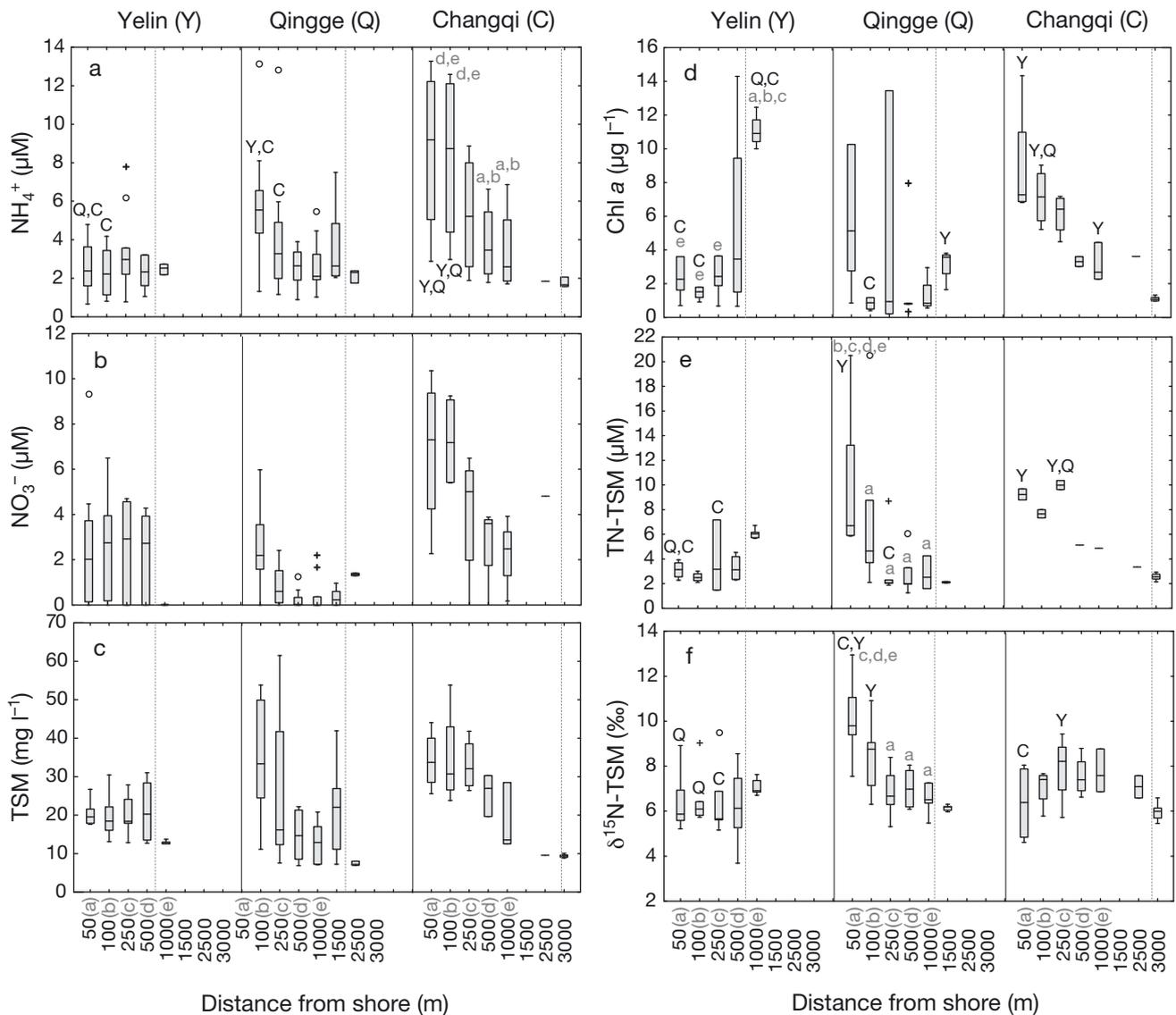


Fig. 3. Medians (midline) with 25 to 75% quartile (box) and non-outlier range (whiskers) of (a) NH_4^+ , (b) NO_3^- , (c) TSM, (d) chl *a*, (e) TN-TSM and (f) $\delta^{15}\text{N-TSM}$ over a distance gradient at the 3 study sites. Samples ($n \geq 4$) were taken at different days and tidal levels during the study period. The dotted vertical line represents the position of the reef crest. Outliers are marked as o; extremes are indicated as +. Corresponding results of the ANOVA tests are presented in Table 3. Results of the Tukey's post hoc test identifying significant differences ($p < 0.05$) between individual stations (50 to 1000 m) are incorporated into this figure: black capital letters indicate differences between sites at each distance, and small grey letters indicate differences in distance within a given site. For example, for panel (a), NH_4^+ concentrations at the 50 m stations were significantly lower at Yelin (Y) than at Qingge (Q) and Changqi (C), while at Changqi, NH_4^+ concentrations were significantly higher at (a,b) the 50 and 100 m stations than at (d,e) the 500 and 1000 m stations

filtered treatment at Qingge, there was an exceptionally strong chl *a* increase after incubation at the 100 m station. At Changqi, the post-incubation chl *a* values decreases from 0 to 500 m, then increased again from 500 m outwards. At Yelin, 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 at Yelin was at the 500 m station, while there

was a significant chl *a* decrease in the bags with filtered water at most stations of Yelin. The chl *a* increase in the bags with unfiltered water was at most stations higher than in those with filtered water. However, the initial chl *a* concentrations in bags with unfiltered water were also 2.2-fold higher at Qingge and 1.3-fold higher at Yelin than those in bags with filtered water, whereas initial concentrations of both treatments were similar at Changqi. The increase in

Table 3. Results of the 2-way-ANOVA as well as the Tukey's post hoc test for site and distance of the water quality parameters (for definitions see Tables 1 & 2) in coastal waters that are presented in Fig. 3. Results of the Tukey's post hoc test for an interaction between site and distance for the 50 to 1000 m stations are presented in Fig. 3 using small and capital letters. Yl: Yelin, Qg: Qingge, Cq: Changqi. Significance level: ns: $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

| Variable | Source of variability | df | F | Sig. level | Tukey's post hoc test |
|------------------------------|-----------------------|----|------|------------|-------------------------------|
| NH ₄ ⁺ | Site | 2 | 14.6 | *** | Cq ≠ Yl & Qg |
| | Distance | 4 | 3.91 | ** | 50 m ≠ 250 m & 500 m & 1000 m |
| | Site × Dist | 8 | 2.75 | ** | See Fig. 3a |
| NO ₃ ⁻ | Site | 2 | 11.1 | *** | Cq ≠ Yl & Qg |
| | Distance | 4 | 2.22 | ns | |
| | Site × Dist | 8 | 1.93 | ns | See Fig. 3b |
| TSM | Site | 2 | 5.66 | ** | Cq ≠ Yl |
| | Distance | 4 | 3.44 | * | 50 m ≠ 1000 m |
| | Site × Dist | 8 | 1.77 | ns | See Fig. 3c |
| Chl a | Site | 2 | 4.34 | * | Cq ≠ Qg |
| | Distance | 4 | 1.16 | ns | |
| | Site × Dist | 8 | 4.86 | *** | See Fig. 3d |
| TN-TSM | Site | 2 | 5.16 | * | Cq ≠ Yl & Qg |
| | Distance | 4 | 2.74 | * | 50 m ≠ 500 m |
| | Site × Dist | 8 | 3.52 | ** | See Fig. 3e |
| δ ¹⁵ N-TSM | Site | 2 | 11.9 | *** | Yl ≠ Qg & Cq |
| | Distance | 4 | 1.08 | ns | |
| | Site × Dist | 8 | 4.99 | *** | See Fig. 3f |

chl a concentrations in the bags over the incubation period generally tended to be higher as ambient median NH₄⁺ concentrations increased.

There were no significant differences in the TN-TSM and δ¹⁵N-TSM values in the bags between the filtered and unfiltered treatments. Therefore, the TN and δ¹⁵N results of both treatments were combined (Fig. 5). Except at 250 m at Yelin, TN contents were significantly elevated after the incubation period (Fig. 5a). At Qingge and Changqi, the TN increase was most pronounced close to the shore, where TN contents were on average 4- and 5-fold higher, respectively, than the initial values, and the increment decreased in offshore waters. At Yelin, TN contents were at similar levels over the distance gradient. Overall, the TN-TSM increase was significantly higher at Changqi than at Yelin and Qingge (Tukey's test; $p < 0.001$) and at 50 m than 500 m (Tukey's test; $p = 0.03$). The δ¹⁵N-TSM in the bags showed a significant increase at all stations of Qingge and Changqi and at the 50 and 100 m stations of Yelin after incubation (Fig. 5b). The increase was significantly higher at Qingge and Changqi than at Yelin (Tukey's test; $p < 0.001$) and at the 2 nearshore stations compared to the stations further offshore (Tukey's test; $p < 0.001$). The strongest δ¹⁵N increase was at Qingge at 50 m distance from the shore, where mean values

Table 4. Correlation of individual water column parameters (for definitions see Tables 1 & 2) at Yelin (Y), Qingge (Q) and Changqi (C) as indicated by the correlation coefficient (r^2) and level of significance determined by the Spearman rank order correlation analysis. Significance level: ns: $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

| | — NH ₄ ⁺ — | | — NO ₃ ⁻ — | | — TSM — | | — Chl a — | | — TN-TSM — | | — δ ¹⁵ N-TSM — | |
|------------------------------|----------------------------------|------------|----------------------------------|------------|---------|------------|-----------|------------|------------|------------|---------------------------|------------|
| | r^2 | Sig. level | r^2 | Sig. level | r^2 | Sig. level | r^2 | Sig. level | r^2 | Sig. level | r^2 | Sig. level |
| Distance | | | | | | | | | | | | |
| Y | 0.04 | ns | -0.07 | ns | -0.42 | * | 0.6 | ** | 0.55 | * | 0.28 | ns |
| Q | -0.38 | ** | -0.40 | ** | -0.49 | ** | -0.05 | ns | -0.64 | ** | -0.75 | *** |
| C | -0.71 | *** | -0.69 | *** | -0.82 | *** | -0.90 | *** | -0.89 | *** | -0.22 | ns |
| NH ₄ ⁺ | | | | | | | | | | | | |
| Y | | | 0.59 | *** | 0.12 | ns | 0.41 | ns | 0.07 | ns | 0.02 | ns |
| Q | | | 0.39 | ** | 0.01 | ns | -0.18 | ns | 0.52 | * | 0.35 | * |
| C | | | 0.88 | *** | 0.66 | *** | 0.44 | * | 0.8 | *** | -0.34 | ns |
| NO ₃ ⁻ | | | | | | | | | | | | |
| Y | | | | | 0.39 | * | -0.50 | * | -0.48 | * | -0.56 | ** |
| Q | | | | | 0.02 | ns | 0.57 | ** | 0.32 | ns | 0.34 | * |
| C | | | | | 0.55 | ** | 0.46 | * | 0.8 | *** | -0.34 | ns |
| TSM | | | | | | | | | | | | |
| Y | | | | | | | -0.52 | * | -0.18 | ns | -0.28 | ns |
| Q | | | | | | | 0.17 | ns | 0.5 | * | 0.35 | * |
| C | | | | | | | 0.76 | *** | 0.81 | *** | 0.28 | ns |
| Chl a | | | | | | | | | | | | |
| Y | | | | | | | | | 0.75 | ** | 0.41 | ns |
| Q | | | | | | | | | 0.27 | ns | 0.38 | ns |
| C | | | | | | | | | 0.83 | *** | 0.39 | ns |
| TN-TSM | | | | | | | | | | | | |
| Y | | | | | | | | | | | 0.57 | ** |
| Q | | | | | | | | | | | 0.77 | *** |
| C | | | | | | | | | | | 0.04 | ns |

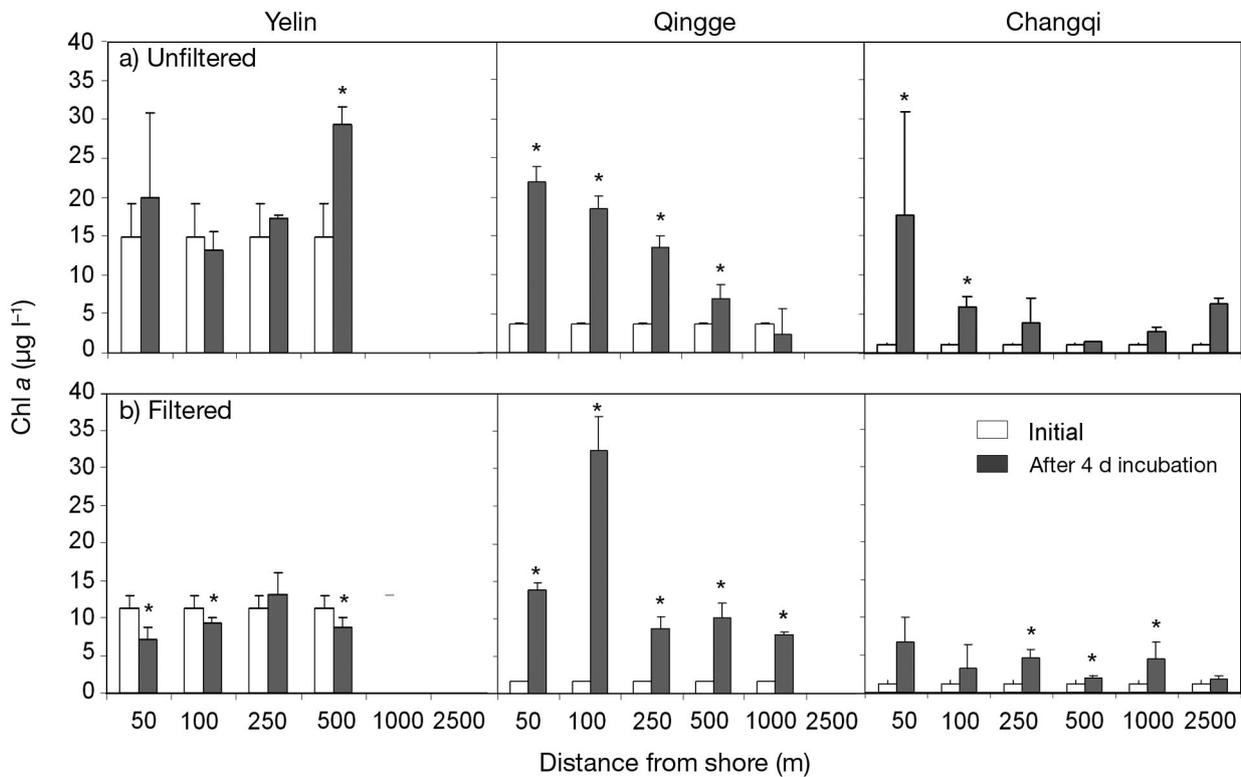


Fig. 4. Chl *a* concentration (mean \pm SD) in the dialysis bags at the time of deployment (initial) and at retrieval after 4 d over a distance gradient at the 3 study sites with (a) unfiltered ($n = 3$) and (b) 50 μm filtered water ($n = 4$). Significant differences ($p < 0.05$) in chl *a* concentrations between the 2 time intervals are indicated with * based on Student's *t*-test

increased from 6 to 13%. At Changqi, the $\delta^{15}\text{N}$ increase at the nearshore stations was comparatively lower (from 6 to $\sim 9\%$) but remained high along the transect, with values $>8\%$ after incubation even at the station 2500 m from the shore. Therefore, the $\delta^{15}\text{N}$ -TSM growth increment at the offshore stations was significantly higher at Changqi than at Qingge and Yelin (Tukey's test for 500 m distance; $p < 0.001$). At Qingge and Changqi, increases in TN and $\delta^{15}\text{N}$ in the bags over the incubation period were higher as ambient median NH_4^+ concentrations increased, whereas the opposite trend was observed at Yelin.

DISCUSSION

Nitrogen and its isotopic composition in aquaculture effluents

Elevated mean DIN concentrations in aquaculture ponds and drainage channels, as compared to coastal waters, reflect high nitrogen enrichment. Remineralization of organic matter, dominated by ammonification and excretion of NH_3 by the cultured animals,

results in a predominance of NH_4^+ in the DIN pool. This finding is concordant with other studies that report high dissolved nitrogen concentrations in shrimp ponds (Briggs & 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 examined in detail by Herbeck et al. (2012).

High $\delta^{15}\text{N}$ values of NH_4^+ in aquaculture ponds and drainage channels ($\sim 17\%$; Table 2) confirm the assumption that aquaculture effluents are enriched in ^{15}N (e.g. Jones et al. 2001, Costanzo et al. 2004, Lin & Fong 2008). Three reasons account for the high $\delta^{15}\text{N}$ in pond effluents. (1) The nitrogen in ponds originates from animals of a relatively high trophic level in combination with fish and shrimp feed that contains fish meal and small fishes. Typically, $\delta^{15}\text{N}$ increases by $\sim 3\%$ moving up each trophic level (Minagawa & Wada 1984). Therefore, remineralization of the ^{15}N -enriched surplus feed and excretions from farmed animals results in relatively high $\delta^{15}\text{N}$ - NH_4^+ in ponds, despite fractionation during organic

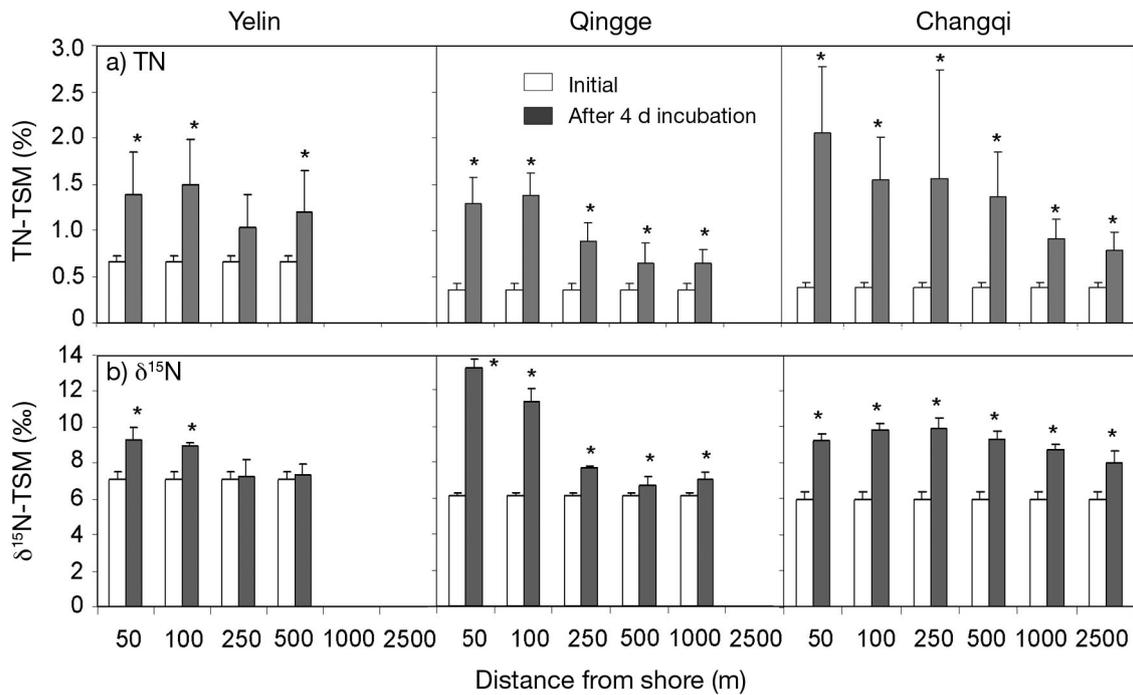


Fig. 5. (a) TN and (b) $\delta^{15}\text{N}$ of the TSM in the dialysis bags (mean \pm SD) at the time of deployment (initial) and at retrieval after 4 d over a distance gradient at the 3 study sites ($n = 7$). Data for bags with filtered and with unfiltered water were combined because there were no significant differences in TN and $\delta^{15}\text{N}$ between the treatments. Significant differences ($p < 0.05$) in TN and $\delta^{15}\text{N}$ between the 2 time intervals are indicated with * based on Student's t -test

matter decomposition. (2) Fractionation during volatilization of NH_3 and nitrification causes further ^{15}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 the mean $\delta^{15}\text{N}\text{-NO}_3^-$ of 6.7‰ in the ponds of the study area. (3) NH_4^+ uptake by primary producers causes further ^{15}N enrichment of the remaining NH_4^+ , while planktonic organic matter is comparatively lower (e.g. Montoya 2008). This process is of considerable importance as TSM in aquaculture ponds consists mostly of phytoplankton (Jackson et al. 2003, Herbeck et al. 2012). 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 a preference of plankton for NH_4^+ can be expected. Vizzini & Mazzola (2004) report similar $\delta^{15}\text{N}\text{-TSM}$ values of $\sim 8\%$ in fish farm effluents in Italy.

A few exceptionally low $\delta^{15}\text{N}$ values of $< 5\%$, especially of NO_3^- in fish ponds, suggest contributions of artificial fertilizers. Fertilizer is added to ponds mainly at the beginning of crop production to stimulate the growth of phytoplankton, which serves as feed for the young animals raised. However, $> 75\%$ of the ponds and drainage channels had $\delta^{15}\text{N}\text{-NH}_4^+$ val-

ues of $> 14\%$ and $\delta^{15}\text{N}\text{-TSM}$ values of $> 7\%$. Those elevated $\delta^{15}\text{N}$ signatures in our study contradict previous assumptions that shrimp pond effluent TSM ($\delta^{15}\text{N} \approx 6\%$) is generally less enriched in ^{15}N than e.g. sewage TSM ($\delta^{15}\text{N} \approx 10\%$) (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 previously reported values generally mark the lower end of possible $\delta^{15}\text{N}$ values. This variability could be related to differences in operation characteristics of the farms, e.g. in terms of food sources containing different $\delta^{15}\text{N}$ and/or pond water maintenance. High pH values, for example, may support volatilization of NH_3 causing ^{15}N enrichment of NH_4^+ .

Concentrations of DIN, chl *a* and TSM as tracers for effluent dispersal

In Changqi and Qingge, high concentrations of NH_4^+ and NO_3^- close to shore compared to the offshore sites and a DIN composition similar to that found in aquaculture effluents indicate land-derived nitrogen enrichment from the large shrimp and fish pond areas. The higher NH_4^+ and NO_3^- concentrations in coastal waters of Changqi relative to Qingge

relate to annual nitrogen export from aquaculture ponds, which is ~4-fold higher in Changqi than in Qingge (Table 1). At both sites, DIN concentrations decreased in the offshore direction to concentrations similar to those found close to shore at the control site Yelin. This results on the one hand from dilution with nutrient-poor oceanic waters (Herbeck et al. 2012) and on the other hand from biological nitrogen uptake. High chl *a* concentrations at the stations with high NH_4^+ and NO_3^- concentrations close to shore in Qingge and Changqi indicate significant stimulation of planktonic primary production. Chl *a* concentrations declined with decreasing nutrient availability in the offshore direction to concentrations close to $1 \mu\text{g l}^{-1}$, the maximum found in other reef areas (Furnas et al. 1990, Liston et al. 1992, Van Duyl et al. 2002, Otero & Carbery 2005). The exceptionally high back-reef water chl *a* concentrations indicate that autochthonous matter was a major fraction of the TSM at most stations. This is further corroborated by $\delta^{13}\text{C}_{\text{org}}$ values between 19 and 21‰ and a C:N ratio of ~7 (data not shown), which are typical for marine phytoplankton (e.g. Fischer 1991).

In contrast, relatively high concentrations of NH_4^+ and especially NO_3^- at the offshore stations at Yelin and significantly increasing chl *a* concentrations in the offshore direction at this site are likely due to transient exposure to the river plume of the WWE. This plume extends far offshore only during and after strong rain events, which export nutrients, estuarine phytoplankton and eroded soils from the estuary's catchment into coastal waters and stimulate coastal primary production (Herbeck et al. 2011). Depending on tidal currents, the river plume is occasionally deflected in a northward direction, influencing the outer coastal stations at Yelin as confirmed by a salinity range of 12 to 33. This is further confirmed by the comparatively higher NO_3^- portion of the DIN at the offshore stations in Yelin, which is similar to that of the WWE during rain events (Herbeck et al. 2011), as well as $\delta^{13}\text{C}_{\text{org}}$ values of ~-24‰ and a C:N ratio >10 (data not shown) that indicate an enhanced contribution of allochthonous organic matter in the TSM (Unger et al. 2012). Therefore, elevated DIN and chl *a* concentrations at those stations are likely not related to effluents released from aquaculture ponds in the Yelin 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. 2012). Even though the Qingge and Changqi sites were not situated close to river mouths, they are likely to be occasionally affected by water drainage from land, especially after rain events, although to a

much lower extent than at Yelin. The exceptionally low $\delta^{13}\text{C}_{\text{org}}$ values of ~-23‰ at the nearshore stations at Qingge and Changqi (data not shown) indicate a mixture of phytoplankton and allochthonous particles, which could have been e.g. shrimp or fish food remains or phytoplankton from the ponds. In general, the very high TSM concentrations as compared to other back-reefs indicate a high degree of turbidity in the study area, especially at the nearshore stations of Changqi and Qingge (Fig. 3c).

Overall, the observed spatial trends in NH_4^+ , NO_3^- , TSM and chl *a* concentrations point to eutrophication close to shore at Qingge and Changqi, caused by pond aquaculture, and at the offshore stations in Yelin, due to river plume exposure. However, these trends were often not statistically significant (Fig. 3). This was mainly because of high short-term variability in concentrations due to variable tidal mixing intensity, rain-related water discharge from land, nutrient supply from aquaculture ponds and uptake by primary producers, which masked spatial gradients. Variability in chl *a* values may moreover be associated with variable light conditions and grazing affecting phytoplankton abundance.

TN and $\delta^{15}\text{N}$ of suspended matter as indicators for biotic responses to anthropogenic nitrogen

The TN and $\delta^{15}\text{N}$ of TSM mirrored the spatial trends observed for NO_3^- , NH_4^+ , TSM and chl *a*. A good correlation of TN-TSM and chl *a* values (Table 4) is a strong indicator that the high TN-TSM values mainly reflect high *in situ* production and/or storage of excess nitrogen by phytoplankton, both pointing to high DIN availability close to shore in Changqi and Qingge and at the offshore stations at Yelin. However, high TN-TSM values could also be a result of a mixture containing phytoplankton and other nitrogen-rich particles, such as particulate fish food remains or other allochthonous particles, as mentioned earlier.

Irrespective of the effects of fractionation during planktonic nitrogen uptake leading to lower $\delta^{15}\text{N}$, the TSM close to shore in Qingge is characterized by high $\delta^{15}\text{N}$ values of up to 13‰, strongly suggesting that assimilated DIN originates primarily from aquaculture effluents (Fig. 3, Table 2). The decreasing trend of $\delta^{15}\text{N}$ -TSM offshore suggests a decreasing impact of effluents and the admixture of ^{15}N -depleted marine water. At Changqi, high $\delta^{15}\text{N}$ -TSM values at the 250 m station (8.2‰) and decreasing values offshore can also be related to uptake of vari-

able amounts of the ^{15}N -enriched DIN of pond effluents, with the exception of the much lower median $\delta^{15}\text{N}$ -TSM at the 50 m station (6.3‰). In fact, TSM in 2 shrimp ponds directly adjacent to this station also had exceptionally low $\delta^{15}\text{N}$ signals of 2.8‰ and 4.3‰, probably due to recent application of lighter nitrogen fertilizers. It is therefore conceivable that the 50 m station received effluents from those fertilized ponds, while all other stations at Changqi were rather influenced by aquaculture effluents exported from the tidal creek, which received effluents enriched in ^{15}N from fish and shrimp ponds. In Yelin, median $\delta^{15}\text{N}$ -TSM values <6‰ near the shore do not indicate any distinct influence from aquaculture effluents. However, maximal values of 9‰ at these stations point to occasional influence from effluents from the few ponds in that area. A higher median $\delta^{15}\text{N}$ -TSM close to $\delta^{15}\text{N}$ values in estuarine TSM (~8‰; Herbeck et al. 2011) points to exposure of the most offshore station to DIN and TSM from the river plume of the WWE.

Although the temporal variability of TN and $\delta^{15}\text{N}$ in TSM was less than that of DIN and chl *a* concentrations, they revealed only a few statistically significant trends along individual transects. Most likely, this was due to a fast response of small-cell planktonic organisms to nutrients, which therefore do not show a signal over longer time scales. Besides that, variable amounts of allochthonous matter in the bulk of the TSM and variable fractionation rates as related to differences in the phytoplankton species composition may restrict the comparability of the 3 sites and impair the informative value of TN and $\delta^{15}\text{N}$ in suspended matter for *in situ* processes.

Phytoplankton bioassay

Chl *a* increments over the incubation time in the phytoplankton bioassays with unfiltered water displayed similar trends as the water quality parameters and TN and $\delta^{15}\text{N}$ of suspended matter (Figs. 3 & 4). These results reflect highest nutrient availability close to shore decreasing in the offshore direction in Changqi and Qingge but reveal an opposite trend at Yelin. While a clear linear decrease in chl *a* increments was observed in Qingge, this did not occur at Changqi. It is likely that phytoplankton growth in the dialysis bags at Changqi was temporarily light limited due to rain-related flush-out of particles from the adjacent tidal creek during the incubation time. This is corroborated by especially high TSM concentrations in the surface water of between 25 and 55 mg l^{-1}

during the experiment at this site (Fig. 3c). Differences in the light climate due to tide-related variations in incubation depth, variable amounts of particles in the water column and potential self-shading of phytoplankton in the bags may have had a certain effect on phytoplankton growth rates in the bioassays. It is likely that, for example, the comparatively larger chl *a* increase at the 2500 m station compared to that of the 1000 m station at Changqi can be attributed to the comparatively lower incubation depth of the floating bags during peak light intensities at mid-day and a lower TSM concentration in the surface water at the 2500 m site (Fig. 3c). However, increases in chl *a* in the bioassays at Qingge and Changqi were overall much larger close to shore where DIN concentrations and light limitation were at a maximum. Better light conditions at these stations may have further boosted phytoplankton growth and would have even increased the relevance of the experiment. Therefore, we generally regard the effect of light as inferior to the effect of nutrient exposure. The chl *a* increase (up to 31 $\mu\text{g l}^{-1}$) was also generally much higher than in most other published phytoplankton bioassays, reporting increases from 0.2 to <4 $\mu\text{g l}^{-1}$ over a 4 d incubation (Dalsgaard & Krause-Jensen 2006). This indicates substantially higher nutrient availability and much better growth conditions despite comparatively high TSM concentrations in our study area.

In the treatment with the filtered phytoplankton, growth increments were also high at Qingge and Changqi, while chl *a* concentrations, in contrast to this, decreased at Yelin, implying degradation of the phytoplankton by the end of the incubation time. The higher chl *a* increase observed in bags with unfiltered than with filtered water was opposite to the results of a phytoplankton bioassay carried out in the Mediterranean Sea (Pitta et al. 2009). There, grazing within the bags with unfiltered water inhibited any significant chl *a* increase as a response to nutrients from fish farm wastes. In contrast to mainly oligotrophic environments, top-down control of phytoplankton by grazers seemed not to be of relevance in the shallow back-reef systems of Hainan, probably because the phytoplankton in the study area is mainly composed of species >50 μm (Maier 2010), which are not as easily grazed as the smaller-celled primary producers of the Mediterranean (Pitta et al. 2009). In fact, lower initial chl *a* concentrations in the bags with the filtered water, as compared to those with unfiltered water, indicates that filtration caused a removal of both zooplankton and large phytoplankton.

The significant increase of TN and $\delta^{15}\text{N}$ in the phytoplankton bioassays at all stations in Qingge and Changqi suggests that the whole back-reef area is impacted by aquaculture effluents. The enormous increase in $\delta^{15}\text{N}$ in the bioassays, to values comparable 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. This also applies to the nearshore areas of Yelin despite the relatively low abundance of ponds at this site and the lack of evidence from water quality parameters. The results indicate that $\delta^{15}\text{N}$ and TN in bioassays are very sensitive tracers of nitrogen inputs. Despite the well documented fractionation during N assimilation of the phytoplankton, the $\delta^{15}\text{N}$ -TSM values in the bags rose significantly. This very strong increase shows that fractionation effects of the phytoplankton did not impair the significance of the results. Nevertheless, possible differences in species-specific fractionation rates make a comparison between sites difficult. However, the significant trends along individual transects, where the same water with identical plankton assemblages was used to fill the sample bags, support the suitability of the applied method. Unlike chl *a*, TN and $\delta^{15}\text{N}$ in the phytoplankton bioassays are also less affected by surrounding light conditions. Unchanged $\delta^{15}\text{N}$ in the bioassays after incubation at the offshore stations at Yelin indicates additional nitrogen sources, such as agriculture, urban wastes and aquaculture ponds and floating net cages, which impact the area via the outflow of the WWE (Herbeck et al. 2011).

The growth increments of TN and $\delta^{15}\text{N}$ over the incubation were substantially higher in our phytoplankton bioassays compared to similar bioassay studies, which reported maximum relative growth increments of TN of 0.7 to 1.1% and of relative increases in $\delta^{15}\text{N}$ of 0.8 to 1.3‰ (García-Sanz et al. 2011, Lin & Fong 2008). Also, the spatial extent of the present study (>2500 m) exceeded those from these studies. The strong increase in TN and $\delta^{15}\text{N}$ 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 other regions. This indicates that TN and $\delta^{15}\text{N}$ in bioassays with primary producers serve not only as suitable qualitative but also as quantitative indicators of nutrient enrichment from aquaculture effluents. Overall, the modified set-up of the phytoplankton bioassay provided an appropriate tool for shallow back-reef areas under a microtidal regime, and thus, this method is highly

recommended, especially for areas that lack the occurrence of any macroalgae that could be used for bioassays.

CONCLUSION

Different approaches were applied to study the spatial extent of nitrogen dispersal released into back-reef areas from 3 coastal aquaculture pond areas of different size. The present work is the first study to show high $\delta^{15}\text{N}$ of ammonium and nitrate in aquaculture effluents, thereby confirming the suitability of $\delta^{15}\text{N}$ as a tracer for pond effluents. Furthermore, the work is also novel in that it demonstrates that phytoplankton bioassays can be used to trace nutrients released from aquaculture ponds. The bioassay approach overcame the high short-term variability found in standard water quality parameters, such as DIN and chl *a*, as well as *in situ* TN- and $\delta^{15}\text{N}$ -TSM that often mask the significance of spatial gradients. Despite decreasing concentrations of DIN in offshore direction at Qingge and Changqi, $\delta^{15}\text{N}$ increases in the phytoplankton bioassays after incubation revealed persisting exposure to effluents at a distance of 1000 and 2500 m, respectively. These results verify the suitability of $\delta^{15}\text{N}$ in phytoplankton bioassays as a very sensitive indicator for tracing aquaculture effluents in coastal waters. Significant $\delta^{15}\text{N}$ increases in combination with the enormous chl *a* increase in the phytoplankton bioassays prove nutrient-rich pond effluents to cause eutrophication in the entire back-reef areas of those sites and possibly even further offshore. Eutrophication is likely reinforced by the limited tidal mixing in those partly enclosed coastal areas. Thus, we conclude 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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