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Effects of non-indigenous species mariculture on benthic ecosystems in Bohai Sea, China

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CONTENTS

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Summary

Marine aquaculture, or mariculture, is one of the fastest growing food-producing sectors. By 2020, 32.3% of aquatic food will come from mariculture (FAO 2011). This strong expansion of mariculture industry has brought significant environmental impacts to coastal ecosystems (Silva et al., 2012), such as sediment organic enrichment and eutrophication (Holmer et al., 2005; Kalantzi and Karakassis, 2006), chemical pollution from pharmaceuticals, organics, bactericides and metals (Antunes and Gil, 2004; Cabello, 2006; Sapkota et al., 2008), and changes in the biodiversity and community structure of benthic fauna (Tomassetti et al., 2009; Vezzulli et al., 2008). Culturing non-indigenous species (NIS) is an important sector of aquaculture industry but also includes ecological risks. Though many studies focused on the environmental impact of those invasive NIS, the NIS for aquaculture use (including mariculture) is often neglected and specific effects of NIS on the ecosystem functioning remain underdocumented. Moreover, it is important for sustainable mariculture application to identify indicators for any change in the ecosystem (i.e. at abiotic, biotic and functional levels).

Given this context, the general aim of this PhD study is to assess the impact of the mariculture on the recipient ecosystems in the context of using NIS as cultured species. The outcome of this research will provide scientific information for the decision makers to apply for a better management of mariculture. This PhD study targets the environmental variables, the meiobenthos community, and benthic trophic interactions. It focusses on the group of meiobenthos because of their crucial roles in

connecting primary producers and higher level of consumers in the marine food web. Also, meiobenthos are good candidates to assess the status of ecosystem under stress due to their small sizes, short generation times and closer associations with sediments (Kennedy and Jacoby, 1999).

The objectives of this thesis were triple: (1) to unravel the response of environmental variables, meiobenthic communities and their trophic ecology towards mariculture activities, (2) to investigate the specific effect of different mariculture installations/techniques on the benthic environment, and 3) to evaluate the measured variables as contribution to an early warning system of NIS mariculture. As China plays an important role in the world mariculture production, we selected two typical mariculture cases in the Bohai Sea, China, i.e. open-water suspension farming of *Argopecten irradians* (non-indigenous) (Chapter 2, 3, 4) as well as coastal shrimp farming of *Litopenaeus vannamei* (non-indigenous) and *Marsupenaeus japonicus* (native) (Chapter 2, 5). We analyze the potential effects on the sediments and benthic community, discuss the feasibility to apply indicators for the impact assessment and also to unravel the implications of the use of NIS in mariculture.

We first target the benthic environmental variables in an open-water culture area of scallop *A. irradians* and the pond-culture systems of *M. japonicus* and *L. vannamei* (Chapter 2). The mariculture effects were studied in terms of sediment properties. The sensitivities of physiochemical variables (redox potential - Eh, bulk organic matter, chlorophyll *a*, biochemical composition, and single indices like protein to carbohydrate ratio – PRO: CHO) were examined and compared with

each other. We found that, in the case of scallop farms, the bulk organic matter concentrations were not affected under the scallop farms, while sedimentary Eh showed a slightly but significantly decrease under the scallop farming in sandy shallow (7 m water-depth) station. Biopolymeric carbon (BPC), which indicated the benthic trophic status, significantly increased under the two farms where the farming activities were more intense. In the case of the shrimp farm, bulk organic matter, Chl *a* and BPC showed the temporal pattern that accumulated from May to August during the shrimp farming period, and the patterns were different between *M. japonicus* and *L. vannamei* ponds as well as the early and the late rearing stages. As the Eh values and biochemical organic concentrations all fell within a meso-oligotrophic status, open-water scallop farming has in general no negative effect on the benthic environment. However, during the shrimp rearing stages, the benthic environment of two types of ponds were highly reduced, anoxic and highly eutrophic (even reach hypertrophic). We also found BPC to be a more suitable indicator for mariculture assessment than the other variables measured due to its sensitivity and closer linkage with the food availability to the benthos. The PRO: CHO ratio should be used with care as microbial activity could mask the initial concentrations of proteins and carbohydrates.

Then we turned to focus on benthic organisms. Chapter 3 and Chapter 4 deal with a case-study of open-water farming scallop *A. irradians* in Laizhou Bay. In Chapter 3, we observed a small effect of scallop farming on the structure and diversity of meiobenthos at the higher-taxon level and for copepod assemblages at the species level in the two stations with high scallop production density. Only under the farm in the sandy bottom sediment, meiobenthic community differed from the control site as there was a larger proportion of nematodes. The harpacticoid copepod community structure changed at the scallop farms in the station with the muddy bottom, where its density and number of species were enhanced. This structural change can relate to the enhanced labile organic matter (i.e. BPC). We suggest that the responses of entire meiofauna community and harpacticoid copepod assemblages to scallop farming is location specific and need to be interpreted with other evidence (e.g. environmental variables) when using them to assess the potential environmental impact of scallop farming or any other similar bivalve farming. Then we further investigated the resource use of harpacticoid copepods and nematodes (the abundant groups of meiobenthos) inside and outside those scallop farms, by means of natural abundance of stable carbon and nitrogen isotopes (Chapter 4). The biodeposits of scallop were found to be the most enriched in $\delta^{15}N$ compared to other food sources which made it traceable. The enriched $\delta^{15}N$ in several meiobenthos at the farms together with the mixing model results indicated that biodeposits could be a new food source for most of the harpacticoid copepods and some nematodes. The quantities and the pathways of assimilation differed between the copepod families depending on their feeding behaviour and the receiving environment. Furthermore, with higher levels of polyunsaturated fatty acids (PUFA), in particular docosahexaenoic acid (DHA), the dominant copepod family Canuellidae that abundantly consumed scallop faeces had a higher nutritious quality compared with those in the control sites, indicating a potential positive effect on the local marine food web.

Chapter 5 addresses temporal changes in the meiobenthic community structure and food sources utilization during the shrimp farming stages as well as depicted the trophic relationships among shrimp feeds, meiobenthos and shrimps by stable isotopes analysis and fatty acids profiling. We found that nematode densities were largely associated with benthic eutrophication during *L. vannamei* culture and led to different meiobenthos assemblages through time. At the end of the rearing season, *L. vannamei* ponds showed a high-abundance but low-diversity meiofauna community dominated by nematodes and indicates a disturbed state. *M. japonicus* ponds had higher diversity of meiobenthos over *L. vannamei* ponds, the meiobenthic community structure only changed in the early stage that attribute to the enhancement of nematodes. We also found that natural productivity contributed abundantly to both *M. japonicus* and *L. vannamei* in different forms between stages. Meiobenthos in the early stage, sharing similar diets with shrimps (both fed on primary organic sources), potentially competed with shrimps in terms of resource utilization. Due to the ontogenetic shifts in diet of shrimps, at the late stage, meiobenthos rather became a functional link between primary producers and shrimps as it serves as nutritional food source with high PUFAs to shrimps. Supplementary feed was less consumed by *M. japonicus* and *L. vannamei* but may fuel the benthic food web through other pathways such as promoting secondary production.

Overall, by using *in situ* sampling, abiotic and biotic approaches, biomarkers and stable isotopes mixing models, this PhD research concludes that the mariculture NIS effects on benthic environment are system-specific and depend on several localized factors, e.g. hydrodynamics, the types of sediments, the background organic concentrations. More specifically, open-water scallop *A. irradians* farming seems to be less environmental risky in terms of generating no negative impacts on the benthic ecosystem, while the coastal shrimp ponds farming *M. japonicus* and *L. vannamei* had polluted benthic environment within ponds which is likely due to the overfeeding. The strength of physico-chemical, bio- and functional indicators to assess mariculture effects is also system-specific and lacks generality. However, functional measurement for mariculture assessment is recommended since it can provide a more integrated understanding of ecosystem changes..

Samenvatting

Mariene aquacultuur of maricultuur is één van de snelstgroeiende voedselproducerende sectoren. Tegen 2020 zal 32,3% van het aquatisch voedsel afkomstig zijn van mariculture (FAO 2011). Deze sterke uitbreiding van de maricultuurindustrie heeft aanzienlijke gevolgen voor het milieu, in het bijzonder voor kustecosystemen (Silva et al., 2012). Belangrijke effecten zoals sediment organische aanrijking en eutrofiëring (Holmer et al., 2005; Kalantzi en Karakassis, 2006), chemische vervuiling door geneesmiddelen, organische stoffen, bactericiden en metalen (Antunes en Gil, 2004; Cabello, 2006; Sapkota et al., 2008) en veranderingen in de biodiversiteit en de gemeenschapsstructuur van benthische fauna (Tomassetti et al., 2009; Vezzulli et al., 2008) kunnen verwacht worden. Het kweken van niet-inheemse soorten (NIS) is een belangrijke aandeel van de aquacultuursector maar omvat ook ecologische risico's. Hoewel veel studies gericht zijn op de milieueffecten van invasieve NIS, wordt het gebruik van NIS in aquacultuurtoepassingen (inclusief maricultuur) vaak verwaarloosd en zijn specifieke effecten van NIS op het functioneren van ecosystemen nog steeds niet gedocumenteerd. Bovendien is het belangrijk voor duurzame maricultuurtoepassingen om indicatoren te identificeren voor elke verandering in het ecosysteem (zowel voor abiotische, biotische en functionele factoren).

De algemene doelstelling van dit doctoraatsonderzoek is het onderzoek van de impact van de maricultuur op het ecosysteem waar

NIS gekweekt worden. De resultaten van dit onderzoek zal wetenschappelijke informatie opleveren voor beleidvoerders en de managers van de aquacultuurinstallaties om zich in te zetten voor een beter beheer van de maricultuur. Dit doctoraatsonderzoek richt zich op de omgevingsvariabelen, de meiobenthos-gemeenschap en benthische trofische interacties. Het onderzoek spitst zich toe op het meiobenthos vanwege hun cruciale rol bij het verbinden van primaire producenten en hoger trofische niveaus van consumenten in het mariene voedselweb. Daarenboven is meiobenthos ook een goede indicator om de algemene status van ecosystemen onder stress te beoordelen omdat ze klein zijn, korte generatietijden hebben en in nauwe associatie met het sediment voorkomen (Kennedy en Jacoby, 1999).

De doelstellingen van dit proefschrift zijn drieledig: (1) ontrafelen van de respons van omgevingsvariabelen, meiobenthische gemeenschappen en hun trofische ecologie op maricultuuractiviteiten, (2) om het specifieke effect van verschillende maricultuurinstallaties / -technieken op de benthische omgeving te onderzoeken, en 3) om de gemeten variabelen te evalueren als bijdrage aan een vroegtijdig waarschuwingssysteem voor de impact van NIS-maricultuur.

Omdat China een belangrijke rol speelt in de wereldwijde productie van maricultuur, werden twee typische maricultuur installaties geselecteerd in de Bohai Zee, China, waar een open-water suspensieteelt van *Argopecten irradians* (niet-inheemse soort) (hoofdstuk 2, 3, 4) en een kustgarnaalkwekerij van *Litopenaeus vannamei* (niet-inheemse soort) en *Marsupenaeus japonicus* (inheems) (hoofdstuk 2, 5) plaatsvindt. De mogelijke effecten op de sedimenten en op de benthische gemeenschap, de haalbaarheid om indicatoren toe te passen voor de effectbeoordeling en ook om de implicaties van het gebruik van NIS in de maricultuur te ontrafelen, werden onderzocht.

In eerste instantie werden de bentische milieuvariabelen in een open cultuurgebied van Sint-jakobsschelp *A. irradians* en de vijverculturssystemen van *M. japonicus* en *L. vannamei* (hoofdstuk 2) onderzocht. De maricultuureffecten werden bestudeerd in termen van sedimenteigenschappen. De gevoeligheden van fysicochemische variabelen (redoxpotentiaal - E_h, bulk organisch materiaal, chlorofyl a, biochemische samenstelling en enkele indices zoals eiwit:koolhydraatratio - PRO: CHO) werden onderzocht en met elkaar vergeleken. Bij de kweek van de Sint-Jacobsschelp werden de totale organische stofconcentraties niet beïnvloed terwijl sedimentair E_h een lichte maar significante afname vertoonde onder de aquacultuurinstallaties in zandige en ondiepe (7 m waterdiepte) stations. Biopolymere koolstof (BPC), als indicatie van de benthische trofische status, nam aanzienlijk toe onder de twee aquacultuurinstallaties waar de kweekactiviteiten intenser waren. In het geval van de garnalenkwekerij, vertoonden bulk organisch materiaal, Chl a en BPC een temporeel patroon met een toename van mei tot augustus tijdens de garnalenkweekperiode. De patronen verschilden tussen de kweek van *M. japonicus* en *L. vannamei* en er waren tevens verschillen tussen de vroege en de late kweekfasen. Omdat de E_h-waarden en biochemische organische concentraties allemaal binnen een meso-oligotrofe status vielen, heeft de kweek van Sint-jakobsschelp in open water over het algemeen geen negatief effect op de benthische omgeving. Tijdens de opkweekperiode voor garnalen was de benthische omgeving van beide types kweekinstallaties echter sterk gereduceerd, zuurstofloos en zeer eutroof (bereikte zelfs een hypertrofische toestand). We vonden ook dat BPC een meer geschikte indicator is voor de beoordeling van de impact van maricultuur dan de andere gemeten variabelen vanwege de gevoeligheid en nauwere koppeling met de voedselbeschikbaarheid voor het benthos. De PRO:CHO-ratio moet met zorg worden gebruikt, omdat microbiële activiteit de initiële concentraties van eiwitten en koolhydraten kan maskeren.

In de volgende hoofdstukken ligt de nadruk op de bentische organismen. Hoofdstuk 3 en Hoofdstuk 4 behandelen een case-study van de Sint-jakobsschelpkweek in open water (*A. irradians* in Laizhou Bay). In Hoofdstuk 3 hebben we een klein effect van de Sint-jakobsschelpkweek waargenomen op de structuur en diversiteit van het meiobenthos op hoger taxonniveau en op de copepodengemeenschappen op soortsniveau in de twee stations met een hoge productiedichtheid van de Sint-jakobsschelp. Enkel voor de kweek boven de zandbodem verschilde de meiobenthische gemeenschap van de controle omdat er meer nematoden aanwezig waren. De gemeenschapsstructuur van de harpacticoïde copepoden veranderde bij de Sint-jakobsschelpen in het station met het fijne sediment (slibbodem), waar de dichtheid en het aantal soorten werden verbeterd. Deze structurele verandering kan mogelijks verklaard worden door het verbeterde labiele organische materiaal (BPC). Op basis van deze resultaten wordt besloten dat de respons van de gehele meiofaunagemeenschap en harpacticoïde copepodengemeenschappen op de kweek van Sint-jakobsschelpen lokatiegebonden is en moet geïnterpreteerd worden aan de hand van andere omgevingsvariabelen wanneer ze worden gebruikt om de mogelijke milieueffecten van de Sint-jakobsschelp of andere soortgelijke schaaldierenteelt te beoordelen.

Vervolgens werd het gebruik van voedselbronnen door harpacticoïde copepoden en nematoden (de meest abundante meiobenthostaxa) binnen en buiten de kweekzones van Sint-jakobsschelp verder onderzocht, door middel van de natuurlijke abundantie van stabiele koolstof- en stikstofisotopen (hoofdstuk 4). De biodeposits van Sint-jakobsschelp bleken het meest verrijkt te zijn in δ^{15}N vergeleken met andere voedselbronnen waardoor het traceerbaar was. De verrijkte δ^{15}N in verschillende meiobenthos organismen in de kweekopstellingen samen met de resultaten van het mixed model gaven aan dat biodeposits een nieuwe voedingsbron kunnen zijn voor de meeste van de harpacticoïde copepodensoorten en sommige nematodensoorten. De hoeveelheden en de pathways van assimilatie verschilden tussen de copepodenfamilies, afhankelijk van hun voedingsgedrag en de ‘ontvangende omgeving’. Bovendien, met hogere niveaus van meervoudig onverzadigde vetzuren (PUFA), in het bijzonder docosahexaeenzuur (DHA), verkreeg de dominante copepodfamilie Canuellidae, die overvloedig faeces van de Sint-jakobsschelp consumeerde, een hogere voedzame kwaliteit vergeleken met de individuen in de controlelokaties. Dit geeft een mogelijk positief effect op het lokale mariene voedselweb.

Hoofdstuk 5 gaat in op de temporele veranderingen in de meiobenthische gemeenschapsstructuur en het gebruik van voedselbronnen tijdens de garnalenkweekstadia, evenals de trofische relaties tussen garnalenvoeders, meiobenthos en garnalen door gebruik te maken van stabiele isotopenanalyse en vetzurenprofilering. De dichtheden van nematoden waren in sterke mate geassocieerd met benthische eutrofiëring tijdens de *L. vannamei*-cultuur en leidden tot verschillende meiobenthosgemeenschappen na verloop van tijd. Aan het einde van het kweekseizoen vertoonden *L. vannamei*-vijvers een hoge densiteit maar lage diversiteit van de meiofauna-gemeenschap die werd gedomineerd door nematoden en in een verstoorde staat verkeert. *M. japonicus*-vijvers hadden een hogere diversiteit van meiobenthos dan *L. vannamei*-kweekvijvers, de meiobenthische gemeenschapsstructuur veranderde alleen in het vroege stadium wat vooral te wijten is aan een toename van nematoden. De natuurlijke productiviteit droeg in grote mate bij aan zowel *M. japonicus* en *L. vannamei* in verschillende vormen en tussen de kweekfasen. Meiobenthos in het vroege stadium, met een gelijkaardig dieet als de garnalenkweek (beiden gevoed met primaire organische bronnen), kan potentieel concurreren met garnalen in termen van het gebruik van voedselbronnen. Als gevolg van de ontogenetische verschuivingen in het dieet van garnalen, werd meiobenthos in de late fase eerder een functionele link tussen primaire producenten en garnalen omdat het dient als voedingsbron met hoge PUFAconcentratie voor garnalen. Aanvullend voer werd minder geconsumeerd door *M. japonicus* en *L. vannamei* maar kan het benthische voedselweb voeden via andere wegen zoals het bevorderen van de secundaire productie.

Op basis van de *in situ* bemonstering, abiotische en biotische benaderingen, biomerkers en stabiele isotopen in mixing modellen, kan uit dit onderzoek geconcludeerd worden dat de maricultuur NIS-effecten op de bentische omgeving systeemspecifiek zijn en afhankelijk zijn van verschillende lokale factoren, b.v. hydrodynamica, het sedimenttype en de achtergrondconcentratie van organische componenten. Meer in het bijzonder lijkt de open-water kweek van Sint-jakobsschelp *A. irradians* minder risicovol te zijn door het niet genereren van negatieve effecten op het benthische ecosysteem. Dit is in tegenstelling met de kustgarnaalvijvers die *M. japonicus* en *L. vannamei* kweken en hierdoor de benthische omgeving in de kweekvijvers hebben vervuild, waarschijnlijk vanwege de overvloed aan voederen. De kracht van fysisch-chemische, bio- en functionele indicatoren om de maricultuureffecten te beoordelen, is ook systeemspecifiek en kan niet veralgemeend worden. Functionele metingen voor maricultuurbeoordeling worden echter aanbevolen, omdat dit een meer geïntegreerd inzicht van ecosysteemveranderingen kan bieden.

Santrauka

Jūrų akvakultūra yra viena iš sparčiausiai besivystančių maisto pramonės šakų. 2020 metais apie 32 % maisto produktų, išgaunamų iš jūrinių bei kitų vandenų, bus užauginta akvakultūriniu būdu (FAO 2011). Ženklus šio sektoriaus augimas daro pakankamai reikšmingą poveikį pakrančių ekosistemoms (Silva ir kt., 2012). Šio poveikio rezultatas – nuosėdų organinis sodrinimas bei eutrofikacija (Holmer ir kt., 2005, Kalantzi ir Karakassis, 2006), cheminė tarša vaistais, organinių junginių, bakteoricidų ir metalų patekimas į jūrinę aplinką (Antunes ir Gil, 2004, Cabello, 2006; Sapkota ir kt., 2008), pokyčiai bentoso faunos biologinėje įvairovėje ir bendrijų struktūrose (Tomassetti et al., 2009, Vezzulli et al., 2008). Nevietinių rūšių kultūra yra svarbus akvakultūros sektorius, tačiau tam tikrais atvejais sukelia ir ekologinę riziką. Nors daugelis tyrimų vertina invazinių rūšių poveikius aplinkai, nevietinių rūšių eksploatavimas akvakultūros reikmėms dažnai yra ignoruojamas ir specifinis šių rūšių poveikis ekosistemų funkcionavimui lieka nepakankamai ištirtas. Taip pat, siekiant tvaraus akvakultūros plėtojimo, svarbu yra nustatyti kiekvieno ekosistemos pokyčio rodiklius (abiotiniame, biotiniame ir funkciniame lygmenyse).

Atsižvelgiant į šias aplinkybes, šio mokslinio tyrimo tikslas yra įvertinti jūrinės akvakultūros poveikį vietinėms ekosistemoms ūkiuose, kuriuose yra auginamos svetimkraštes rūšys. Šio tyrimo rezultatai suteiks mokslinių žinių tiriamojoje srityje, taip pat pagelbės priimant sprendimus jūrinės akvakultūros valdymo klausimais. Šiame darbe

nagrinėjami aplinkos kintamieji, meiobentoso bendrijos bei jų trofinės sąveikos. Tyrime daugiausia dėmesio yra skiriama meiobentosui, nes ši grupė atlieka svarbų vaidmenį sujungiant pirminius maisto šaltinius ir aukštesnius vartojimo lygmenis trofiniame tinkle. Taipogi meiobentoso vertinimas yra tinkamas rodiklis, kuriuo galima apibūdinti ekosistemos būklę stresinėje bendrijų būklėje dėl jų nedidelio dydžio, trumpo pasikartojimo laiko ir glaudesnių ryšių su nuosėdomis (Kennedy ir Jacoby, 1999).

Šiame darbe keliami trys pagrindiniai tikslai: 1) nustatyti aplinkos kintamųjų, meiobentinių bendrijų ir jų trofinių ryšių reakciją į jūrinės akvakultūros veiklą; 2) ištirti specifinį skirtingų jūrinės akvakultūros įrenginių bei metodų poveikį jūros dugno aplinkai; 3) įvertinti tiriamųjų kintamųjų indėlį į svetimkraščių rūšių akvakultūros ankstyvojo įspėjimo sistemą. Kadangi Kinija vaidina svarbų vaidmenį pasaulinėje jūrinės akvakultūros gamyboje, buvo parinkti du tipiški jūrinės akvakultūros auginimo atvejai Bohajų jūroje (Kinija). Tiriamos rūšys – jūrų šukutės *Argopecten irradians* (svetimkraštė rūšis) (2, 3, 4 skyriai), krevetės *Litopenaeus vannamei* (svetimkraštė rūšis) ir *Marsupenaeus japonicus* (vietinė rūšis) (2, 5 skyriai). Buvo išanalizuotas galimas poveikis nuosėdoms ir dugno bendrijoms, aptartos galimybės taikyti poveikio vertinimo rodiklius ir įvertinti svetimkraščių rūšių naudojimo jūrinėje akvakultūroje pasekmes.

Pirmiausia buvo ištirta jūrų šukučių *A. irradians,* krevečių *M. japonicus* ir *L. vannamei* aplinka (2 skyrius). Jūrinės akvakultūros veiklų poveikis buvo tiriamas pagal nuosėdų savybes. Buvo išnagrinėtas ir palygintas fizikocheminių kintamųjų jautrumas (redokso potencialas

– *Eh*, bendrosios organines medžiagos, *chlorofilas a*, biocheminė sudėtis ir atskiri indeksai, tokie kaip baltymų ir angliavandenių santykis – PRO:CHO). Tyrimo metu nustatyta, kad eksploatuojant jūrines šukutes, organinių medžiagų koncentracija nekito tiriamuosiuose ūkiuose, o nuosėdinis Eh mažėjo nesmarkiai, tačiau reikšmingai, smėlėtoje seklioje tyrimų stotyje (7 m gylyje). Biopolimerinės anglies (BPC) koncentracija, kuri nurodo dugno bendrijų trofinę būklę, padidėjo abiejuose stebėtuose ūkiuose. Krevečių ūkyje organinių junginių *Chl a* ir BPC koncentracija parodė, kad krevečių auginimo laikotarpiu nuo gegužės iki rugpjūčio mėn. buvo stebima organinių junginių laikina kaupimosi tendencija, o rūšių *M. japonicus* ir *L. vannamei* baseinuose buvo stebimos skirtingos medžiagų kaupimo tendencijos ankstyvojo ir vėlyvojo rūšių auginimo etapuose. Kadangi *Eh* reikšmės ir biocheminių organinių medžiagų koncentracijos svyravo meso-oligotrofinės būklės ribose, galima teigti, kad jūros šukučių auginimas neturėjo neigiamos įtakos dugno bendrijų aplinkai. Tačiau krevečių auginimo metu dviejų tipų tvenkinių dugno bendrijų aplinka buvo labai susilpnėjusi, t. y. anoksinė ir labai eutrofinė (net pasiekė hipertrofinę). Taip pat buvo nustatyta, kad BPC yra tinkamesnis jūrinės kultūros vertinimo rodiklis, kuris yra jautresnis ir turi artimesnį ryšį su maisto patekimu į dugno bendrijų aplinką, palyginti su kitais kintamaisiais. Atkreiptinas dėmesys į tai, kad PRO:CHO santykis turėtų būti vertinamas atsargiai, nes mikrobinis aktyvumas gali užmaskuoti pradines baltymų ir angliavandenių koncentracijas.

Kitame etape dėmesys buvo nukreiptas į dugno organizmus. 3 ir 4 darbo skyriuose aptariamas atvirame vandenyje auginamų jūros šukučių

A. irradians atvejis Laizhou įlankoje. 3 skyriuje pateikiami pastebėjimai apie nedidelį jūros šukučių auginimo poveikį meiobentoso struktūrai bei įvairovei aukštesnio taksono lygmeniu. Taip pat pateikiami pastebėjimai apie jūros šukučių auginimo poveikį irklakojų vėžiagyvių santalkoms rūšių lygmenyje abiejose stotyse, kuriose buvo didelis jūros šukučių auginimo tankis. Šiame skyriuje taip pat yra pateikiama informacija apie aptiktus meiobentoso skaičiaus skirtumus tarp kontrolinių vietovių ir tirtų baseinų su smiltingomis dugno nuosėdomis, kuriose aptiktas didesnis apvaliųjų kirmelių skaičius. Baseinuose su dumblinu dugnu, kuriuose buvo auginamos jūros šukutės bei kuriuose jūros šukučių skaičius buvo ženkliai padidintas, buvo stebimi irklakojų vėžiagyvių bendrijų struktūriniai pokyčiai. Šie pokyčiai gali būti siejami su padidėjusia lengvos organinės medžiagos, t. y. BPC, koncentracija. Remiantis gautais rezultatais darytina prielaida, jog meiofaunos bendruomenių ir irklakojų vėžiagyvių santalkų atsakas į jūros šukučių auginimą yra priklausomas nuo vietovės. Todėl vertinat jūros šukučių auginimo poveikį aplinkai turi būti stebimi papildomi faktoriai – aplinkos kintamieji. Papildomai buvo ištirta, kaip yra vartojami maisto ištekliai irklakojų vėžiagyvių ir apvaliųjų kirmelių bendrijose jūros šukučių fermų viduje bei išorėje panaudojant natūralios stabilios anglies ir azoto izotopus (4 skyrius). Buvo nustatyta, kad jūros šukučių bionuosėdų komponentai, palyginti su kitais maisto šaltiniais, yra labiausiai praturtinti $\delta^{15}N$ izotopu, kurio padidinta koncentracija padarė jį matomą. Tyrimų duomenys surinkti keliose fermose. $\delta^{15}N$ izotopu praturtintas meiobentosas bei izotopų mišrių modelių modeliavimo rezultatai parodė, kad bionuosėdos gali būti naujas maisto šaltinis daugumai irklakojų vėžiagyvių ir kai kurių apvaliųjų kirmelių bendrijoms. Buvo nustatyta, kad maisto medžiagų asimiliacijos kiekiai ir būdai skyrėsi tarp vėžiagyvių šeimų priklausomai nuo jų mitybos elgsenos ir juos supančios aplinkos. Dominuojančios vėžiagyvių rūšies *Canuellidae* gyviuose, kurie gausiai vartojo jūros šukučių fekalijas, buvo aptikta gausesnė polinesočiųjų riebiųjų rūgščių (PUFA), ypač dokozaheksaeno rūgšties (DHA) koncentracija. Galima daryti išvadą, kad tirti vėžiagyviai yra didesnės maistinės vertės palyginti su tirtais individais išorinėse kontrolinėse vietovėse. Šie rezultatai rodo galimą teigiamą jūros šukučių auginimo poveikį vietiniams jūrų maisto tinklams. 5 skyriuje aptariami meiobentinių bendrijų maisto medžiagų vartojimo bei bendrijų struktūros laikini pokyčiai krevečių auginimo etapuose. Taip pat grafiškai atvaizduoti trofiniai ryšiai tarp krevečių maisto medžiagų, meiobentoso ir krevečių panaudojant stabiliųjų izotopų analizę ir riebiųjų rūgščių profiliavimą. Buvo nustatyta, kad apvaliųjų kirmelių bendrijų tankiai yra labiausiai susiję su dugnine eutrofikacija *L. vannamei* krevečių auginimo metu, dėl ko buvo stebimos skirtingos meiobentoso santalkos laike. Pasibaigus auginimo sezonui *L. vannamei* krevečių auginimo vietose buvo nustatyta didelė meiofaunos gausa, kurios įvairovė buvo skurdi su dominuojančiu apvaliųjų kirmelių paplitimu, kuris atspindi sutrikusią būklę. Telkiniai su *M. japonicus* rūšimis turėjo didesnę meiobentoso įvairovę palyginti su telkiniais, kuriuose auginamos *L. vannamei*, tačiau meiobentoso bendrijos kito tik ankstyvojoje stadijoje ir yra sietinos su apvaliųjų kirmelių gausos didėjimu.

Taip pat buvo nustatyta, kad natūralus produktyvumas buveinėse gausiai prisidėjo prie *M. japonicus* ir *L. vannamei* įvairiomis formomis skirtinguose jų gyvavimo etapuose. Ankstyvojoje stadijoje meiobentosas minta tokiomis pat maisto medžiagomis kaip ir krevetės (pirminiais organiniais šaltiniais) ir potencialiai konkuruoja su krevetėmis išteklių naudojimo požiūriu. Dėl ontogenetinių krevečių mitybos pokyčių vėlyvoje vystymosi stadijoje meiobentosas tampa funkcine jungtimi tarp pirminių maisto medžiagų generuotojų ir krevečių, nes meobentosas yra maistinis šaltinis, aprūpinantis krevetes didelėmis PUFA koncentracijomis. *M. japonicus* ir *L. vannamei* mažiau suvartoja papildomų maisto medžiagų, tačiau gali aprūpinti bentoso mitybos tinklą kitais būdais, pvz., skatinant antrinę maisto medžiagų gamybą.

Pritaikius *in-situ* mėginių ėmimo metodus, abiotinius ir biotinius metodus, biologinius žymenis ir stabilius izotopų mišriuosius modelius, šiame moksliniame tyrime prieita išvada, kad nevietinės rūšys, eksploatuojamos jūrinėje akvakultūroje, daro sistemiškai specifinį poveikį aplinkai ir priklauso nuo kelių lokalių veiksnių, tokių kaip hidrodinamika, nuosėdų tipai, organinių medžiagų foninės koncentracijos. Atvirųjų vandenų moliusko *A. irradians* auginimas yra mažiau neigiamai veikiantis aplinką, t. y. nepaveikia neigiamai bentoso ekosistemos, kai tuo metu pakrančių krevečių telkiniuose, kuriuose aptinkamos *M. japonicus* ir *L. vannamei* rūšys, buvo stebima užteršta dugno aplinka greičiausiai dėl maisto medžiagų pertekliaus. Fizikinių-cheminių, biologinių ir funkcinių rodiklių, skirtų jūrinės akvakultūros poveikiui įvertinti, patikimumas yra sistemiškai specifinis bei neapibendrintas. Tačiau funkcinis jūros akvakultūros vertinimas yra rekomenduotinas, nes jis gali padėti geriau suprasti ekosistemų pokyčius.

摘要

海水养殖是近些年来发展最为迅速的产业之一。预计到 2020 年，全球约 32.3%的水产品来自海水养殖（FAO，2011）。然而，该产业的发展给沿海生态系统带来重大的环境影响（Silva et al., 2012），包括沉积物和水体富营养化（Holmer et al., 2005; Kalantzi and Karakassis, 2006），化学药物、有机物、杀菌剂和重金属污染（Antunes and Gil, 2004; Cabello, 2006; Sapkota et al., 2008），底栖动物生物多样性与群落结构的变化（Tomassetti et al., 2009; Vezzulli et al., 2008）。养殖外来种是水产养殖业的重要组成，占水产养殖总产量 25%，在水产品供应提供重要作用；但存在着生态风险。目前关于外来物种的研究多集中于入侵物种对本土物种的竞争及其对环境的影响，而水产养殖产业（包括海水养殖）中引入的外来种的生态影响常常被忽视，尤其是外来种对生态系统功能的影响仍然缺乏足够的研究数据。因此，建立环境评价指标（包括环境因子、生物群落和生态系统功能水平层面的指标）对于可持续发展的海水养殖环境监测很重要。

本博士论文研究通过评估外来种海水养殖对生态系统（环境因子、小型底栖生物群落和底栖食物网）的影响，期望为决策者提供海水养殖管理相关的科学依据。小型底栖生物群落连接初级生产者和更高层次的消费者，在底栖食物网中有着重要位置。此外，小型底栖生物由于体积小、生命周期短、与沉积物的联系密切，是评估生态系统受胁迫状况的良好指标（Kennedy and Jacoby, 1999）。

本论文基于以下三个目的展开：（1）揭示海水养殖对环境因子、小型底栖生物群落及底栖食物关系的影响；（2）研究不同海水养殖模式对底栖环境（环境因子、小型底栖生物群落和食物关系）的影响；（3）评估所选的研究指标预警外来种海水养殖影响的指示效能。中国在世界海水养殖生产中的有不

可取代的地位，而中国海水养殖种引入的外来种种类多，分布广，但相关研究却相对缺乏。因此，我们选取了中国渤海两个典型的海水养殖案例——海湾扇贝 *Argopecten irradians*（外来种）的筏式养殖（于第 2，3，4 章讨论）以及凡纳滨对虾 *Litopenaeus vannamei*（外来种）和日本对虾 *Marsupenaeus japonicus*（本地种）的虾塘养殖（于第2，5章讨论）。论文结果分析了海水养殖对沉积物和底栖生物群落的影响，探讨了不同层面的指标应用于生态系统功能监测的可行性，并讨论这些指标对外来物种海水养殖进行环境监测的可能性。

我们首先分别分析了海湾扇贝筏式养殖以及日本对虾和凡纳滨对虾的池塘养殖对环境因子的影响，以及不同环境因子对海湾扇贝筏式养殖和虾塘养殖活动响应的敏感性（第 2 章）。环境因子包括理化指标（氧化还原电位、颗粒有机质、叶绿素a、生化组成、蛋白质与碳水化合物比值PRO：CHO）。（1）在海湾扇贝养殖的案例中，首先我们发现，海湾扇贝养殖不影响沉积物总有机碳氮含量。氧化还原电位则只在沙质浅水站位（水深 7 米）的扇贝筏架下受到影响——沉积物氧化还原电位呈现轻微但显著降低。生物可利用碳（Biopolymeric carbon，BPC）反映了底栖营养状况。在养殖密度较大的两个海湾扇贝养殖区中，沉积物 BPC 显著增加。（2）在对虾养殖池中，沉积物总有机碳氮、叶绿素 a、BPC 呈现出随养殖时间积累趋势。日本对虾虾池和凡纳滨对虾虾池的积累趋势不同。两种虾池相比，养殖前期和养殖后期这些指标也显著差异。（3）氧化还原电位值和生物可利用碳浓度显示，扇贝养殖水域的底栖环境处于寡营养状态，说明海湾扇贝筏架养殖并未对底栖环境造成显著的负面影响。而两种虾塘底栖环境严重恶化，最终呈现缺氧和高度富营养化。（4）由于BPC对环境变化响应快以及与底栖生物食物来源的密切联系，该指标相比其他指标更适合作为海水养殖评估。PRO：CHO比值在环境监测中应谨慎使用，因为微生物活动可能会降低该指标敏感度。

进而我们关注于海水养殖对小型底栖生物群落的影响。第 3 章和第 4 章为海湾扇贝筏架养殖区的案例。在第 3 章中，海湾扇贝养殖对小型底栖动物（高分类阶元水平）和底栖桡足类（物种水平）的群落结构和多样性的影响不大，

只在沙质底的站位中，扇贝养殖导致线虫的丰都和比例显著增加。在泥质底站位，我们观察到扇贝养殖致使底栖桡足类密度和物种数量显著增加，群落结构发生变化。这种结构变化与生物可利用碳（如 BPC）含量的积累趋同。我们建议，小型底栖生物群落和桡足类群落对扇贝养殖的响应因底栖环境的本底而异，不具有普适性，若使用这两个生物指标评估扇贝或其他贝类海水养殖对环境的潜在影响时，需要使用其他参数（例如环境因子）补充解释。

然后，我们利用碳氮稳定同位素技术，进一步分析了三个扇贝养殖场内外的主要小型底栖动物桡足类和线虫的食物来源（第 4 章）。我们发现，扇贝的粪便中 $\delta^{15}N$ 值显著高于其他食物来源的 $\delta^{15}N$ 值。而扇贝养殖场中几种小型底栖动物的 $\delta^{15}N$ 显著高于对照站位。同位素混合模型的结果表明，扇贝的粪便可能成为大部分底栖桡足类和某些线虫的食物来源。不同科的桡足类之间摄入扇贝粪便的数量和途径，因其各自的摄食行为和生存环境而异。此外，我们还测定优势类群 Canuellidae 科桡足类的脂肪酸含量，发现养殖站位的多不饱和脂肪酸（PUFAs），特别是二十二碳六烯酸（DHA）含量比对照站位显著增高，这由于其对扇贝粪便的摄食。由于底栖桡足类是鱼类的重要食物来源，其高质量脂肪酸含量的提高，对当地水域食物网有积极影响。

在第 5 章里，我们通过稳定同位素和脂肪酸技术，探究了不同对虾养殖阶段的底栖生物群落结构和食物来源，并描述了虾类饲料、底栖生物群落和对虾之间的营养关系。我们发现在凡纳滨对虾养殖过程中，由于线虫丰度与底栖富营养化密切相关，致使小型底栖生物群落产生变化。在养殖季节结束时，凡纳滨虾塘呈现出以线虫为主的高丰度、低多样性的小型动物群落，并呈现为受扰动状态。与凡纳滨对虾池塘相比，日本对虾塘中底栖生物的多样性较高，养殖早期线虫数量增加。在底栖食物网的研究中，天然食物源（底栖微藻、浮游植物和小型底栖生物）在养殖早期和晚期对日本对虾和凡纳滨对虾均有不同程度的贡献。在早期阶段，小型底栖生物与对虾（均为初级消费者）具有相似的食性，可能与对虾形成竞争食物的关系。由于对虾的食性随生长发生变化，在对虾养殖的后期，小型底栖生物被对虾大量摄食。我们还发现小型底栖生物含有

丰富的 PUFAs，是高营养饲料。相比之下，日本对虾和凡纳滨对虾较少消耗人为投入的饲料（日本对虾：杂鱼；凡纳滨对虾：豆粕、卤虫；商业饲料）。这些饲料可能通过其他途径进入底栖食物网，如细菌代谢。

本论文采用环境因子、小型底栖生物群落和稳定同位素混合模型，首先发现海水养殖外来种对底栖环境的影响具有系统特异性，并且取决于若干生境特征，例如水动力学、沉积物类型和背景有机物含量。海湾扇贝筏式养殖没有对底栖生态系统产生负面影响，而养殖日本对虾和凡纳滨对虾的滨海虾塘则呈现有机物积累的底栖环境。然后，理化指标、生物指标和生态系统功能作为环境评价的指标，也不具有通用性。最后，研究生态系统功能（例如能量流动）能够为海水养殖评估提供更全面的认识。

Chapter 1: General introduction

1.1 Aquaculture and mariculture

1.1.1 Aquaculture worldwide

Aquatic food provides animal proteins and is increasingly consumed in human diets in the past decades (Tacon and Metian, 2013). The world per capita fish consumption has been estimated to increase beyond 20 kg/year in recent years (FAO, 2016). However, the traditional supplies of aquatic food products, i.e. capture fishery, appear to have levelled off around 90 million tonnes/year over the last two decades (FAO (2016); Fig 1.1). Thus, the reliance on aquaculture has become stronger and the industry has expanded continuously (Fig 1.1). In 2015, the global aquaculture production reached 76.6 million tonnes, contributing 45.8% of the total aquatic production, which is 5.8 times more than 25 years ago (FAO, 2017). Until 2020, 50% of the global aquatic food will derive from aquaculture; 32.3% of which will come from marine aquaculture, or mariculture (FAO, 2011).

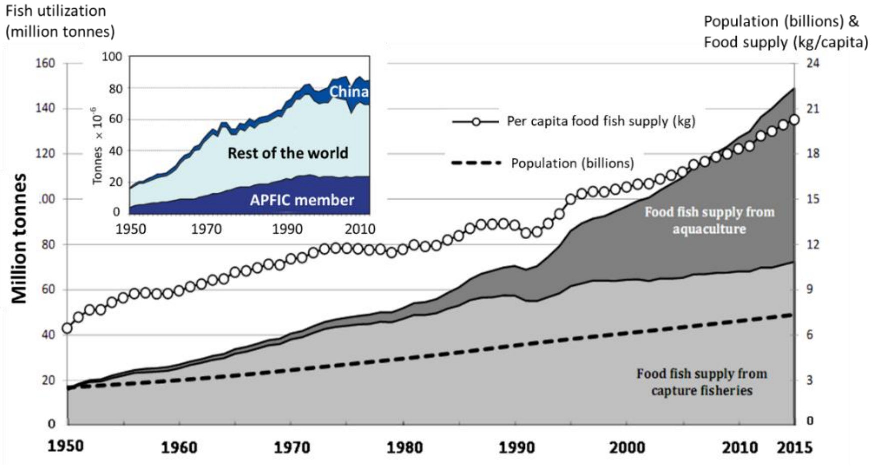

Figure 1.1 Trends of world capture fisheries and aquaculture production (Modified from FAO, 2017; APFC, 2014)

The Asia-Pacific region, in particular Asia (representing 88.9% of the global aquaculture production), remains the major aquaculture global producer, with production from mariculture increasing from 7.6 million tonnes in 1994 to 21.9 million tonnes in 2014 (Subasinghe, 2017). More than 200 species of aquatic animals are cultured with diverse farming forms (APFC, 2014). Among those cupped oysters, Japanese carpet shell, whiteleg shrimp, scallops, and other marine molluscs are the top five cultured organisms in the marine environment by quantity (Subasinghe, 2017).

1.1.2 Mariculture in China

China has a long history of mariculture that can be traced back 2000 years ago (Li et al., 2011). China also dominates the global mariculture production since the late 1980s. The marine production largely increased after 1985 (production: 1.42 million tonnes; area: 0.27 million hectares) and reached 19.63 million tonnes of production in an area of 2.16 million hectares in 2016. The forms of mariculture are diverse, with mariculture industries being present in both open-water (e.g. suspended culture which include long-line and cage culture; see Box 1.1) as well as based in coastal land (e.g. ponds). Suspended cultures produce the largest yield, accounting for 37.6% of the total production in 2016, followed by bottom sowing (see Box 1.1) (35.3%) and pond culture (15.2%) (MOA, 2017).

Mariculture areas are unevenly distributed along the 18593 km long Chinese coastline. Shandong province, located along the Yellow Sea and Bohai Sea, is the most important mariculture province, featuring the highest mariculture production among nine other coastal provinces in the last 5 years (Wartenberg et al., 2017), and producing more than 25% of marine aquatic products in 2016 (MOA, 2017).

There are 36 marine and brackish water species categories recorded in MOA 2003-2014 (Tang et al., 2016). The predominate mariculture production focuses on the low trophic-level species: mollusks, macroalgae and crustaceans (Fig 1.2). In 2016, the annual production of mollusks (i.e., any of the phylum Mollusca of invertebrate animals with a soft unsegmented body usually enclosed in a calcareous shell; of which bivalves - oyster, clam, scallop, or mussel are the main) increased to 14.21 million tonnes (72% of total mariculture output), followed by macroalgae (2.17 million tonnes); crustaceans ranked the third with 1.56 million tonnes production (Fig 1.2; MOA, 2017).

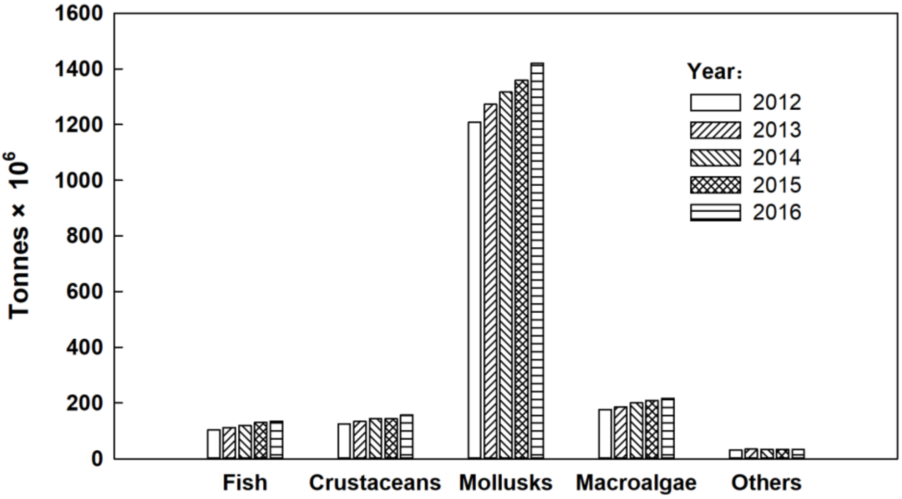

Figure 1.2 Mariculture species in Chinese marine coastal waters during 2012-2016. Data from MOA (2012-2016)

Among cultivated mollusks, scallop is one of the most popularly cultured one that occupied 0.47 million hectares (34.9% of total mollusks mariculture area) with a production of 1.86 million tonnes in 2016. The northern Yellow Sea and the Bohai Sea contributed more than 90% of the total production in China (MOA, 2017). Scallop farming has expanded rapidly since 1980s and increased by 320-fold during 1984-2016, accounting for 87% of world scallop production in 2016 (FAO, 2017; MOA, 2017). High demand of scallop is expected to further accelerate the expansion of this mariculture in the current production areas (Han et al., 2013).

Box 1.1 Main types of mariculture system

- *Pond* culture is land-based and built in tidal and intertidal mudflats near estuaries, bays, creeks, lagoons and salt marshes. Ponds are mostly used for farming shrimp and crab, but also produces mollusk, finfish, and macroalgae. Coastal ponds contain brackish or salt water and are usually constructed of soil (the so-called earthen pond) (Jia and Chen, 2001). The major pond culture management includes the water exchange with natural environment and addition of fertilizers and feeds.

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- *Suspension longline culture* is conducted in open-waters (open sea or coastal embayment and estuaries). This system requires moorings and rigging; The cultured organisms are placed in trays or nets hanging in long-lines or floats, so that they can suspend below the water surface. The cultured species include filter-feeding organisms (scallop, oyster, mussel) and algae (kelp and laver) (Jia and Chen, 2001). No feeding costs arise during the grow-out phase.

- *Bottom sowing* or bottom culture can be conducted in open-water or in coastal ecosystems (e.g. mudflat). The cultured species are usually deposit feeders (e.g. sea cucumber, abalone, cockle, Japanese scallop). The hatchery-reared juveniles are released and grow up on the bottom of the natural environment. Neither physical structures nor feed are involved (Wartenberg et al., 2017).

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- *Cage culture* is similar to suspended longline culture in the sense that structures are also moored in the open water, but with a different structure. The cultured organisms (mostly finfish) are enclosed in a cage or basket where water can pass freely (Masser, 1988). Feeds are periodically applied in the system. Cage cultures are commonly positioned in the inshore coastal areas. However, there is a recent trend to move the cage in off-shore waters in order to take advantage of enhanced water exchange (Holmer, 2009).

Another noteworthy mariculture sector in China is the shrimp farming in the land-based coastal ponds. Chinese shrimp farming was initiated in the 1970s and experienced a rapid increase during the 1980s (total shrimp production increased by beyond 400 times during 1978-1991). In the early 1990s, shrimp pond mariculture underwent a recession due to the outbreak of shrimp diseases in *Penaeus chinensis*, but the industry largely expanded again since 1998 (growing from 0.14 to 1.27 million tonnes during 1998-2016) (Cai and Wang, 1999; Miao, 2005; MOA, 2017). The introduction and widely applied culturing of *Litopenaeus vannamei* is one of the important reasons for this expansion (Miao, 2005). Land-based pond is the most important culture method of shrimp production in coastal areas (FAO, 2010). There are three categories for marine shrimp farming in terms of cultured area and stocking density, as practiced in China: extensive (traditional), semi-intensive, and intensive (Table 1.1). Although the government is focusing on developing techniques for intensive shrimp farming, small scale semi-intensive farming is currently still the mostly practiced form of shrimp mariculture in China (Wu, 2012).

Table 1.1 Shrimp farming strategies. Modified from Tacon and Metian (2013)

	Extensive	**Semi-intensive**	**Intensive**
Rearing units	Large earthen ponds or enclosures up to 100 ha	Earthen ponds of 1-20 ha	Earthen ponds/plastic or concrete tanks (1-20 ha/100-1500 m^3)
Water exchange	< 5%	5-20%	5-40% (outdoor)/< 5% (indoor)
Stocking rate	< 5 ind. m^{-2}	12-25 ind. m^{-2}	40-2000 ind. m^{-2}
Labor input	< 0.1 person/ha	0.1-0.5 person/ha	0.4-3 person/ha
Feeding regime	none or fertilization	Fertilization and feed	Fertilization and feed
Feed conversion ratio	0.9-1.3	1.2-1.75	1.4-3.0
Shrimp production	500-1000 kg/ha/year	1000-3000 kg/ha/year	10000-340000 kg/ha/year

1.2 Potential effect of mariculture on coastal marine ecosystem

Mariculture, like any other human activity, affects the environment, due to its reliance and links to natural waters, sediments and food webs. The degree of concern about impairing the natural resources is in proportion to the extent of resource exploitation and development of the industry (Pillay, 2008). In this context, the increasing emphasis on mariculture has been questioned on environmental grounds and accompanied by potential problems (Silva et al., 2009); many of these are associated with habitat destruction (Naylor et al., 2000), loading of organic waste and eutrophication (Holmer et al., 2005), transferring of diseases (Meyer, 1991) and biological invasions (Arismendi et al., 2009). Although such irreversible changes in coastal marine environments were caused by the lack of management of mariculture activities, we should also realize that not all environmental consequences of mariculture are negative, for instance, cultured bivalves can be extractive and improve water quality in nutrient enriched areas (Shumway et al., 2003). It is also known that the influence of mariculture largely depends on the characteristics of the selected location, e.g. hydrodynamics, physical and chemical features (Giles et al., 2006; Sarà, 2007), as well as the culturing condition, e.g., reared species and their densities (Callier et al., 2007; Sarà, 2007).

1.2.1 How mariculture causes environmental stress: two case studies

Mariculture comes in multiple versions; two of which, water-based farms and land-based ponds, are well-recognized polluters worldwide, causing different types of environmental stress (Pillay, 2008). To illustrate that, the present PhD research will analyze two cases: bivalve mariculture (water-based) and coastal shrimp ponds (land-based), which are both widely applied worldwide, and especially in China.

Case 1: Bivalve mariculture

Suspended cultured bivalves, including mussels, scallops and oysters, generally change the environment in two ways: 1. material processing - feeding, excretion and defecation; 2. physical alteration - structures introduced by mariculture, such as anchoring facilities. The material processing procedure makes bivalve species "keystone" species in the ecosystem (Fig 1.3): they filter the particles (e.g. phytoplankton and detritus) from the water column and then produce dissolved ammonium and biodeposits (i.e. faeces and pseudofeaces). When bivalve populations are abundant, such activities exert "top-down" control of phytoplankton and also "bottom-up" effects through biodeposition on the sea bottom (i.e. benthic environment) (Dame, 1996; Sousa et al., 2009). Although bivalve mariculture does not require any external feed inputs that cause additional nutrient loadings, their capacity to largely relocate particulate organic matters from the water column to benthic sediments (so-called biodeposition) has raised a lot of concerns, especially when bivalve species are present at high densities (Dumbauld et al., 2009; McKindsey et al., 2011).

Biodeposits of bivalve species can be rich in labile organic contents and of good nutrient value with low C:N ratios (Kautsky and Evans, 1987; Miller et al., 2002). With a fast sinking velocity, bivalve farming areas usually have enhanced organic flux to the benthic environment (Dame, 1996; Newell, 2004). Studies found that mussel farms increased sedimentation by a factor of 3 to 12 (Dahlbäck and Gunnarsson, 1981; Grenz et al., 1990; Hartstein and Stevens, 2005). Dahlbäck and Gunnarsson (1981) reported that mussels in mariculture areas deposited more than 1000 g organic carbon m^{-2} per year. This is also the case in the scallop farms in China (Wang et al., 2018; Zhou et al., 2006).

There is a paradox that bivalve farming is facing: bivalve may help with the assimilation of nutrients in the water due to their large filtration of water. On the other hand, the extensive production of biodeposits can cause an organic enrichment in the sea floor and affect benthic communities (including macrobenthos, meiobenthos, microphytobenthos, macrophyte and bacteria) by modifying: (1) the physicochemical characteristics in the water or sediment, e.g. lower the oxygen concentrations, change the sulfide pathway that lead to sulphate reduction, change in nutrient ratio (Fig 1.3) (Hargrave et al., 2008) and (2) the quantity and quality of the food sources for benthic organisms (Callier et al., 2013; Dubois et al., 2007).This has potential to further deteriorate the ecological status of benthic environment (Holmer et al., 2005). In order to ensure the sustainability of this industry, more information about the relationship between biodeposits production and its influence on the benthic environment is needed (Callier et al., 2006).

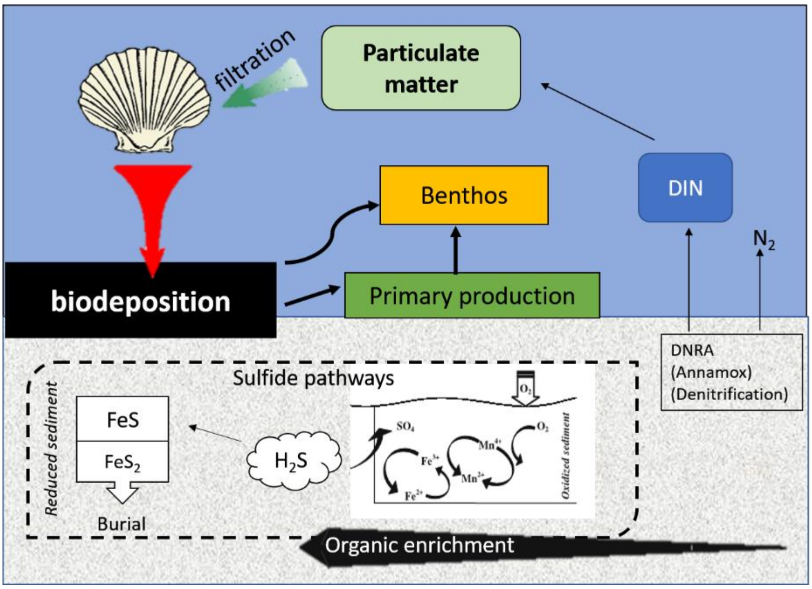

Figure 1.3 Scheme of the biological processes of scallops and their potential ecological impacts. DNRA = Dissimilatory nitrate reduction to ammonium (DNRA). DIN = dissolved inorganic nitrogen; Particulate matter includes phytoplankton and detritus. Note: denitrification and annamox may be inhibited due to a lack of available NO_3^- (Castine et al., 2012).

Case 2: Coastal shrimp ponds

Coastal shrimp ponds are built at the expense of destroying natural habitats (such as wetlands) (Ha et al., 2014; Polidoro et al., 2010; Senarath and Visvanathan, 2001), as well as their associated ecosystem services, e.g. carbon sequestration, elimination of greenhouse gas (Chmura et al., 2003; Mcleod et al., 2011).

Other than that, the accumulation of nutrients (both organic and inorganic) is also a key environmental concern in the majority of coastal shrimp ponds (Funge-Smith and Briggs, 1998; Vismann, 1996; Yang et al., 2017). Most shrimp ponds are maintained through the external supply of feed and fertilizer. Fertilizer is used to stimulate the natural productivity of phytoplankton and microphytobenthos in the ponds, while feed provides extra food sources for the shrimps in case the natural primary production is not sufficient. There are various feed types that range from farm-made (e.g., soymeal and fishmeal) to commercially formulated feed (Gamboa-Delgado, 2014). However, only a small proportion of these nutrients are assimilated by shrimps (New, 1987). As the efficiency of feed utilization is only 4-27.4% (Chen et al., 2016; Su et al., 2009), more than 70% of nutrients in the unconsumed feed together with the shrimps' faecal materials become waste products. These products contain high concentrations of organic carbon and nitrogen, ammonium, urea, bicarbonate and phosphate (Pillay, 2008). The fate of these substances depends on whether the leftover feed and faeces gets buried in the sediment or is transformed into dissolved inorganic nutrients or gas after reworking by different groups of microbes in the pond bottom (Fig 1.4). In either way, the nutrients accumulate in the pond; once they reach the tolerance thresholds, adverse effects on water and sediments appear in the form of harmful algae blooms and deterioration of pond quality, that further cause the shrimp disease and reduce production (Hu et al., 2014). Moreover, the discharge of the untreated pond effluents further pollutes the adjacent coastal habitats, causing eutrophication and posing threats to other marine organisms (Boyd, 2003; Feng et al., 2004). However, in well-managed ponds, waste of feed can be minimized (Boyd, 1995). One of the most important ways to minimize waste discharge is the proper estimation of feed input based on the feeding behavior of cultured shrimps and the pond food web (Martinez-Cordova et al., 1998).

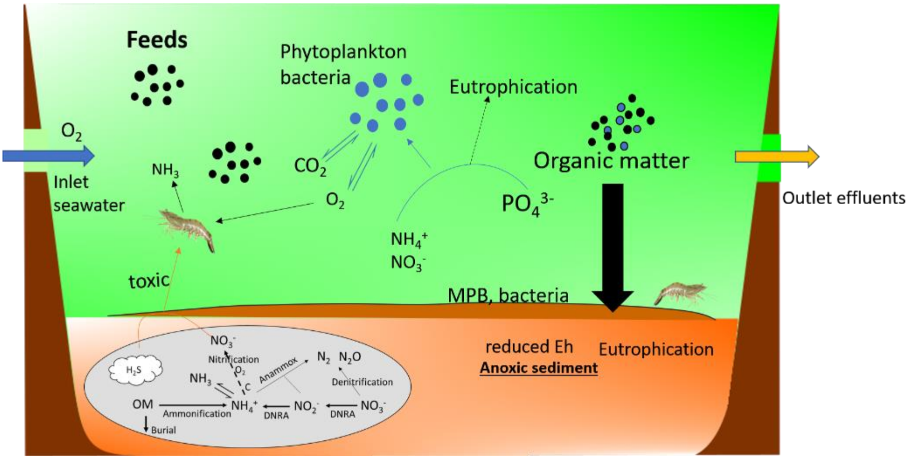

Figure 1.4 Scheme of biochemical processes in shrimp ponds. Modified from Funge-Smith and Briggs (1998). The dashlines indicate reations occuring under oxic condition or oxic layer. When the organic loading is high and the oxygen supply is lower than the compustion, the sediment became anoxic. Nitrification stops due to an oxygen deficiency. During the hydrosulfide production, free sulfides inhibit nitrification and therefore, the N_2 production - denitrification and annammox may be inhibited due to a lack of available NO_3^- (Castine et al., 2012). Dissimilatory nitrate

reduction to ammonium (DNRA) and ammonification is increased, stimulating primary production on one hand, and being toxic to shrimps on the other hand.

1.2.2 Potential impacts of mariculture on the benthic environment

Stressors (or causes) from the two above cases are different but the effects are both directed towards the **benthic ecosystem**. As organic enrichment of aquatic ecosystems increases, the balance between pelagic and benthic metabolism appears to shift to become dominated by benthic processes (Holmer et al., 2005).

Box 1.2 Benthic environment

The benthic environment is defined as "physically distinct areas of the seabed that are associated with the occurrence of particular species" (Harris and Baker, 2012). This definition refers to both abiotic (i.e. physical substrates) and biotic elements (i.e. benthic communities including primary producers and consumers). The benthic environment is an important location of various biogeochemistry cycles (Levinton and Levinton, 1995). It serves crucial functions such as space for shelter, feeding, and breeding for benthic communities and fishes (Kritzer et al., 2016). The benthic environment is currently under stress and risks due to human activities (Diaz and Rosenberg, 2008). It is urged to protect benthic communities and habitats so that biological diversity and ecological functioning and the associated services can be maintained (Giere, 2013).

The accumulation of organic enriched mariculture waste can affect the benthic environment at three levels: sediment physiochemical properties (abiotic), organisms' characteristics (biotic), and ecosystem functioning.

1.2.2.1 Potential benthic effect of mariculture at the abiotic level

Organic waste from mariculture degrades more easily than the natural particulate matter in coastal areas since it contains a large proportion of labile organic matter and stimulates the microbial activity when settling on the sediments (Holmer et al., 2005; Kausty and Evens, 1987). This causes changes in sedimentary geochemical cycles and thus affects the physio-chemical properties of sediments (Holmer et al., 2005). The texture of sediments may be altered. For instance, the intensive organic loading can turn fine sand bottoms to muddy deposits (Kaspar et al., 1985; Netto and Valgas, 2010).

Due to the development of microbial activities, a direct consequence of organic enrichment is oxygen consumption, which was found to be higher under finfish and bivalve farms and in the bottom of culture ponds (Burford and Longmore, 2001; Christensen et al., 2003; Giles et al., 2006; Papageorgiou et al., 2010). In areas experiencing low turbulence and high organic input, the oxygen consumption rate may become greater than that of renewal by water exchange and thus the sediment can become anoxic (Pillay 2008). When the oxygen is limited, other terminal acceptors will be used for organic decomposition, generating reduced sediments with low redox potential and producing associated reduced products, e.g. H_2S, ketones, aldehydes, amines and mercaptans (Avnimelech and Ritvo, 2003). Other than that, nitrogen and phosphorus are also commonly abundant in both open-water farms and coastal ponds (Boyd, 1995; Martin et al., 1998).

1.2.2.2 Potential benthic effect of mariculture at the biotic level

Organic enrichment can primarily affect the microscopic biota such as microphytobenthos, bacteria, protozoa, metazoa, etc. For instance, the abundance and biomass of bacteria and heterotrophic protists was found to increase significantly by the feed pellets from fish farms (Bongiorni 2005). Primary production by microphytobenthos was stimulated under some fish and mussel farms (Franzo et al., 2014; La Rosa et al., 2001), while others found reduced production of microphytobenthos under mussels farms (Christensen et al., 2003; Sdrigotti and Umani, 2002).

Organic loading can affect the benthic infaunal communities (i.e. benthos) in their number of species – S, abundance – A, and biomass – B (SAB). This was first described in Pearson and Rosenberg (1978) by studying the temporal and spatial changes of macrobenthic communities along an organic gradient (Fig 1.5). It is further concluded that the negative effects on macrobenthos is rather caused by the formation of hypoxic and anoxic conditions and the toxic effects of hydrogen sulfide as the organic matter increased (Gray et al., 2002; Hargrave et al., 2008). As the organic matter accumulates from natural to moderate levels, the small opportunistic species (e.g. polychaetes such as *Capitella capitata*) present and enhanced with the organic gradient (Pearson and Rosenberg, 1978). Once the organic matter increased to a higher level, the sediment becomes anoxic and even the tolerant taxa cannot survive, and at the end, only single cell organisms such as microbes are able to present (Fenchel and Riedl, 1970; Hamoutene, 2014). Studies on macrofauna from aquaculture sites often found the changes of macrobenthic communities associated with the organic loading and oxygen supply (Brook et al., 2003; Chamberlain et al., 2001; Keeley et al., 2012; Mangion et al., 2017; Wildish et al., 2001). As with the general model, moderate organic enrichment leads to density enhancement of total meiobenthos (Castel et al., 1988), nematodes (Lambshead, 1986; Moore and Bett, 1989) and epi- and endobenthic copepods (Shiell and Anderson, 1985). As oxygen concentrations decrease, most of the species will disappear (harpacticoid copepods usually disappear before nematodes), except few tolerate species (e.g. some sulfide tolerant nematodes) that live in anoxic environment and feed on decaying materials and bacteria (Giere, 2009). The mariculture impact studies on meiobenthos usually reported a large reduction in meiobenthos density and diversity under the fish farms which generate reduced and anoxic conditions (Grego et al., 2009; Mazzola et al., 1999, 2000; Mirto et al, 2000; La Rosa et al., 2001). Other study found no effect of mussel farming on either the density nor diversity of meiobenthos which they attribute to high hydrodynamics that alleviate the organic accumulation (Danovaro et al., 2004).

Food availability is also an important driver of benthic community. Rosenberg (1987) and Rosenberg (2001) further suggested that the SAB of benthic communities is enhanced by the food availability. In mariculture sites, the downward organic flux potentially increases the food loading on the benthic environment, which may facilitate some trophic group of benthos (e.g. deposit feeding benthos) (Callier et al., 2013).

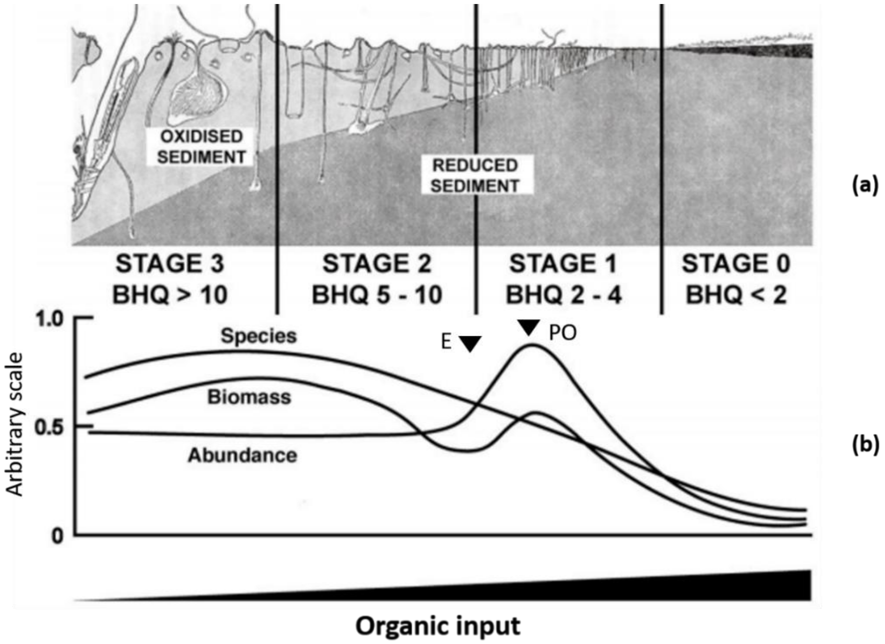

Figure 1.5 The Benthic Habitat Quality (BHQ) index (Nilsson and Rosenberg, 1997) (a) and the general model of predicted response of benthic communities along a gradient of organic enrichment (SAB curve; S- number of species, A – abundance; B – biomass; after Pearson and Rosenberg, 1978) (b). Diagram adapted from Rosenberg (2001). PO: peak of opportunistic species; E: ecotone point - An ecotone point is a transition region between two or more diverse communities (Odum and Odum, 1959). In this case, the community in the ecotone point consists of species from both polluted side and unpolluted side of communities (Pearson and Rosenberg, 1978). As the organic matter increased, the oxygen concentrations decrease that changes the oxidation-reduction state of the sediment as well as the habitat quality (upper graph), and the benthic communities in terms of species, biomass and abundance (bottom graph).

1.2.2.3 Potential benthic effect of mariculture at the ecosystem functioning level

Environmental effects of mariculture can also occur cumulatively on a systematic level and affect ecosystem functioning. The notion of ecosystem functioning integrates changes in energy and matter over time and space through biological activity, e.g. food-web characterization (Power, 1992).

There is a broad range of potential effects of mariculture at the functional level. The potential to change the aquatic food web is the most fundamental one. Introducing cultured animals into ecosystems may influence trophic interactions since the natural food sources have been modified (Lefebvre et al., 2009). Faeces and uneaten feed settle on the benthic environment where they may enter different ecosystem levels. The large particles can be consumed by detritus-feeding organisms (Callier et al., 2013; Redmond et al., 2010), while the small particles remain suspended and are assimilated by consumers in the water column such as zooplankton or fish (Vizzini and Mazzola, 2012). Such processes change the energy pathways of ecosystems and potentially shift a system from top-down- to a bottom-up driven.

In addition, parts of nitrogen and phosphorus from mariculture wastes underwent significant transformation by different groups of microbial communities and thus resulting in increased nutrient fluxes. This may induce further cascading effects that are greater than simple shifts in nutrient levels, e.g. release of nitrogen stimulation of primary production (Hatcher et al., 1994), production of reduced materials that are toxic to consumers (e.g., H_2S, ammonia) and cause lethal or sublethal effects on fish and shrimp (Funge-Smith and Briggs, 1998; Vismann, 1996).

1.3 Environmental assessment for mariculture

The environmental threats related to mariculture as stated in section 2 have raised awareness to stimulate the development and implementation of environmental policies to protect and manage coastal marine ecosystems (Borja et al., 2008). With varying monitoring strategies among countries, aquaculture policies focus on environmental legislation and monitoring (Wang, 2001). Therefore, it is necessary to improve the understanding of ecosystem functioning and provide effective methods to decision-makers and the public regarding monitoring organic waste discharge from mariculture (Hooper et al., 2005; Kalantzi and Karakassis, 2006; Lubchenco, 1998).

To determine the impact of mariculture in quantitative terms, many indicators have been proposed, ranging from abiotic monitoring approaches, e.g. physicochemical parameters, to more recently applied bioindicators (i.e. the biological processes, species, or communities which used to assess the environmental quality and how it changes over time) (Anderson et al., 2005; Callier et al., 2008; Hargrave et al., 2008; Holt and Miller, 2011; Wildish et al., 2001). In addition, new techniques such as stable isotopes and fatty acids have been introduced to reveal information on mariculture impacts at the functional level, e.g. identifying the origin and dispersal of farm-derived wastes (Vizzini and Mazzola, 2012; White et al., 2017).

1.3.1 Classic physicochemical approaches

Based on the geochemical cycle of sulfur that drives anoxic condition, redox potential and total sulfide are commonly used as basic monitoring methods for organic enrichment (Hargrave et al., 2008). Studies conducted on mariculture areas classified organic enrichment on sediments into five categories from oxic to anoxic (Figure 1.6). Wildish et al. (2001) developed an empirical regression equation to predict the relationship between total sulfides and redox potential. However, monitoring organic enrichment through redox potential and total sulfide is not always efficient, for instance, these methods failed to detect the organic enrichment in mussel farms (Callier et al., 2007). Anderson et al. (2005) found that the wide variability in sediment characteristics, such as organic matter concentrations, may mask any general trends between farm sites and reference sites, and suggested to consider the natural level of organic matter when developing methodologies to assess and monitor for mariculture sites.

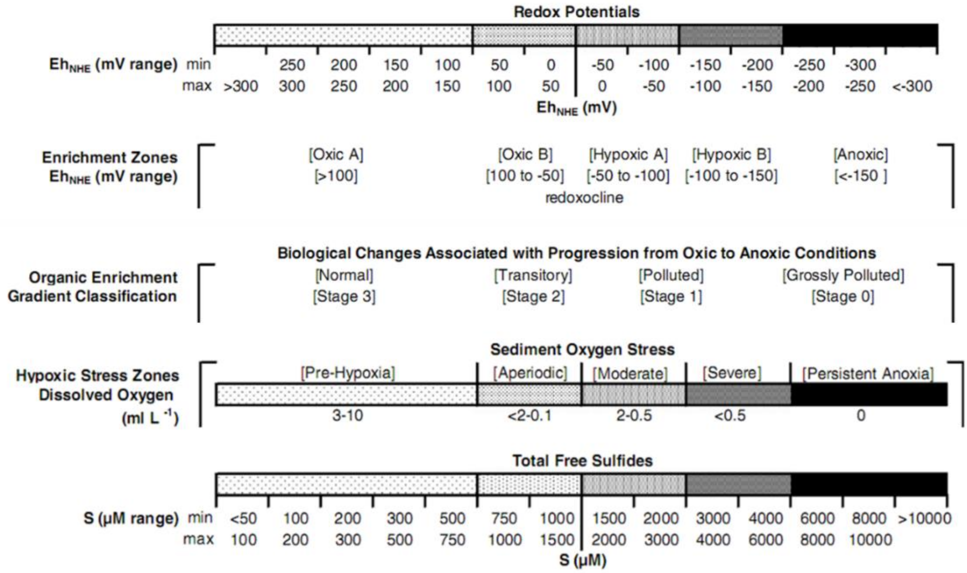

Figure 1.6 Benthic enrichment zonation based on redox potentials and sulfides
Modified from Hargrave et al. (2008).

1.3.2 Benthic trophic status as an indicator

Sedimentary organic matter is the main energy source for benthic communities. The information on the organic matter input to the benthos (i.e. trophic status) has been proposed to evaluate benthic eutrophication resulting from mariculture (Pusceddu et al., 2009; Pusceddu et al., 2011). Measuring the quantity of bulk organic matter (e.g. total organic carbon) is one of the most direct approaches to reflect the benthic trophic status as well as assess organic enrichment (Nixon, 1995). However, due to its conservative nature, changes in bulk concentration is not always detectable when the organic loading is low (Callier et al., 2008; Fabiano et al., 1995). In fact, the benthic trophic status is also a function of organic quality. One approach to measure benthic trophic status is to use of abundance of photosynthetic primary producers, e.g. microphytobenthos (Franzo et al., 2014; Kelly, 1998). Another approach consists of measuring changes in the biopolymeric fraction of organic matter in the sediments, which seems to be more sensitive to the presence of mariculture waste and can reflect the modification of trophic status by mariculture (Mirto et al., 2000; Pusceddu et al., 2009; Pusceddu et al., 2011; Vezzulli et al., 2002).

Box 1.3 Use biopolymeric carbon to indicate benthic trophic status

Biopolymeric carbon (BPC) is the fraction of total organic carbon that potentially available to benthos (Fabiano et al., 1995). It is calculated as the sum of carbon equivalents in proteins, carbohydrates and lipids by using the following conversion factors: 0.49, 0.40, and 0.75 mg C mg^{-1}, respectively (Fabiano et al., 1995). BPC includes the heterotrophic nutrition and is important to benthic environment where large proportions of food for benthos are deposited or detrital organic materials (Pusceddu et al., 2009). BPC can represent the total organic carbon pool, on the other hand responds promptly to the changes in productivity of benthic environment (Fabiano et al. 1995; Pusceddu et al. 2009). The accumulation of BPC increases the oxygen consumption and may be responsible for the hypoxic or anoxic condition in sediment (Pusceddu et al., 2009).

1.3.3 Meiobenthic research as mariculture assessment

Measuring only abiotic variables to identify changes can be somehow problematic (Goodsell et al., 2009; Suter, 2001). Living organisms are considered more appropriate indicators since they integrate abiotic and biotic elements of an ecosystem through their adaptive responses (Casazza et al., 2002). Choosing the right target faunal group is a crucial factor to successfully detect the biological impacts of mariculture (Schratzberger et al., 2000). Compared with macrofauna (i.e. benthos which is retained on a 500 μm mesh sieve), meiofauna (see 3.3.1) is a more accurate target faunal group, because of its higher sensitivity to environmental changes and faster generation turnover (Giere, 2009). Consequently, it has recently been developed as an environmental assessment tool (Kennedy and Jacoby, 1999; Mirto et al., 2012; Mu et al., 2002).

1.3.3.1 Meiobenthos, harpacticoid copepods: the definition

The term of "meiobenthos" (also known as "meiofauna") was introduced by Mare (1942) in order to define a group of benthic metazoans that are smaller than "macrobenthos" but larger than "microbenthos" (Giere, 2009). Their size range varies: from 500μm/1000μm as an upper limit of sieve size and 38 μm/44 μm/63 μm as a lower limit of the sieve (Giere, 2009; Higgins and Thiel, 1988; Mare, 1942; Swedmark, 1964). Some organisms are "permanent" meiobenthos, while others are only members of the meiobenthos during their early stages and later become macrobenthos. Meiobenthos are abundant (on average 10^6 ind m^{-2}) in all marine ecosystems (Higgins and Thiel, 1988). They also show a high diversity in morphology and taxonomy. The most dominate taxa include nematodes, copepods, ostracods, kinorhynchs, tardigrades, gastrotrichs, turbellarians, oligochaetes, polychaetes and rotifers. Gastropods and bivalves are also important groups of the meiobenthos (Gee, 1989).

Harpacticoid copepods are small crustaceans belonging to the order Harpacticoida of the subclass Copepoda and are recognized as the second most abundant meiobenthos taxon in marine sediments after nematodes (McIntyre, 1969). In general, harpacticoid copepods have a linear shape with a wider cephalosome than the urosome. The morphology can however vary in function of the environment (Fig 1.7). Harpacticoid copepods are usually classified into interstitial, burrowing, and epibenthic groups (Hicks, 1983).

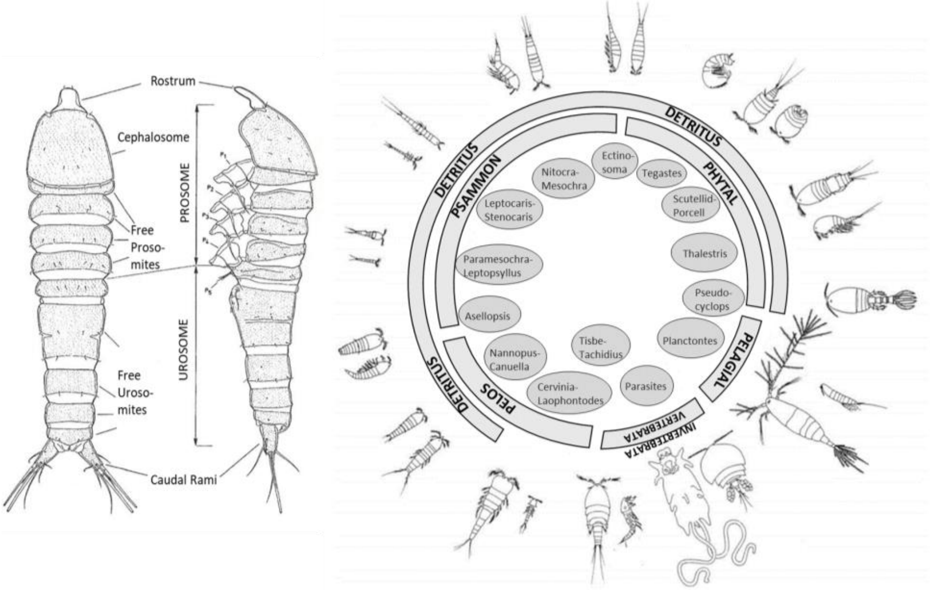

Figure 1.7 Basic body plan of harpacticoid copepods (Huys, 1996) and the morphological adaptions of Copepoda to habitats (Noodt, 1971)

All meiobenthos lives in or on sediments, and sediment granulometry characteristics are thus one of the most important factors driving their distribution patterns (Coull, 1988). For instance, interstitial harpacticoid copepods are abundantly present in sandy bottom, while shallow muddy substrates are dominated by burrowing harpacticoids. Other than granulometry, other abiotic factors, e.g. oxygen level, redox potential, total sulfide and biotic factors, e.g. food availability, also largely affect assemblage of harpacticoid copepods (Giere, 2009).

1.3.3.2 Their roles in marine food web

The energy flow in the marine food web can generally be attributed to three pathways: herbivorous (primary producers - lower consumers - higher consumers), detrital (detritus – decomposers/detritivores), and microbial (microbes - microbivores) (Landry, 2002; Moore et al., 2004). Meiobenthos consumes a wide spectrum of food sources and is involved in all these pathways (Giere, 2009). It is well recognized that detritus is vital for the meiobenthos' nutrients in shallow coastal environments (Schlechtriem et al., 2004). This particularly holds for nematodes; some groups of which can directly consume deposited phytodetritus (Moens et al., 2002; Ólafsson et al., 1999), as well as detritus derived from plants e.g. *Spartina*, seagrass (Carman and Fry, 2002; Lebreton et al., 2012; Vafeiadou et al., 2014).

The herbivorous pathway is strongly linking primary producers and meiobenthos. Many harpacticoid copepod and nematode species strongly rely on microphytobenthos (Buffan-Dubau and Carman, 2000; Cnudde et al., 2015; Montagna et al., 1995). Some can selectively feed on diatoms of a particular size (De Troch et al., 2006). Herbivorous and detrital pathways sometimes crossover with microbial pathway in small food web (Van Oevelen et al., 2006). For instance, microphytobenthos can excrete mucus-enriched extracellular polymeric substances (EPS) (Orvain et al., 2003). This EPS serves as substrate for bacteria which are consumed by copepods and nematodes (De Troch et al., 2006; Rzeznik-Orignac et al., 2008).

The grazing behavior of meiobenthos is a regulator of the ecosystem process e.g. mineralization. Grazing by meiobenthos, the bacteria communities can be enhanced, since their population has been kept in an active growth phase (Nascimento et al., 2013). The mucus excreted by meiobenthos can also attract bacteria to colonize (Moens et al., 2005). Furthermore, the enhanced bacteria together with the bioturbation of meiobenthos speed up mineralization of labile organic matter (Alkemade et al., 1992; De Mesel et al., 2004; Kristensen, 2000). As such, the meiobenthic grazing behavior is crucial to the ecosystem process in the mariculture benthic environment, since mariculture sites are usually characterized by an enhanced bacteria community and microbial loops (La Rosa et al., 2001; McKindsey et al., 2011; Nevejan et al., 2018).

Meiobenthos is also an important food item for macrofauna (e.g. shrimps and crabs) and fish (Gee, 1989). Fish were found to preferentially feed on harpacticoid copepods because of the lower cost of capturing them and their high nutrient levels e.g. calories and fatty acids (See BOX 1.4) (Bell and Tocher, 2009; Gee, 1987; Volk et al., 1984). Harpacticoid copepods thus become important nutrient conveyors to higher trophic levels in aquatic food webs (Iverson, 2009). In this sense, meiobenthos is encouraged to be widely used as mariculture feed (Gee, 1989). Harpacticoid copepods are considered as good candidates but are so far limited because of difficult mass rearing (Chandler, 1986; Nanton and Castell, 1999). More recently, nematodes have been proposed to be applied in the mariculture industry as well (Da Silva et al., 2008; Schlechtriem et al., 2004).

As such, meiobenthos play an important role of connecting the lower and higher levels of aquatic food webs. Consequently, any environmental impact on the assemblages of this group (e.g. abundance, diversity) can cause a cascading effect on ecosystem functioning.

Box 1.4 Fatty acids and essential fatty acids

Fatty acids (FA) are the important unit of lipids, which consist of a carbon and hydrogen chain terminated respectively with a carboxylic acid (COOH) and a methyl group (CH_3) at two ends (Iverson, 2009). There are various forms of FA with different length (14-24 carbons), saturation (0-6 double bonds), and shape. During recent years, the role of FA in aquatic ecosystems has gained growing interest (Brett and Muller-Navarra, 1997). Long-chain (≥20 carbons) polyunsaturated FA (PUFA) with the linolenic (ω3) and linoleic (ω6) groups, particularly 20:5ω3 (eicosapentaenoic acid, EPA) and 22:6ω3 (docosahexaenoic acid, DHA), are well-recognized to regulate the survival, growth, and reproduction of zooplankton (Brett and Muller-Navarra, 1997), harpacticoid copepods (Ederington et al., 1995), benthic macrofauna (Vanderploeg et al., 1996), and fish (Watanabe et al., 1982); i.e. the so-called essential fatty acids (EFA). Those EFA affect the dynamics of phospholipids structure that influence membrane protein functioning (Iverson, 2009; Li et al., 2009). Unfortunately, EFA can only be synthesized by primary producers, heterotrophic protists, and some lower animals (e.g. harpacticoid copepods) (Monroig et al., 2013). Consumers from higher trophic levels have to gain EFA from their food sources. Thus, EPA and DHA have been proposed as important indicators of high-quality food source (Støttrup and Jensen, 1990), especially in mariculture industry (Nanton and Castell, 1999).

1.3.3.3 Meiobenthos as bioindicators

Mariculture can stimulate (Castel et al., 1989) or not affect total meiobenthos densities (Danovaro et al., 2004), but most commonly reduces the abundance of meiobenthos (Grego, 2010; Mirto et al., 2012). These changes are driven not only by an increased food supply (eutrophication due to leftover feeds), but also by the alteration of the sediment characteristics (see 1.2.2.1).

Harpacticoid copepods and nematodes are suitable to assess disturbance on the marine environment because they are dominant and have a high species richness (Sandulli and De Nicola, 1991; Schratzberger et al., 2000). Some genera/species are proposed as putative bioindicators due to their sensitive or tolerant nature. For example, the nematode genera *Richtersia*, *Desmoscolex* and *Halalaimus* are highly sensitive to biodeposition, while *Terschellingia*, *Sabatieria* and *Daptonema* can be favored by organic loading (Mirto et al., 2014; Mirto et al., 2002; Netto and Valgas, 2010; Vezzulli et al., 2008). Similarly, harpacticoid copepod genera with slender shape like *Enhydrosoma* and *Acrenhydrosoma* can survive under hypoxic or anoxic conditions (De Troch et al., 2013; Grego et al., 2014), whereas there are species (e.g. *Amphiascus tenuiremis*, *Microarthridion littorale*, *Tisbe sp.*, *Tigriopus japonicus*) showing a lower tolerance to environmental stressors (Giere, 2009).

Identifying indicator species/genera can provide information on the overall health of marine habitats. However, the identification to a lower taxonomic level can be time-consuming and requires special knowledge and experience, especially for nematodes (Kennedy and Jacoby, 1999). In this sense, a more rapid method that only identifies higher meiobenthos taxa (i.e. taxonomic minimalism) has been developed (Kennedy and Jacoby, 1999; Warwick, 1988). This method is based on the recognition that, even at higher taxonomic levels, the sensitivity to stressors (more specifically organic enrichment) varies among taxa (Giere, 2009). For instance, nematodes are more adaptive to pollution (Peterson and Fry, 1987), while the population of sensitive taxa, e.g., harpacticoid copepods and kinorhynchs, can be suppressed under high organic loading (Grego, 2010; Mirto et al., 2012). So far, multivariate analyses based on meiobenthos assemblages at higher taxonomic levels are the most widely used approach linking meiobenthic community composition to environmental impacts including mariculture (Kennedy and Jacoby, 1999; McKindsey et al., 2011). However, it is also debated whether higher taxa are a sufficient proxy to show species/genera-level patterns especially at a regional scale (Hawkins and Norris, 2000; Heino and Soininen, 2007).

In addition, a simpler method, based on the more robust nature of nematode compared to harpacticoid copepods, is to calculate the ratio of nematode and copepod densities (Ne/Co) (Raffaelli and Mason, 1981). This index has been tested and criticized for the indication of benthic pollution (Coull, 1988; Raffaelli and Mason, 1981; Sandulli and De Nicola, 1991), yet its application in assessing mariculture activities is rather new and feasible (Sutherland et al., 2007).

1.3.4 Applying trophic analysis in mariculture

Understanding the mariculture food web is useful to address resource management in an ecosystem context (Ferriss et al., 2015). Depicting trophic relationships within culture systems, in particular, between nutrient sources (e.g., supplementary feed, waste) and consumers (reared or natural presented), is applicable in assessing the impacts of mariculture ventures (Schroeder, 1983). For instance, determining the utilization and fate of different food sources in shrimp ponds can provide a solution to the issue of overfeeding (Burford et al., 2004). Also, how the benthic food webs respond to bottom-up disturbances, such as biodeposition from marine farms, provides evidence to evaluate such mariculture activity (Callier et al., 2013; Yokoyama et al., 2006).

Stable isotopes as a biomarker (Box 1.5) have been recently used to trace the waste products from finfish mariculture and showed that the waste might enter the macrobenthic trophic route (Callier et al., 2013; Redmond et al., 2010; Vizzini and Mazzola, 2012). However, there is little information either about tracing the wastes from bivalve farms or targeting on the meiobenthic trophic route.

Box 1.5 Stable isotope analysis (SIA) in food web studies

Stable isotopes are atoms that have the same number of protons and electrons but with different numbers of neutrons, and at the same time energetically stable, e.g. $^{2}H/^{1}H$ (Fry 2006). Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are two of the most widely used stable isotopes in food web analysis (Boecklen et al., 2011). Isotopic signature is expressed as δ, which is the deviation between the isotopic ratio of a sample and an international standard and reported in per mille (‰). There are basically two themes when using stable isotopes to trace the trophic process: 1. Mixing, i.e. the isotopic signature of a consumer is the weighted mix of isotopic signatures of its food sources. 2. Fractionation, i.e. the changes of isotopic signatures from any physiochemical reaction, for instance, a relatively constant increase of heavier isotope abundance is observed in a consumer compared with its food source. Such arithmetic difference of isotopic signature between consumer and diet has been termed 'trophic enrichment factor' (TEF) (Newton, 2010). TEFs has been investigated for many taxa and often assumed averagely ca. 0.5‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ (McCutchan et al., 2003; Minagawa and Wada, 1984). However, the value varies and depends on many factors, e.g. taxon, habitat, type of food, length of food chain (Post, 2002; Zanden and Rasmussen, 2001). The best option would be to set up an isotopic food tracer experiment to measure the species/system-specific TEF in each case, but it requires great efforts and time (Caut et al., 2008). The alternative option is to use the TEF from the close taxon/system that has been determined. To calculate the relative contributions of food sources to a consumer, mixing models have been developed which can cope with more than n+1 food sources from n stable isotopes and estimate the range of feasible contributions of sources (Phillips and Gregg, 2003; Yu, 2014). Also, models based on Bayesian theory, such as MixSIR (Moore and Semmens, 2008), SIAR (Parnell et al., 2010), MixSIAR (Stock and Semmens, 2013), FRUITS (Fernandes et al., 2014), have been applied to incorporate the uncertainty of TEF and to deal with the variability of isotopic values of sources and consumers (Phillips et al., 2014). Based on that, stable isotopes analysis (SIA) has been widely used in trophic ecology. Compared with the conventional methods (e.g. direct observation and gut content analysis), SIA integrated the information of diets over a longer period (Fry, 2006). To date, SIA is a powerful tool to determine marine and estuarine food web structure (Peterson & Fry 1987; Michener & Kaufman 2007).

Stable isotopes are also commonly applied to determine the relative contributions of food sources to reared animals as well as trophic interactions between organisms in culture ponds (Feng et al., 2004; Nunes et al., 1997; Yokoyama et al., 2002). However, the trophic interactions in culture ponds can be complex (Moriarty, 1997), for instance, feed can be transformed by autotrophic and heterotrophic activities into microbes and their exudates rather than directly fueling consumers from higher trophic level (Moriarty, 1997; Schroeder, 1983), which bulk stable isotope technique is not adequate to measure (Gamboa-Delgado, 2014). The application of **fatty acids** as biomarker (Box 1.6) is capable to provide information on these pathways, yet it is still a new tool for mariculture food web analysis.

Box 1.6 Fatty acids in food web analysis

FAs (See BOX 1.4) are widely used as biomarker in trophic relationships study because of the specificity of the source and the strong conservatism through the trophic transfer (Napolitano, 1999). The FA profiling technique that assigns specific food sources to consumers is to identify certain FA in consumers which cannot synthesized by themselves (a.k.a. fatty acids trophic markers - FATM) (Smith et al., 1996). Table 1.2 lists the commonly used FATMs in marine resources to indicate the consumers' diet. Compared with SIA, FA analysis can trace some of the pathways in organic flow with a higher resolution, for instance, providing biomarkers for different groups of bacteria, flagellates and dinoflagellates (Dalsgaard et al., 2003; Kelly and Scheibling, 2012; Nelson et al., 2001). This is useful in meiobenthic trophic studies where the microbial pathway is important. Thus, FA profiling have complemented SIA as a second biomarker in meiobenthic ecology research (Cnudde et al., 2015; Leduc et al., 2009; Mascart et al., 2018).

Table 1.2 Commonly used fatty acids trophic markers (FATMs) as tracers of marine food sources to meiobenthos

Source	FATMs	References
Bacteria	Σ 15, 17, Σi-FA+ai-FA, 18:1ω7	(Budge and Parrish, 1998; Stevens et al., 2004; Viso and Marty, 1993)
Diatoms	16:1ω7, EPA	(Dalsgaard et al., 2003; Reuss and Poulsen, 2002)
Dinoflagelletes	DHA, DHA/EPA, 18 PUCA, 18 PUFA+DHA, 18:4ω3, 18:1ω9	(Dalsgaard et al., 2003; Kelly and Scheibling, 2012; Mansour et al., 1999)
Chlorophyta	18.2ω6, 18.3ω3	(Graeve et al., 2002; Kelly and Scheibling, 2012)
Carnivorous feeding	18:1ω9/18:1ω7	(Nelson et al., 2001; Nyssen et al., 2005)

1.4 Non-indigenous species in mariculture

The potential environmental and economic damage caused by **NIS** (see BOX 1.7) have been well documented worldwide especially in aquatic systems (Olenin and Minchin, 2011). These species were introduced to a new range and established themselves (see Box 1.7). Some of the established species may spread and become invasive (Richardson et al., 2000). This can result in negative ecological impacts at variable levels, including polluting the local gene pool (Olenin et al., 2010), transferring diseases (Burreson et al., 2004), changing the physical habitat (Rodríguez et al., 2005), threatening the biodiversity (e.g. shifts in community and loss of keystone species) (Britton-Simmons, 2004; Gophen et al., 1995), altering the energy flow in the local food web (Feng et al., 2018; Marić et al., 2016); and therefore affect the ecosystem services and economics e.g. local native fisheries

(McArdle et al., 1991; Ruiz et al., 1997). Marine coastal ecosystems provide important services to humans while at the same time being highly vulnerable to biological invasions and are therefore receiving increasing concern about the NIS issues (Garcia et al. 2017; Rilov & Crooks, 2009).

Box 1.7 Non-indigenous species (NIS)

Biological introduction, i.e. introducing a species into a new environment, has existed for a long time in coastal and marine ecosystems (Elton, 1958). However, under the context of trading expansion and globalization, geographic barriers preventing the dispersal of species have weakened, which largely facilitates biological introduction (Carlton, 1989; Olenin and Minchin, 2011). There are several terms to describe such species as alien, exotic, introduced, non-native, non-indigenous, and invasive alien. Here we refer to '**non-indigenous species (NIS)**', which are defined as 'species, subspecies, or lower taxa that are outside their natural range (past or present) and outside their natural dispersal potential' (Olenin et al., 2010). These species have been introduced to a given region by intentional/unintentional human activities rather than natural dispersal, except the secondary introduction*(1) of NIS that could occur without humans involved (Olenin et al., 2010). Invasive non-indigenous species, or invasive alien species/alien invasive species/invasive species, are a subset of established*(2) NIS which have spread, are spreading or have demonstrated their potential to spread elsewhere, and have an adverse effect on biological diversity, ecosystem functioning, socio-economic values and/or human health in invaded regions (Olenin et al., 2010).

*(1): A NIS directly arriving to a new location from its native region is considered as "primary introduction", while its subsequent spread from the founding site is called "secondary introduction" (Olenin et al., 2010).

*(2): Established - with a self-perpetuating population, naturalized, feral and breeding successfully or released and breeding successfully (Williamson, 1996).

Aquaculture is an important pathway for the introduction of NIS (Ruiz et al., 1997), since the use of NIS plays a crucial role in the growing aquaculture industry (De Silva et al., 2009; Lin et al., 2015). This applies to mariculture industry (Shelton and Rothbard, 2006). However, accelerating the introduction of cultured NIS has faced a paradox. On one hand, cultured NIS are usually selected based on traits such as high growth rate, wide ranges of diets, high reproduction ability and larger tolerance to the environment (Yakupitiyage and Bhujel, 2005). Those traits compensate to some extent the decline of the production associated with local species (e.g. diseases; See Box 1.9) (Lee, 2010). On the other hand, the introduction of a species into a new environment is supposed to influence the local ecosystem processes, with some of the traits of NIS accelerating such impacts on ecosystem processes, ultimately altering the functioning of the whole ecosystem (Newell, 2004). Although not all the introduced species have obvious impact for the moment, or can manage to escape, establish and cause serious problems, it is difficult to predict the future problematic species (Hill, 2009). To date, most NIS impacts studies focus on the successful invaders which have caused more obvious effects, but the NIS introduced by aquaculture are often neglected (Padilla et al., 2011).

Here we describe two of the most popular NIS cultured in China: the bay scallop *Argopecten irradians* in open-water mariculture and the whiteleg shrimp *Litopenaeus vannamei* in coastal ponds. Although there is no established population (i.e. self- perpetuating population that naturalized, feral and breeding successfully) for these two species in China as far as we know, the environment assessment and monitoring are still necessary (see the Box 1.8 and Box 1.9).

Box 1.8 Bay scallop (*A. irradians*) mariculture in China

Scallop farming in China started in the late 1960s. The native species Chinese scallop, *Chlamys farreri* Jones & Preston was the major cultured scallop back then. However, *C. farreri* underwent massive summer mortalities in 1998, and the bay scallop *A. irradians* (See BOX 1.13) gradually became the dominating cultivated species in China (Fig1.8). To date, *A. irradians* has largely supported the Chinese scallop industry by accounting for 58% of total scallop production in 2012 (Guo and Luo, 2016). Culturing bay scallops has some advantages over the native *C. farreri*, for instance, the shorter turn-around time due to faster growth rate (Wang et al., 2013). The open-water mariculture cycle usually starts in May and bay scallops can reach market size (50-60 mm) around November, which saves almost half of the time compared with culturing *C. farreri*. However, a high biodeposition was found in areas with *A. irradians* mariculture, inducing mass downward fluxes (ca. 1140 tonnes per day) from the water column to the benthic environment (Wang et al., 2018). This deposition rate is higher compared with those reported bivalves, e.g. *Mytilus edulis* (Kautsky and Evans 1987; Jansen et al. 2012), *Choromytilus chorus* (Jaramillo et al. 1992) and almost 10 times higher than the values for the *C. farreri* (Li et al., 2009; Wang et al., 2018; Zhou et al., 2006).

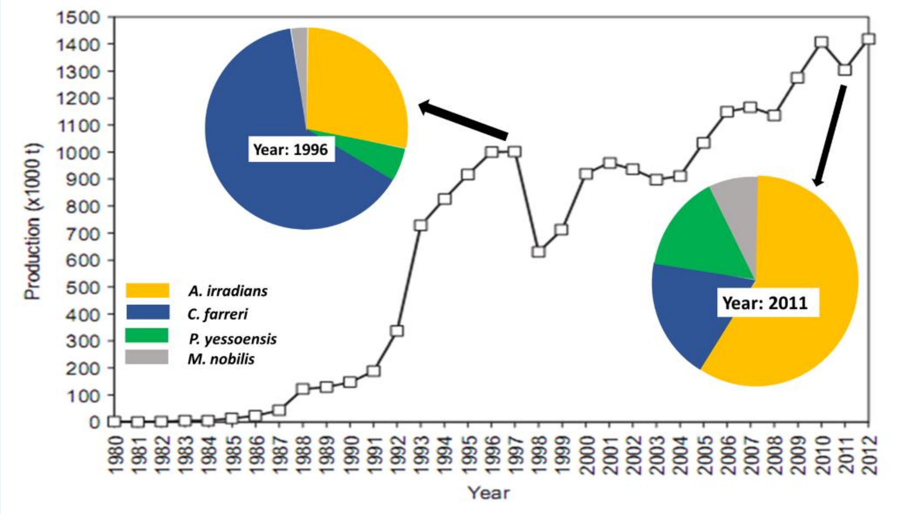

Figure 1.8 Mariculture production of scallops in China during the period 1980-2012 and the production of each species (*Chlamys farreri*, *Argopecten irradians*, *Patinopecten yessoensis*, *Mimachlamys nobilis*) in 1996 and 2011. The sharp decrease in 1998 is caused by massive mortality of *Chlamys farreri*. Adapted from Yu (2014) and Guo and Luo (2016)

Box 1.9 Whiteleg shrimp *L. vannamei* coastal ponds in China

The Pacific white shrimp (*Litopenaeus vannamei*) (See Box 1.10) is the most famous NIS cultured in China. Chinese shrimp farming largely relies on *L. vannamei*, which accounts for 74.3% (grown in brackish water) and 43.6% (grown in freshwater) of the total shrimp production in China in 2011 ((Lin et al., 2015); Fig 1.9). Due to their less aggressive behavior, high tolerance to environment (e.g. temperature, salinity), *L. vannamei* is easy to culture in very high densities (Briggs et al., 2004). Disease problem is a major issue to collape the shrimp farming industry (Kautsky et al., 2000). *L. vannamei* is considered to be more disease resistant than the indigenous species (*Fenneropenaeus chinensis*) and also any other white shrimp (Wyban and Sweeny, 1991; Briggs et al., 2004). One of the main advantages of culturing *L. vannamei* is the commercial availabity of specific pathogen free (SPF) stocks (Briggs et al., 2004). This group has passed through a disease screening process and is free for pathogens (such as white spot syndrome virus (WSSV), yellow head virus (YHV), Taura syndrome virus (TSV)) that cause the common shrimp diseases (Funge-Smith and Briggs, 2005). Also, many works have been done on selecting the Specific Pathogen Resistant (SPR) strains for *L. vannamei* and applied for the practical use (Funge-Smith and Briggs, 2005). However, *L. vannamei* are commonly cultured with maximal feed addition, which leads to serious environmental problems because of an overload of food and its leftovers (See 2.1). Also, *L. vannamei* has been demonstrated as one of the most voracious species in terms of food consumption among marine shrimp (Chavanich et al., 2016). Thus, it can pose a threat to marine areas if it manages to escape and establish reproductive populations (Lin et al., 2015).

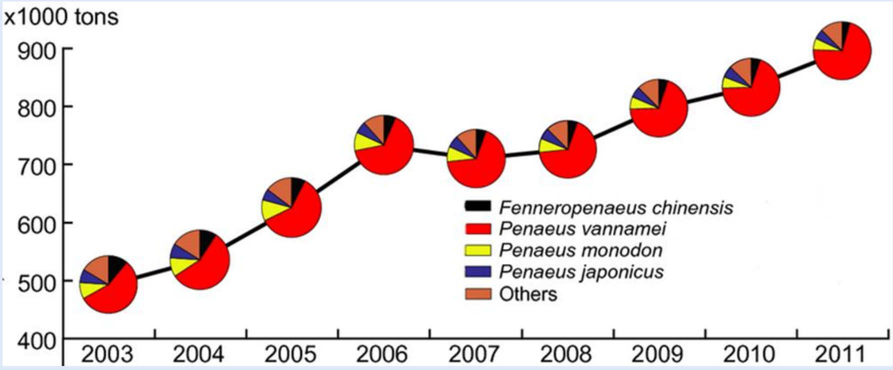

Figure 1.9 Mariculture production of shrimp in China during 2003-2011 (line), and production proportion of each species per year. Modified from Lin et al., (2015)

1.5 Study areas

This thesis focusses on two locations with different mariculture systems located along the Bohai Sea, China (Fig 1.10). The Bohai Sea is a part of the West Pacific and situated in the north-east of China. It is a semi-closed sea covering an area of 77,000 km^2 and only opens into the Yellow Sea through the Bohai Strait (Pan and Wang, 2012). As it is surrounded by highly industrialized regions and suffering from low water exchange, the Bohai Sea is threatened by human activities (Cao et al., 2007), especially in the south and the west of the Bohai Coast region, including Laizhou Bay and Bohai Bay (Gao and Li, 2012; Zhuang et al., 2014).

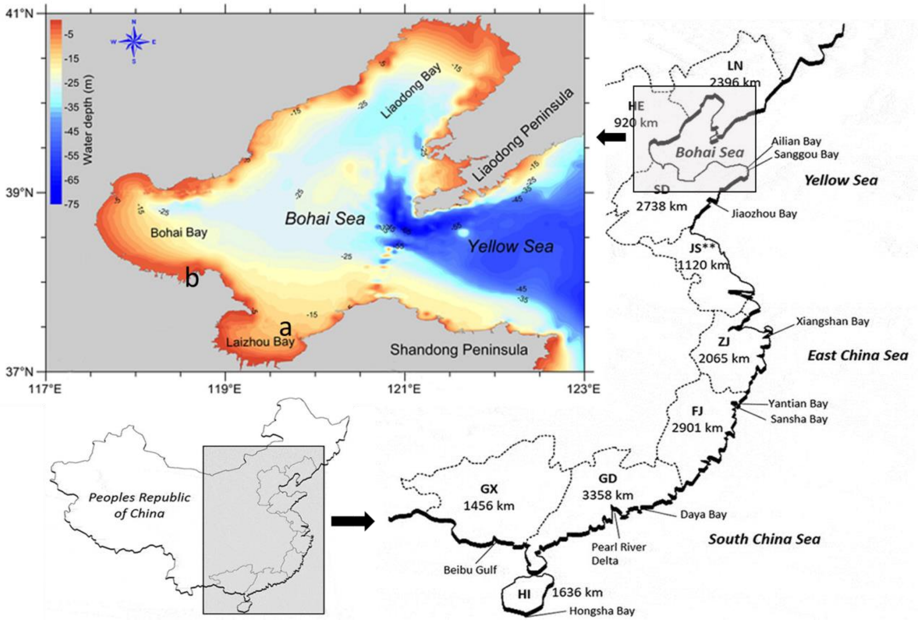

Figure 1.10 Two study sites, a: scallop open-water mariculture area; b: shrimp farming ponds, are located in Laizhou Bay and the coast of Bohai Bay, respectively, along the north of the Chinese coastline. Adapted from Hu et al. (2013) and Wartenberg et al. (2017)

1.5.1 Laizhou Bay

Laizhou Bay (area: c.a. 7000 km^2, coastline length: c.a. 320 km, mean depth <10 m, max. depth: c.a. 18 m) is located in the southeast of the Bohai Sea, northwest of Shandong Penisula (Huang et al., 2003). There are several rivers flowing into the bay. Except for the Yellow River, most of the other rivers are small and seasonal (Liu et al., 2004). The bottom of the bay is relatively flat and characterized by fine sediments (clay + silt) (Zhuang et al., 2014). Laizhou Bay is an important spawning, breeding and feeding ground for many species, e.g. flatfish, but it is currently under the pressure of intense human activities (Jin et al., 2013). The nutrient levels in the water column have changed dramatically in the past 30 years, with a ten-fold increase in dissolved inorganic nitrogen (DIN) but a ten-fold decline in dissolved inorganic phosphorus concentrations (Li et al., 2013; Liu et al., 2004; Liu et al., 2011). The main component of DIN changed from ammonium to nitrate due to anthropogenic pollution (Shan et al., 2000). The suspended non-indigenous scallop *A. irradians* mariculture is one of the key industries in the region, started in 1986 and has lasted for c.a. 30 years. Until 2011, Laizhou bay had a total culture area of c.a. 28 thousand ha, concentrated in the south bay, with a production of 144470 tonnes (Li et al., 2013). The location of this study was specifically focused on the *A. irradians* mariculture area (Fig 1.10). This area has high quality of seston for zooplankton and scallops as diatoms dominate the phytoplankton during the summer and autumn (Liu et al., 2004).

BOX 1.10 Bay scallop farming method in Laizhou Bay

Bay scallops are typically cultivated with the suspended long-line method (Fig. 1.11). About 100 to 400 individuals of scallops are placed on a lantern net divided into around 10-20 equally spaced cells by round plastic disks. 70-80 lantern nets are hung vertically on a suspended horizontal long-line, 5 to 15 m above the bottom and about 2 m under the seawater surface. For one scallop farm, there are 30-150 lines parallel to and about 3-10 m apart from each other, forming an area of c.a. 5-7 ha. Scallop farming activities are conducted from May to November each year without any provision of additional feed. Scallops are harvested when they reach a commercial size of 6 cm. One lantern net can produce c.a. 20 kg scallop.

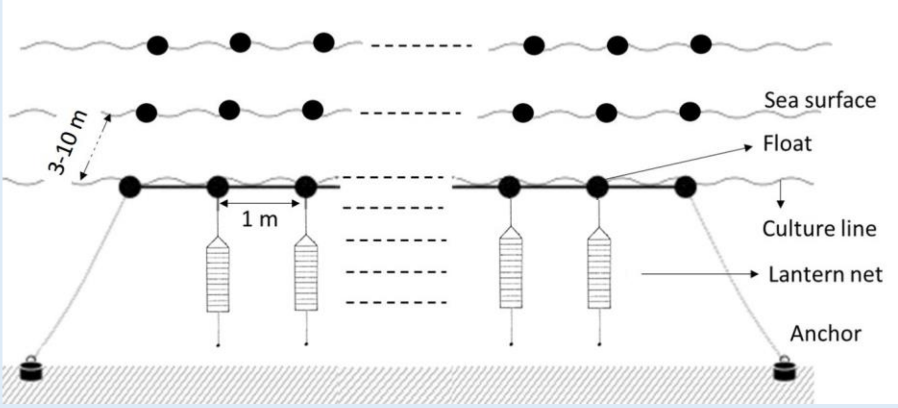

Figure 1.11 The long-line suspended method culturing bay in Laizhou Bay, China

1.5.2 The coast of Bohai Bay

Bohai bay, the second largest bay in the Bohai Sea, is situated along the western region of Bohai Sea, covering a total area of 15914 km^2 with an average depth of 12.5 m, and has a soft muddy bottom (Zou et al., 1985). It extends over one of the most densely populated zones, i.e. Hebei, west of Shandong, and the two megacities Beijing and Tianjin (Gao and Li, 2012). Because of the rapid port expansion, industrialization, urbanization, and development of mariculture along the coast, Bohai Bay receives extensive sewage and faces serious environmental problems such as algae blooms (Cao et al., 2007; Mao et al., 2009). The coastal ponds are distributed along the whole coast and are especially condensed in the southern part (Cao et al., 2007). In an area of shrimp farming, the Chemical Oxygen Demand (COD) level was found to be more than 200 times higher than in the adjacent area (Feng, 1996; Xie and Yu, 2007). Both the NIS *L. vannamei* and the native shrimp *Marsupenaeus japonicus* (BOX 1.13) are extensively cultured in the semi-intensive ponds.

BOX 1.12 Penaeid shrimp farming along Bohai Bay

Penaeid shrimp farming is commonly practiced in Shandong province, especially along the Bohai Bay (Zhao et al., 2016). *Litopenaeus vannamei* and *Marsupenaeus japonicus* are two popular cultured penaeid shrimp in this area. *Penaeus. manodon* and *Fenneropenaeus chinensis* are also being farmed occasionally. The farming methods vary according to shrimp species. *L. vannamei* is reared in three types of systems, semi-intensive/intensively managed earthen ponds (stocking density: between 15 and 30 post larvae (PL) m^{-2}) and super-intensive indoor farms (stocking density: > 500 PL m^{-2}). Among them, the earthen ponds were the most widely practiced method. *M. japonicus* only reared in the extensive managed earthen pond. Thus, the study in this thesis focuses on the earthen pond method rearing *L. vannamei* and *M. japonicus*. Farming *M. japonicus* is relatively new to this area (c.a. 5-10 years), while earthen pond rearing *L. vannamei* has been practiced for at least 10 years (Zhao et al., 2016).

Prior to the rearing activity in April, ponds were prepared by several cycles of draining and drying. Between the cycles, the sludge from the pond bottom is manually removed and the bottom soil is ploughed to oxidize the remains of organic matter and other reduced substances. 4-5% rotenone is used to kill the predators of shrimps and then ponds undergo a sterilization by treated with the calcium oxide. After the above steps, the ponds were filled with water from the adjacent canal that connect to Bohai Bay. The water pass through a 2 mm mesh bag to prevent the entry of predators and competitors. Usually the inorganic fertilizers (triple super-phosphate or ammonium phosphate) are applied at 20 to 100 kg ha^{-1} to improve the natural productivity of the ponds. After the preparation processes, two types of ponds are stocked with hatchery reared shrimps to post-larvae stage (mean total length 10-18 mm), with densities of 3-5 *M. japonicus* m^{-2} and 15-30 *L. vannamei* m^{-2}, respectively. Both species are reared from May to August for 12-14 weeks. Usually another culture crop is applied after August until November. After that, the ponds stay empty until next April.

M. japonicus and *L. vannamei* are fed differently. In the early stage (first 30 days), no external feed was added for juvenile *M. japonicus*. Commercial raised amphipods (*Corophium*) are introduced to the ponds at a rate of 10-30 kg ha^{-1}, which are expected to establish and populated in the shrimp ponds (i.e. transplanted *Corophium*), serving as a food source to adult *M. japonicus* later on. The addition of small fishes and mollusks (the so-called trash fish) occurs when the shrimps reach a size of 40-50 mm (i.e. late stage), at a rate of 75-150 kg/ha/day. For *L. vannamei* ponds, juvenile shrimps are fed with smashed soybeans daily at about 2 kg/ha/day and wet feed of processed frozen *Artemia* at about 7 kg/ha/day, and then later on the diet is shifted to formulated pellet feed at 20-30 kg/ha/day.

Water exchange for both types of ponds was ensured through the tidal dynamics by canals connected to the Bohai Bay. For *M. japonicus*, the water was exchange is up to 20% once in the early stage and 10-20% 2-4 times per month in the late stage. For *L. vannamei*, there is nearly no exchange of water in the early stage, while in the late stage, the water was changed up to 30% once a month. Other than the above principal, water renewal can also occur whenever the farmers consider it as necessary, for example, the water should be changed when its color turns to be blue-green or grey-dark green with less transparency, indicating a bloom of cyanobacteria or green algae.

Note: Pellet feed contains only 10% moisture and the trash fish more than 75% (Haan, 2012) and the frozen *Artemia* more than 90% (Lavens and Sorgeloos, 1996).

Box 1.13 Cultured species in this thesis

- *Argopecten irradians* (Lamarck, 1819) or bay scallop is an edible saltwater bivalve species, which belong to the family Pectinidae. It is originated from the shallow coastal marine habitats along the east coast of United states of America (USA), from Massachusetts to Florida (Clarke, 1965). It was an important economic bivalve species species in USA that largely supported commercial fisheries during 1870's-1980's (MacKenzie Jr, 2008). However, the production of bay scallop dropped by 85% since 1985 in the USA (Yu 2014). Nowadays, the center of global bay scallop production has been transferred to China (Yu, 2014).

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- *Litopenaeus vannamei* (Boone, 1931) or whiteleg shrimp, a marine shrimp belonging to family Penaeidae. Its original distribution is eastern Pacific Ocean, from northern Peru to the Mexican state of Sonora (Wyban and Sweeney, 1991). Now it is the most popular commercial shrimp cultured worldwide, including Brazil, USA, Pacific islands, and Asia (Briggs et al., 2004). This species has yielded 3 million tonnes in 2014, 78% of which were in Asia (Fernández de Alaiza García Madrigal et al., 2017; Saumena, 2015). It is recognized as "the biggest relocation of a single species in the history of the planet" (Walker and Mohan, 2009).

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- *Marsupenaeus japonicus* (Spence Bate, 1888) (or kuruma shrimp) is another member of family of Penaeidae. It has a wide native range encompassing the Indo-West Pacific Ocean (Hayashi, 1996; Liu, 2008; Quigley et al., 2013). It is one of the most valuable mariculture species in many Asian countries (Chen, 1990). China started to farm *M. japonicus* in the late 1980s, and it has become one of the main cultured penaeid shrimp species since then (Li et al., 2014).

1.6 Objectives and thesis outlines

The general **aim** of this PhD study is to assess the impact of the mariculture on the recipient ecosystems in the context of using NIS as cultured species. The outcome of this research will provide scientific information for the decision makers to apply for a better management of mariculture. We were targeting the environmental variables, the meiobenthos community, and benthic trophic interactions.

Three specific **objectives** are put forward:

1. to unravel the response of environmental variables, meiobenthic communities and their trophic ecology towards mariculture activities.
2. to investigate the specific effect of different mariculture installations/techniques on benthic environment.
3. to apply the indication and implication of the measured variables to contribute to an early warning system.

To accomplish these objectives, specific topics were addressed in four chapters that composing the core structure of this thesis.

Chapter 2 targets the benthic environmental variables in an open-water culture area of scallop *A. irradians* (NIS) and the pond-culture systems of *L. vannamei* (NIS) and *M. japonicus* (native species). The sensitivities of traditional variables such as physiochemical characteristics and bulk organic matter were examined and compared with the indicators of the benthic trophic status such as sedimentary biochemical composition.

After that, the chapter focuses on benthic organisms. **Chapter 3** and **Chapter 4** deal with a case-study of farming scallop *A. irradians* in a semi-closed bay. In **Chapter 3**, we focused on the response at biotic level (i.e. meiobenthos community structure and harpacticoid copepod assemblages). The responses of meiobenthos to the biodeposition of scallops were identified on two taxonomy levels: meiobenthic community at the higher taxon level and harpacticoid copepod assemblages at family level. We also used the data of BPC and its composition (i.e. proteins, carbohydrates and lipids) from **Chapter 2**. We tried to interpret the responses of the meiobenthos with the sediment characteristics. In **Chapter 4**, stable isotopes analysis was used to trace the fate of biodeposits from scallop farms and to quantify the trophic interactions between biodeposits and two meiobenthic groups: harpacticoid copepods and nematodes. Furthermore, fatty acids provided insight in possible changes in quality of the harpacticoid copepods (as a food source) in such mariculture area. **Chapter 5** addressed the case of coastal pond farming of *L. vannamei* and *M. japonicus*. The responses of meiobenthic communities were documented and related to different farming practices. Stable isotopes and fatty acids were also used to investigate the trophic interactions among primary producers, bacteria, different supplementary shrimp feeds, meiobenthos and shrimps.

To conclude, an overall discussion (**Chapter 6**) is presented, summarizing the relevant results of this thesis and discussing their contributions to the mariculture assessment. The main conclusions were used to discuss further questions that were raised and need to be further explore. It should be noted that a comparison of the biological traits of native and non-indigenous species is not included in this thesis. Also, the biological traits of the cultured species cannot be differentiated from the mariculture effects.

Chapter 2: Effects of mariculture on sedimentary characteristics and trophic status: two case studies of open-water scallop farms and coastal shrimp ponds in the Bohai Sea region, China

Abstract

Two mariculture systems in the Bohai Sea, China were used as study cases, the open-water scallop farms (suspended-longline method farming *Argopecten irradians*) and the land-based coastal shrimp ponds rearing *Penaeus japonicus* and *Litopenaeus vannamei*. In this study, the variability in sedimentary pH, redox potential (Eh), chlorophyll *a* contents (Chl *a*), and the quantity and biochemical composition of organic matter by the scallop and shrimp culture activities were investigated. In the case of scallop farms, bulk organic matter (total organic carbon and total nitrogen) and Chl *a* were not affected by scallop farming. Eh was slightly reduced in the sandy shallow farm. The response of the biopolymeric carbon (BPC) was in line with the scallop farming intensity, showing a significant accumulation under two scallop farms with larger production density. In the shrimp ponds, Eh showed a more reduced status at the end of rearing cycle. The bulk organic matter, Chl *a* and BPC all accumulated during the rearing periods with different extent in terms of types of shrimp ponds as well as the early and late rearing stages. Eh and biochemical organic concentrations did not reach environmentally critical thresholds in scallop farms, suggesting no general adverse effects of scallop farming on the benthic environment. However, shrimp mariculture generated highly reduced-anoxic and highly eutrophic (even reached hypertrophic) conditions in the pond bottom. We also found BPC as relevant proxy to trace the change of trophic status induced by mariculture activities.

2.1 Introduction

Global marine aquaculture (hereafter mariculture) production has grown considerably since the past decades, reaching a value of 26.7 million tons in 2014 (FAO, 2016). Thus, marine coastal environments have developed into important mariculture grounds, from offshore waters to coastal lands, with a variety of cultured species and practices (FAO, 2017; Primavera, 2006). It has been well known that natural habitats are threatened by these mariculture activities in different forms. For instance, the rapid growing coastal/oceanic water mariculture and coastal land-based ponds are posing different impacts on the environment including water quality deterioration and contaminants, (Hargreaves, 1998), spread of diseases (Bondad-Reantaso et al., 2005; Meyer, 1991), and drug residue (Grave et al., 2008; Schnick, 2001), and biological invasion (Naylor et al., 2001).

The open-water mariculture introduces large densities of bivalves in suspended culture; which is a common practice in marine coastal zones (Callier et al., 2008). There is a growing interest to evaluate potential environmental effects of bivalve farming as high deposition of faeces and pseudofaeces (i.e. biodeposits) have been observed at culture sites (Crawford et al., 2003; Dame, 1996; Newell, 2004). These carbon- and nitrogen-rich biodeposits (Kautsky and Evans, 1987) can alter the physical and biochemical of sediments when accumulate on the seabed (i.e. the so-called benthic environment) (McKindsey et al., 2011). However, studies investigated effects of mariculture on sediment characteristics reported different conclusions, ranged from minimal (Danovaro et al., 2004) to significant impacts that include the changes in nutrient levels (Callier et al., 2008) and generating anoxic sediments (Christensen et al., 2003).

While the open-water mariculture takes place in the natural open water environment, marine shrimp farming is conducted in land-based semi-isolated compartments, such as earthen bottom ponds (hereafter earthen ponds). This earthen ponds are perceived to be less invasive to the marine coastal environment (Paez-Osuna, 2001). However, this practice was recently found to have severe negative impacts on the environment (e.g. Hatje et al. (2016); Ribeiro et al. (2016); Wu et al. (2014)), e.g. the nutrient accumulation and subsequent eutrophication within ponds, which caused by accumulation of uneaten feed, can further pollute the adjacent ecosystem such as saltmarsh and mangrove (Funge-Smith and Briggs, 1998; Wu et al., 2014; Yang et al., 2017). Since the pond sediments provide nutrients and shelter for benthic algae and organisms (Gamboa-Delgado, 2014; Moriarty, 1997), the health status of the pond bottom is vitally important for the production of shrimps (Dall et al., 1991). Therefore, benthic environment monitoring and assessment are increasingly important strategies in shrimp farming management.

A variety of indicators has been proposed to assess the benthic environment of mariculture ranging from the traditional physicochemical variables, e.g. redox potential (Eh), nitrogen and phosphate (Kumar et al., 2012; Nixon, 1995; Stefanou et al., 2000), to proxies that take into account the quality of organic matter for the benthic organisms (or the so-called trophic status) such as biopolymeric carbon (BPC) - the proportion of organic carbon potentially available to benthos (Bianchelli et al., 2008; Fabiano et al., 1995; Pusceddu et al., 2009), protein to carbohydrate ratio (PRO:CHO), and the sedimentary bioavailable fraction of organic matter (Pusceddu et al., 2009; Silva et al., 2017). These proxies have been tested in several mariculture systems (Karakassis et al., 2000; Mirto et al., 2012; Pusceddu et al., 2011).

However, the extent of environmental effects is often correlated to various factors, e.g. the characteristics of habitats (i.e. hydrodynamics, water depths, and sediment characteristics such as granulometry), the reared species (e.g. bivalves, finfish, shrimps), and the management practices (i.e. intensive, semi-intensive, extensive) (Callier et al., 2008; Miron et al., 2005; Sarà, 2007). Therefore, it is important to assess the suitability and consistency of those indicators at the habitat- or system-specific scale (Pusceddu et al., 2011) which can be used to apply across different ecosystem types (Borja et al., 2009; Keeley et al., 2012).

Over the past few decades, rapid development in China has significantly affected its coastal habitats. The southwest Bohai coastal regions, including Laizhou Bay and Bohai Bay, represent such coastal habitats, as it features low water exchange and strong anthropogenic activities (Gao et al., 2012; Zhuang et al., 2014). Eutrophication in Laizhou Bay and Bohai Bay has been observed (Feng, 1996; SOA, 2006-2009) and mariculture is one of the reasons for the eutrophication (Cao et al., 2007; Cui et al., 2005). While most of the environmental evaluations in this area have been focusing on the examination of the water column, especially in terms of trophic status (Hui et al., 2004; Kan et al., 2010; Wu et al., 2013), there is still a lack of study on the benthic system.

By considering that management of environmental friendly mariculture requires scientific-based information for decision making, the present study aims to assess the potential effect of coastal marine aquaculture on the recipient environment (particularly focusing on sediment) as well as to test the suitability of abiotic indicators. The study was conducted in two different mariculture systems in the southeast of

Bohai Sea: open-water suspended farms, rearing scallop *Argopecten irradians*, in Laizhou Bay and two types of semi-intensive shrimp farming ponds, rearing shrimps *Penaeus japonicus* and the *Litopenaeus vannamei*, respectively, in the coast of Bohai Bay. We hypothesize that (1) scallop farming changes the sediment characteristics and benthic trophic status; (2) different patterns of temporal variability in sediment characteristics and benthic trophic status are presence in *Marsupenaeus. japonicus* and *L. vannamei* ponds; (3) the environmental variables from different categories (i.e. physiochemical variables, bulk organic matter, and biochemical concentration) show various sensitivity to detect the mariculture effects or temporal/ spatial variations.

2.2 Materials and methods

2.2.1 Study area and sampling sites

2.2.1.1 Open-water scallop farming in Laizhou Bay

The scallop farming area is located in Laizhou Bay (37°00’-38°30’ N, 118°45’-120°30’ E, southern Bohai Sea, Shandong Province, northern coast of China; Fig. 2.1a). The mean depth of the bay is 9 m (max. depth about 18 m) and the total area is of approximately 700000 ha (Zhuang et al., 2014). A northeast oriented dominant current with a mean velocity of 20 cm s^{-1} runs perpendicularly to the shoreline (Zhao and Chen, 2001).

The major cultured bivalve – bay scallop *Argopecten irradians* is non-indigenous to this area. The species is cultivated using suspended-longline culture method (see details in Chapter 1 Section 1.5). The scallop farms were deployed since 1987 at a distance of 5-20 km away from the shore with a total area of 500 ha. The farms yielded about 144470 tons in 2011 (Li, 2013). Scallops are cultured during 6 months period in a year starting from May to November without adding any feed.

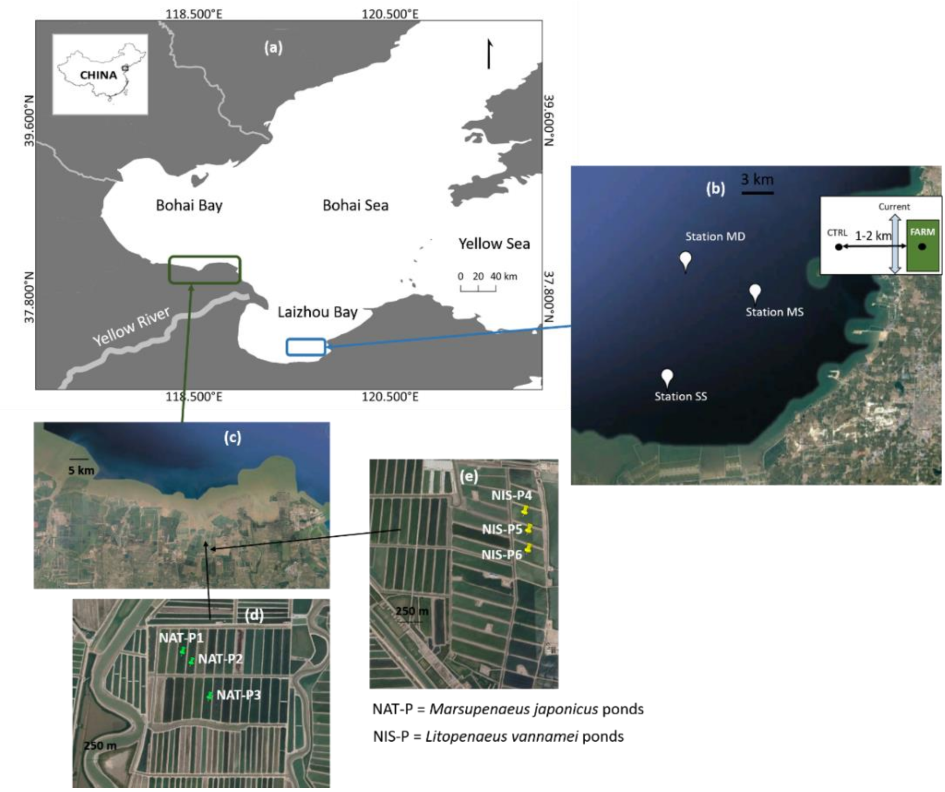

Figure 2.1 The location of the study areas in Laizhou Bay and Bohai Bay (a) with indication of the sampling sites in the scallop farming area (b) and the shrimp ponds (c) – detailed view of the *Marsupenaeus japonicus* ponds (d) and *Litopenaeus vannamei* ponds (e).

For the scallop farming area, samples were collected at three stations with different characteristics: Stn SS (short for sandy shallow; 26-31% silt-clay, 69-73% fine sand; 7 m water depth; distance from the coast 5 km), Stn MS (short for muddy shallow; 57-61% silt-clay, 40-43% sand; 7 m water depth; distance from the coast 10 km), and Stn MD (short for muddy deep; 55-60 silt-clay, 40-45% sand; 12 m water depth; distance from the coast 18 km). Each station included a scallop farm site (FARM) and a control site (CTRL). Each pair of farm and control sites was located at a similar depth and displayed a similar sediment texture (Appendix I Table S1). Farm sites were located in the center of the scallop farms directly under the culture lines. The scallop production densities were different among sampled farms where Farm MS had lower production density than those of Farm SS and Farm MD (Farm MS: c.a. 100 ind. m^{-2}; Farm SS & MD: c.a. 200 ind. m^{-2}). Each control site positioned about 1.5-2 km away from the farm and also at least 2 km away from any other scallop farms. Control was exposed laterally to the predominant current that flowed through the farm (Fig 2.1b). Visual observation and preliminary analyses revealed that the control was not affected by the scallop biodeposition.

2.2.1.2 Shrimp earthen ponds in Bohai Bay coast

Cultivation of native shrimp (NAT) *Marsupenaeus japonicus* and non-indigenous shrimp (NIS) *Litopenaeus vannamei* in the earthen ponds are commonly applied along the southwest coast of Bohai Sea, Dongying, Shandong Province, China (38°00’26.81 N, 118°33’57.61 E; Fig 2.1a). Farming of *L. vannamei* in earthen ponds has been practiced for more than 10 years in this area, while *M. japonicus* farming is relatively new to this district (c.a. 5-10 years). Details about the shrimp rearing cycles and feeding practices in this study area are described in Chapter 1 (See Chapter 1 Section 1.5.2). Briefly, one shrimp rearing cycle lasts around 3.5 months, following the four stages: (1) Preparation for rearing and stocking: the pond sediments are ploughed and the sludges are removed. The ponds are prepared by adding rotenone to kill shrimp predators and calcium oxide to sterilize the pond. Inorganic fertilizers are applied in order to improve the natural primary production (at 25 to 100 kg/ha).

Post-larval-stages of shrimps (about 18 mm) are allocated in the ponds with densities of 3-5 shrimps m^{-2} for *M. japonicus* and 15-30 shrimps m^{-2} for *L. vannamei*. (2) The early rearing stage (from May to early June): in the first 30 days, commercially cultivated amphipods (*Corophium*) are added once to *M. japonicus*' ponds which are expected to propagate in the pond sediments for the rest of the rearing time. *M. japonicus* is reared without external feeds, whereas *L. vannamei* is fed with fermented smashed soybean and processed frozen *Artemia*. (3) The late rearing stage (from middle June to August): *M. japonicus* is fed with small fishes and mollusks (i.e. trash fish) while *L. vannamei* is fed with formulated feed. (4) After harvesting: another culture cycle is usually applied after August until November. After that, the ponds stay empty until April of the following year.

Three *M. japonicus* ponds (NAT P1, P2, P3) and three *L. vannamei* ponds (NIS P4, P5, P6) were randomly chosen from the *M. japonicus* farm and *L. vannamei* farm, which were about 2 km apart from each other (Fig 2.1c, d, e). The background, feeding information and pond sediment granulometry are listed in Table 2.1 and Appendix I Table S2. Water intaking and discharging of both ponds were conducted from canals that connect to the Bohai Bay. There are different water exchange strategies for *M. japonicus* and *L. vannamei* ponds. For *L. vannamei* ponds, there is no water renewal in the first 30 days (the early stage), while the water is exchanged at most 30% once a month in the late stage. For *M. japonicus* ponds, the water is renewed at most once for the first 30 days and 2-4 times at a volume of 10-20% per month at the late rearing stage. During the first rearing cycle, the monthly precipitation were 35 mm, 68 mm, 189 mm and 131 mm in May, June, July and

August, respectively (data from http://data.cma.cn/).

Table 2.1 Background information of three *Marsupenaeus japonicus* ponds (NAT-P) and three *Litopenaeus vannamei* ponds (NIS-P) in Bohai Bay, China.

Shrimp	*M. japonicus*			*L. vannamei*		
Ponds	NIS-P4	NIS-P5	NIS-P6	NAT-P1	NAT-P2	NAT-P3
Pond size (ha)	4	4	4	5	5	5
Depths (m)	1.2-1.6	1.2-1.6	1.2-1.6	1.2-1.6	1.2-1.6	1.2-1.6
Age of the pond (yr)	c.a. 20	c.a. 20	c.a. 20	c.a. 10	c.a. 10	c.a. 10
Stoking density (ind. m^{-2})	3-5	3-5	3-5	15-30	15-30	15-30
Grow-out period per crop (weeks)	12-14	12-14	12-14	12-14	12-14	12-14
Rearing crops	2	2	2	2	2	2
Feed*						
Corophium (kg)	50-100	50-100	50-100	-	-	-
Wet feed – trash fish (kg)	35000	35000	35000	-	-	-
Wet feed - Frozen *Artemia* (kg)	-	-	-	1000	1000	1000
Pellet feed – Smashed soybean (kg)	-	-	-	300	300	300
Pellet feed - Formulated feed (kg)	-	-	-	8400	8400	8400

* Note: Pellet feed contains only 10% moisture and the trash fish more than 75% (Haan, 2012) and the frozen *Artemia* more than 90% (Lavens and Sorgeloos, 1996).

2.2.2 Sample collections

For scallop farming area, two samplings were carried out in May 2016 prior to scallop farming and October-November 2016 during heavy biodeposition stage (Wang et al. 2015 observed that biodeposition rates of bay scallops in Laizhou Bay peaked in November). For shrimp sites, three sampling actions were conducted at three occasions: before shrimp farming (January 2016), the early stage (May 2016; shrimp body length between 15 and 30 mm), and the late stage (August 2016; shrimp body length between 70 and 80 mm), except for sedimentary pH and Eh that failed at T0 due to practical reasons.

In situ measurements of temperature, salinity, dissolved oxygen (DO) and pH were measured in the water column (Scallop: c.a. 1 m above the sea bottom; Shrimp: 20 cm above the pond bottom) by a YSI 6600 multiparameter water quality meter. Sediment samples were collected by SCUBA divers in scallop farming area and by a hand-operated core sampler for the shrimp ponds. Three sampling points (e.g. Farm SS-1, SS-2, SS-3; NAT P1-1, P1-2, P1-3) serving as three replicates were applied in each site. From each sampling point, three virtually undisturbed sediment cores were obtained using one large PVC core (inner diameter: 15 cm) and two plexiglass cores (both with 3.6 cm inner diameter). Each core was processed immediately after the cores were brought out. For sedimentary Eh and pH, electrodes were immersed in the top 1 cm of the sediment of a large PVC core and connected to pH and mV meters (Orion 230A+ Meter and Orion Star A221 pH portable Meter). The calibration of the redox probe was conducted prior to sediment core collection following the instruction of Wildish et al. (1999): the probe was calibrated against the reference electrode in a Zorbell redox buffer solution. During the sampling, redox electrode was periodically checked with Standard Zobell's solutions to ensure its accuracy. The pH electrode was calibrated daily using three NIST scale pH buffers (pH 4.01, 7.00 and 10.01). For Chl *a*, the top 0-1 cm sediment layer of a plexiglass core was sliced, homogenized, stored at -20 °C and measured within two weeks after sampling. For the other variables (e.g. TOC, TN, protein, carbohydrate, and lipid), the top 0-1 cm sediment from the other plexiglass core was sliced and immediately frozen at -20 °C for further analysis (see 2.2.3).

2.2.3 Biochemical parameters of sedimentary organic matter

Chl *a* content was measured in 2.5 g of wet sediment using a fluorescence method after extraction with 90% acetone (Yentsch and Menzel, 1963). For the measurement of TOC and TN percentage, sediments were oven dried (60 °C), grounded, and treated with 1M HCl for 24 h to remove carbonates. TOC and TN were measured with an Element Analyser Flash 2000 (Thermo Fisher Scientific). The C/N ratio was calculated from the TOC and TN levels. Sediment granulometry was measured by laser diffraction with a Malvern Mastersizer 2000 particle analyzer (Malvern Instruments, UK).

The biochemical composition of the organic matter in the sediments (carbohydrate, protein, and lipid contents) was measured photometrically following the procedure of Fabiano and Danovaro (1994). About 0.2-0.3 g of dried and grounded sediment was used as a replicate for each type of analysis, and pre-combusted sediments (450 °C, 4h) were used as blanks to avoid any interference due to the unknown matrix of the sediment. Carbohydrate, protein and lipid contents were converted to the carbon equivalents by multiplying with the conversion factors of 0.40, 0.49, and 0.75 mg C mg^{-1}, respectively, which commonly used for the estimation of coastal marine sediments (Fabiano et al., 1995; Mirto et al., 2010; Pusceddu et al., 2007b). Their sum was reported as biopolymeric carbon (BPC). Chl *a* content was converted to carbon equivalents using a mean value of 40 μg C μg^{-1} Chl *a* (Pusceddu et al. 1999; Pusceddu et al. 2007b). The algal carbon contribution to BPC was calculated as Chl *a* carbon equivalents to BPC content.

2.2.4 Statistical analysis

For the scallop farming area, three factors were considered: (1) Time - fixed factor with two levels: before scallop farming (T0) and during heavy biodeposition stage (T1), (2) Station (Stn) - random factor with three levels: Stns SS, MS, and MD, and (3) Site - fixed factors with two levels: farm and control. For shrimp ponds, three factors were applied: (1) Stage – fixed factor with three levels: before shrimp farming (T0), the early and late stage, (2) Shrimp (type of shrimp pond) – fixed factor with two levels: *M. japonicus* (NAT) and *L. vannamei* (NIS) ponds, and (3) Pond – random factor nested in the type of shrimp pond: NAT P1, P2, P3 and NIS P4, P5, P6.

Due to a small sample size, permutation analyses of variance (PERMANOVA) were used instead of ANOVA. Environmental variables were analyzed using PERMANOVA with the above three factors and the tests were based on Euclidean distance matrixes with 4999 random permutations of the appropriate units under reduced models (Anderson and Braak, 2003). The homogeneity of multivariate dispersion was checked with a distance-based test for homogeneity of multivariate dispersion (PERMDISP). When significant differences were observed, post-hoc comparisons were performed using PERMANOVA pair-wise tests type III.

Three-way PERMANOVA was conducted as multivariate analysis of the sediment trophic status. Highly correlated environmental variables ($r > 0.9$) were removed and the remained variables for analysis were TN, TOC, Chl a, proteins, carbohydrates and lipids for scallop farming and TN, Chl a, proteins, carbohydrates, and lipids for shrimp rearing). A canonical analysis on principal coordinates (CAP) was subsequently performed to visualized the variations among factors. PERMANOVA and CAP analyses were conducted under the same setting described above.

PERMANOVA, PERMDISP, and CAP were performed with Primer V6 (Clarke and Gorley, 2006), using the PERMANOVA+ add-on package (Anderson et al., 2008).

2.3 Results

2.3.1 Physicochemical variables in the water column

In the scallop farming area of Laizhou Bay, the water temperature and the salinity were 20.47-21.70°C and 30.66-31.05 at T0 (i.e. May) and were 11.40-12.21°C and 29.70-30.07 at T1 (i.e. Oct-Nov) during our sampling time. DO and pH in the water column varied among stations and sampling times (Table 2.1; Table 2.2). DO was higher in T1 than T0 at the three stations (Table 2.1; pairwise tests $p < 0.05$). Water pH was slightly lower in T1 compared to those in T0 e.g.in Stns SS and MS (Table 2.1; Table 2.2; pairwise tests $p < 0.05$).

In the shrimp ponds, water temperature varied between 16.61-21.61°C in the early stage (May) and increased to 27.89-31.23°C in the late stage (August) during our sampling periods. Salinity was higher in *L. vannamei* ponds than in *M. japonicus* ponds (*L. vannamei*: 44.53-53.59; *M. japonicus*: 30.26-36.49) and increased throughout the rearing stages (Table 2.1; Table 2.2; pairwise tests $p < 0.05$). Water DO decreased through the rearing stages in all ponds (Table 2.1; Table 2.2; pairwise tests $p < 0.05$). Water pH was slightly higher in *M. japonicus* ponds (7.84-8.99) than in *L. vannamei* ponds (7.28-8.22) during the rearing stages (Table 2.2).

Table 2.2 The values (Mean±SD, N = 3**) of physicochemical variables of the water column in the scallop farming area in Laizhou Bay and in the shrimp ponds in Bohai Bay coast, China.** Scallop farms: T0 = before scallop farming, T1 = high biodeposition period; SS = sandy shallow station, MS = muddy sallow station, MD = muddy deep station; CTRL = control site; FARM = scallop farming site; Shrimp ponds: T0 = before shrimp farming; Early = the early rearing stage, Late = the late rearing stage; NAT P = *Marsupenaeus japonicus* ponds; NIS P = *Litopenaeus vannamei* ponds.

	Site	Temperature	Salinity	Dissolved oxygen	pH
		℃		mg L^{-1}	-
Scallop farms	T0-SS-CTRL	21.69±0.00	31.00±0.00	7.21±0.09	8.22±0.02
	T0-SS-FARM	21.73±0.04	31.04±0.01	7.16±0.19	8.23±0.03
	T0-MS-CTRL	21.02±0.01	30.74±0.00	7.76±0.04	8.09±0.03
	T0-MS-FARM	21.02±0.06	30.72±0.00	7.68±0.09	8.08±0.01
	T0-MD-CTRL	20.96±0.42	30.66±0.01	7.84±0.07	8.11±0.01
	T0-MD-FARM	20.98±0.44	30.66±0.00	7.77±0.02	8.11±0.01
	T1-SS-CTRL	11.41±0.01	29.83±0.01	8.87±0.55	8.13±0.02
	T1-SS-FARM	11.46±0.05	29.94±0.01	8.69±0.01	8.13±0.05
	T1-MS-CTRL	12.06±0.06	29.99±0.06	8.32±0.28	8.03±0.03
	T1-MS-FARM	11.93±0.01	30.04±0.02	8.41±0.01	8.00±0.01
	T1-MD-CTRL	12.12±0.11	29.73±0.02	8.20±0.20	8.16±0.05
	T1-MD-FARM	12.15±0.05	29.70±0.01	8.04±0.33	8.16±0.06
Shrimp ponds	EARLY-NAT-P1	18.50±0.02	36.38±0.04	7.42±0.17	8.20±0.12
	EARLY-NAT-P2	18.09±0.17	35.80±0.06	6.49±0.00	8.19±0.04
	EARLY-NAT-P3	18.35±0.81	36.30±0.16	6.66±0.18	8.47±0.49
	LATE-NAT-P1	30.67±0.17	35.79±0.16	4.23±0.17	7.91±0.11
	LATE-NAT-P2	28.63±0.64	31.27±0.44	6.68±0.15	7.95±0.08
	LATE-NAT-P3	29.57±0.19	30.38±0.19	4.95±0.54	8.06±0.06
	EARLY-NIS-P4	21.44±0.22	46.27±0.16	7.98±0.49	8.09±0.11
	EARLY-NIS-P5	16.96±0.55	44.55±0.02	7.54±0.14	7.61±0.28
	EARLY-NIS-P6	19.81±0.93	48.59±0.16	7.99±0.76	7.64±0.20
	LATE-NIS-P4	29.33±0.01	47.55±0.10	0.79±0.16	7.79±0.01
	LATE-NIS-P5	29.13±0.24	45.21±0.11	3.16±0.30	7.90±0.06
	LATE-NIS-P6	30.59±0.92	52.44±0.99	3.50±2.84	7.85±0.12

2.3.2 Physicochemical variables in the sediment

In scallop farming area in Laizhou Bay, sedimentary pH was characterized by a significant interaction effect of Time × Station (Table 2.2). All three stations showed significant higher pH at T1 than those of T0 (Table 2.2; Figure 2.2) except for T0 of Stn SS which showed higher pH values than the other stations. For the sedimentary Eh, an interaction effect of Time × Station × Site was found on Eh (Table 2.2). The pairwise post-hoc tests showed significant lower Eh in CTRL than those of FARM in Stn SS (Table 2.2; Figure 2.2).

In the shrimp ponds, an interaction effect of Stage × Pond (Shrimp) was found on sedimentary pH indicating that pH in NAT P3 was slightly higher in late stage compared with those in the early stage (Table 2.2; Figure 2.2). Eh of the pond sediments showed negative values (indicating a reduced status) during the rearing periods with even lower values in the late stage than those in the early stage (Table 2.2: Stage $p < 0.01$; Figure 2.2). Variation of Eh also found among ponds that NIS P6 had less reduced status than two other NIS ponds (Table 2.2: Pond (Shrimp) $p < 0.01$).

Table 2.3 Results of PERMANOVA (permutation analyses of variance) tests of environmental variables in top 0-1 cm sediments between factors from a scallop farming area in Laizhou Bay and in two types of shrimp ponds in Bohai Bay coast, China. Factors: Scallop – Time (T0 vs T1), Station (Stn SS, MS and MD), and Site (FARM vs CTRL); Shrimp – Stage (T0, early and late), Shrimp (NIS vs NAT), Pond (NAT P1, P2, P3, NIS P4, P5, P6). Only interaction effect is shown if there is any. Post-hoc comparison is conducted within the fixed factor. The significance of single factor and interactions are shown in Appendix I Table S3. TN = total nitrogen; TOC = total organic carbon; Chl *a* = Chlorophyll *a*; PRO = proteins; CHO = carbohydrates; LIP = lipids; BPC = biopolymeric carbon; Algae C to BPC = Chl *a* carbon equivalent to biopolymeric carbon.

Variable	df	MS	Pseudo-F	Interaction	Factor	Post-hoc comparison
Scallop						
TN	2	0.28	19.195***	Time × Station	Time	Stn SS: T0 < T1
TOC	2	0.22	6.4567**	Time × Station	Time	Stn SS: T0 > T1; Stn MD: T0 < T1
Chl *a*	2	0.44	6.5722**	Time × Station	Time	SS, MS, MD: T0 > T1
PRO	2	0.04	3.3068*	Time × Station × Site	Site	T1: Farm SS > Ctrl SS; Farm MD > Ctrl MD
					Time	Ctrl SS: T0 > T1; Farm SS, Ctrl MD, Farm MD: T0 < T1
CHO	2	0.19	10.852**	Time × Station × Site	Site	T1: Farm SS > Ctrl SS; Farm MD > Ctrl MD
					Time	Ctrl SS: T0 > T1; Farm SS, Ctrl MS, Farm MS, Farm MD: T0 < T1
LIP	2	0.97	37.423***	Time × Station	Time	Stns SS: T0 < T1; Stn MS: T0 > T1
BPC	2	0.08	6.9519**	Time × Station × Site	Site	T1: Farm SS < Ctrl SS; Farm MD < Ctrl MD
					Time	FARM SS, Farm MS, Farm MD: T0 < T1
Algae C to BPC	2	0.92	12.43***	Time × Station	Time	Farm SS, Farm MS, Farm MD: T0 > T1
Eh	2	4875.80	5.7385*	Time × Station × Site	Site	T1: Farm SS < Ctrl SS
pH	2	3.72E-04	6.5576**	Time × Station	Time	Stns SS, MS, MD: T0 < T1
Shrimp						
TN	2	0.02	11.904**	Stage x Shrimp	Shrimp	NAT: T0 = Early < Late; NIS: T0 < Early = Late

					Stage	Early: NAT < NIS
TOC	2	0.07	3.8186*	Stage x Shrimp	Shrimp	NAT: T0 = Early < Late; NIS: T0 < Early = Late
					Stage	Early: NAT < NIS
Chl *a*	2	0.09	4.9074*	Stage x Shrimp	Shrimp	NAT: T0 < Early = Late; NIS: T0 < Early = Late
					Stage	T0, Late: NAT < NIS
PRO	2	0.36	4.7107*	Stage x Shrimp	Shrimp	NAT: T0 < Early < Late; NIS: T0 < Early = Late
					Stage	Early: NAT < NIS
	8	0.07	4.2022**	Stage x Pond (Shrimp)	Stage	NAT ponds - P1, P3: T0 < Early < Late; P2: T0 = Early < Late; NIS ponds - P4, P6: T0 < Early < Late; P5: T0 = Early < Late
CHO	2	1.34	16.09**	Stage x Shrimp	Shrimp	NIS: T0 < Early = Late
					Stage	T0, Early, Late: NAT < NIS
	8	0.08	2.8476*	Stage x Pond (Shrimp)	Stage	NAT ponds - P1, P2: T0 = Early < Late; P3: T0 < Early < Late; NIS ponds - P4, P6: T0 < Early = Late; P5: T0 < Late < Early
LIP	1	33.07	74.57**	none	Shrimp	NAT < NIS
BPC	2	0.62	10.247**	Stage x Shrimp	Shrimp	NAT: T0 < Early < Late; NIS: T0 < Early = Late
					Stage	T0, Early, Late: NAT < NIS
Algae C to BPC	2	1.9403	34.058***	Stage x Shrimp	Stage	NAT: T0 < Late < Early; NIS: T0 = Early < Late
					Shrimp	Early: NAT > NIS; Late: NAT > NIS
pH	4	0.18	5.034**	Stage x Pond (Shrimp)	Stage	NAT P3: Early < Late
Eh	1	0.53	23.036**	none	Stage	Early < Late
	4	0.059	6.5053**	none	Pond (Shrimp)	NIS: P4 = P5 < P6

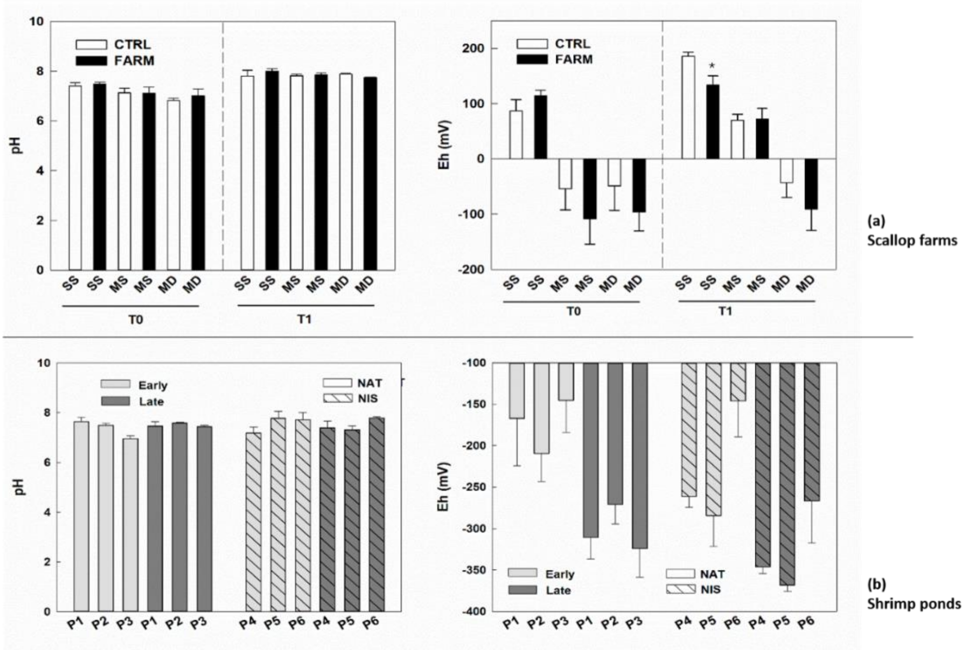

Figure 2.2 Variations of pH and redox potential (Eh) in top 0-1 cm sediment (mean values and standard deviations) from (a) scallop farming area, Laizhou Bay and (b) shrimp ponds, Bohai Bay coast, China. Scallop farms: T0 = before scallop farming, T1 = high biodeposition period; SS = sandy shallow station, MS = muddy sallow station, MD = muddy deep station; CTRL = control site; FARM = scallop farming site; Significant pairwise comparisons between each FARM and CTRL are indicated with an asterisk (* $p < 0.05$). Shrimp ponds: T0 = before shrimp farming; Early = the early rearing stage, Late = the late rearing stage; NAT = *Marsupenaeus japonicus*; NIS = *Litopenaeus vannamei*; P 1, 2, 3 represent *M. japonicus* ponds; P 4, 5, 4 represent *L. vannamei* ponds.

2.3.3 Bulk organic sedimentary variables

In scallop farming areas, TN, TOC and C/N in the sampled sediments showed an interaction of Time and Station (Fig 2.3; Table 2.3). Compared with T0, Stn SS had significantly higher TN but significantly lower TOC and C/N in T1 (Fig. 2.3; Table 2.3).

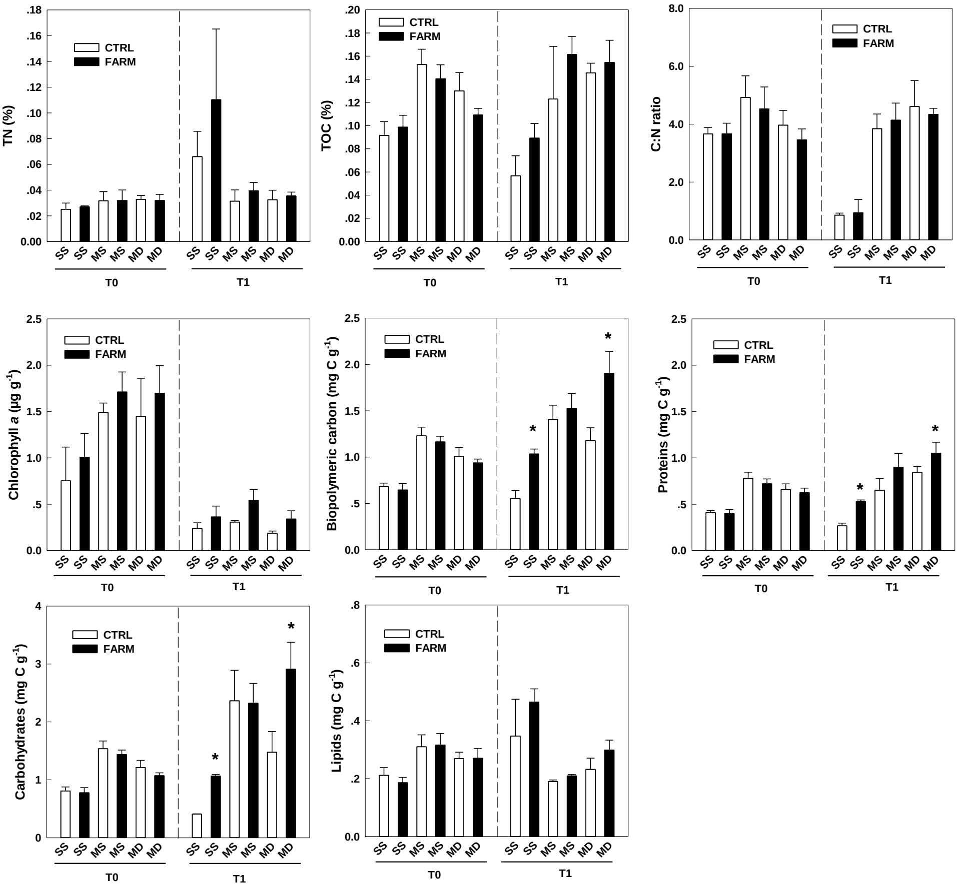

Figure 2.3 Mean values and standard deviation (SD) of the bulk organic variables and biochemical variables (mean values and standard deviations) in the top 0-1 cm sediments from scallop farming area, Laizhou Bay, China. Significant differences between each pair of farm and control site (PERMANOVA pairwise test $p < 0.05$) are indicated by an asterisk. T0 = before scallop farming, T1 = high biodeposition period; CTRL = control site; FARM = scallop farming site; TN = total nitrogen, TOC = total organic carbon.

In the sediment of shrimp ponds, a significant interaction effect of Stage × Shrimp was found on TN and TOC (Table 2.3). Specifically, in *M. japonicus* ponds, TN and TOC were significantly higher at the late stage than at T0 and the early stage (Table 2.3; Fig 2.4). TN and TOC in the early and late stage in *L. vannamei* ponds had higher values than those at T0 (Table 2.3; Fig 2.3).

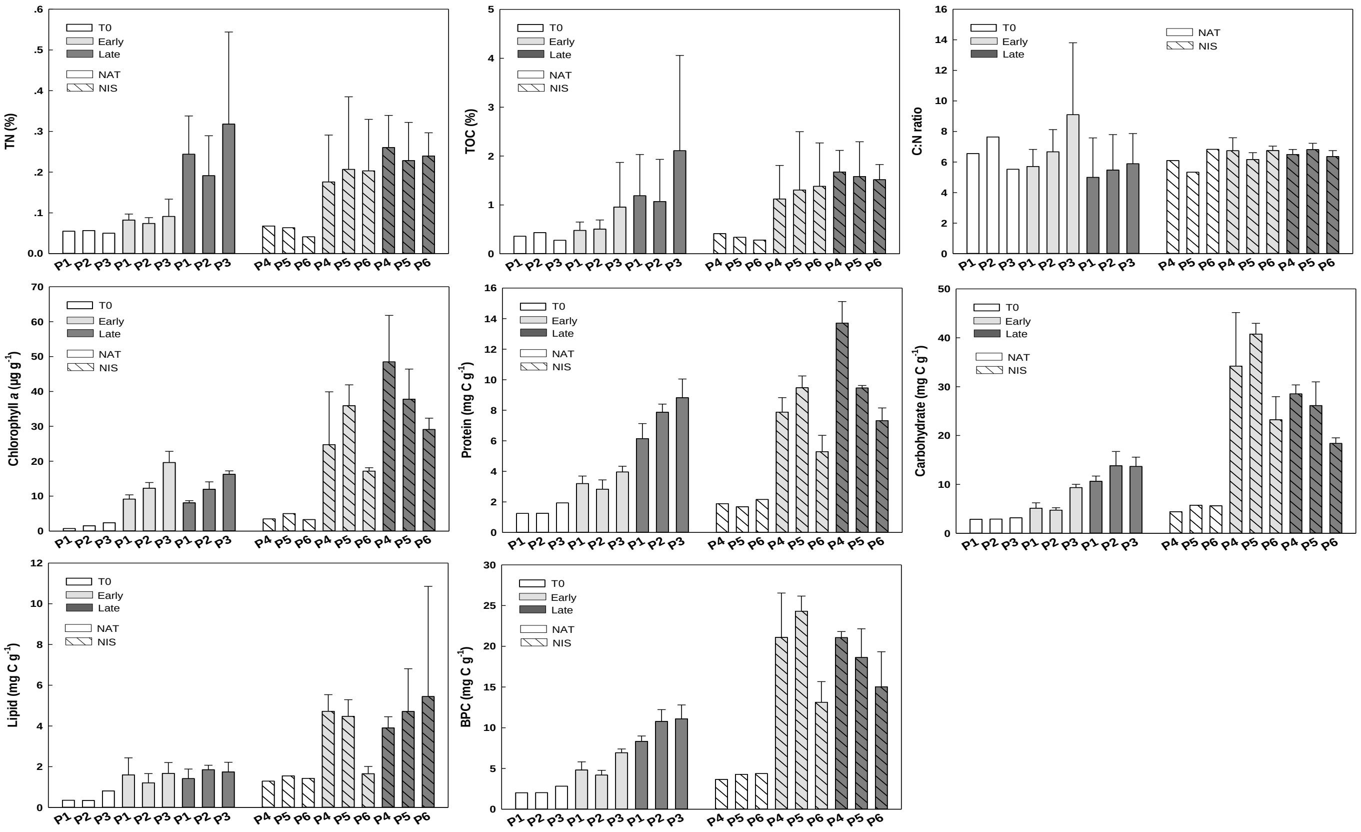

Figure 2.4 Mean values and standard deviations (SDs) of the bulk organic variables and biochemical variables in the top 0-1 cm sediments from two types of shrimp ponds, Bohai Bay coast, China. NAT = *Marsupenaeus japonicus*; NIS = *Litopenaeus vannamei* ponds; TN = total nitrogen; TOC = total organic carbon; BPC = biopolymeric carbon; P 1, 2, 3 represent *M. japonicus* ponds; P 4, 5, 6 represent *L. vannamei* ponds.

2.3.4 Biochemical variables in the sedimentary OM

In the scallop farming area, an interaction effect of Time × Station was found on sedimentary Chl *a* levels (Table 2.3). All stations showed a decrease of Chl *a* from T0 to T1 (Table 2.3; Fig 2.3). BPC, proteins and carbohydrates in the sampled sediments were affected by the interaction of three tested factors: Time × Station × Site (Table 2.3). Further pairwise comparison between each FARM and CTRL showed significantly higher levels of BPC, proteins, and carbohydrates under the FARMs in Stns SS and MD (Table 2.3; Fig 2.3). Only an interaction effect of Time × Station was observed on lipids (Table 2.3). Thus, the higher levels of BPC in the farmed sediments was due to the accumulation of proteins and carbohydrates.

All above measured variables were higher in the sediment of shrimp ponds, especially during rearing stages (Fig 2.3). An interaction effect of Stage × Shrimp was observed on Sedimentary Chl *a* and BPC. Chl *a* level from both *M. japonicus* and *L. vannamei* pond sediments was initially low (Fig 2.3: T0) but increase significantly about 8.9- and 6.6-folde in the early stage, respectively (Table 2.3; Fig 2.4). There was however no significant difference between the early and the late stage in Chl *a* for both types of ponds. BPC showed different patterns between two types of ponds. In *M. japonicus* ponds, BPC levels increased throughout the rearing stages from T0 to the early and to the late stage (Table 2.3; Fig 2.4), while in *L. vannamei* ponds, BPC only increased significantly from T0 to the early stage, and subsequently remained stable in the late stage (Table 2.3; Fig 2.4). In the early stage, the increasing level of BPC of *M. japonicus* ponds was mostly due to the accumulation of proteins and lipids (except for P3 showed carbohydrates accumulated as well), while for *L. vannamei* ponds the accumulation of proteins, carbohydrates and lipids caused the higher BPC level (Table 2.3). In the late stage, the increased BPC content in *M. japonicus* pond bottom was attribute to the higher proteins and carbohydrates (Table 2.3).

Furthermore, we observed significant higher algal fraction of biopolymeric carbon in T0 than T1 for three stations in scallop farming area (Table 2.3; Fig 2.5a). For shrimp farming, algal carbon contribution to the BPC pool largely increased in the early stage in the *M. japonicus* ponds and present a higher value than those in *L. vannamei* ponds. In the late stage this algal fraction showed a slight decrease and a slight increase in *M. japonicus* and *L. vannamei* ponds, respectively (Table 2.3, Fig 2.5a). The proteins to carbohydrates ratio (PRO:CHO) above 1 is the suggested threshold for the eutrophic benthic environment (Dell'Anno et al., 2002; Silva et al., 2017; Venturini et al., 2012). We found PRO:CHO was smaller than 1 in sedimentary samples of both scallop farms and shrimp ponds (Fig 2.5b). Moreover, we did not observe any pattern between BPC and PRO: CHO (Fig 2.5b).

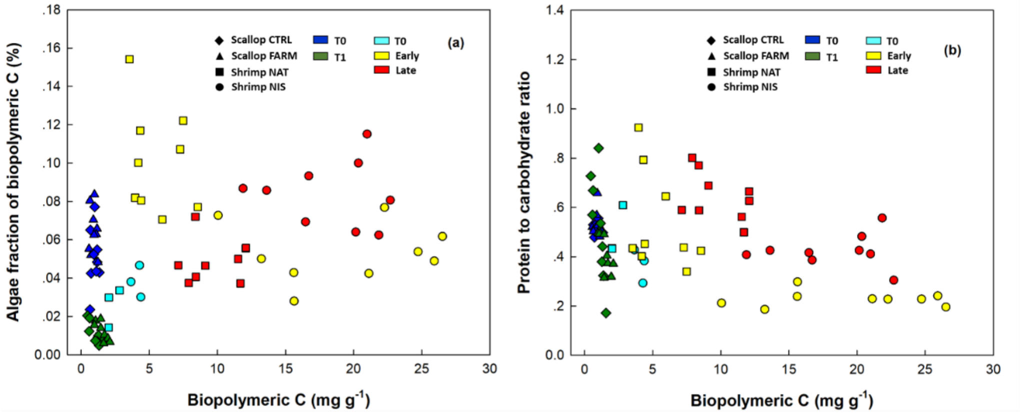

Figure 2.5 Algal contribution to biopolymeric carbon (BPC) and BPC concentration in all sampled sediments (a) and protein to carbohydrate ratio (PRO/CHO) and biopolymeric carbon (BPC) concentration in all the sampled sediments in scallop farms from Laizhou Bay and shrimp ponds from Bohai Bay coast, China.

2.3.5 The trophic status in the sediment

The CAP analysis revealed a clear separation of sampling time and station when applied to sediment OM variables (TN, TOC, Chl *a*, PRO, CHO and LIP) for scallop farms (Table 2.4: PERMANOVA, Time × Station: $p < 0.05$). The samples at T0 grouped together based on the higher Chl *a* concentration (Fig 2.6a). At T1, samples from Stn SS (characterized with higher levels of TN and lipids) were separated from those of Stns MS & MD. No significant effect of Time ×Station × Site was observed (Table 2.4).

For shrimp ponds, the CAP results showed the separation according to the different rearing stages and types of shrimp ponds (Table 2.4: Stage × Shrimp $p < 0.05$; Fig. 2.6b). More specifically, for *M. japonicus* ponds, the feature of sedimentary OM significantly shifted in the late stage, while sediments OM fractions already underwent a significant change in the early stage *L. vannamei* ponds (See pairwise comparisons in Table 2.4). *M. japonicus* and *L. vannamei* ponds clustered together at T0, and differed from each other since the early stage (Fig. 2.6b).

Table 2.4 Permutational analysis of variance (PERMANOVA) testing for organic matter variations among factors in the multivariate environmental asset in sediment in scallop farming area from Laizhou Bay and in shrimp ponds from Bohai Bay coast, China. df = degree of freedom; MS = mean square; ns = non-significant difference; *= $p < 0.05$; **= $p < 0.001$; ***= $p < 0.001$; P-F = Pseudo-F. Highly correlated environmental variables (r > 0.9) were removed from the analysis. Scallop farms: T0 = before scallop farming, T1 = high biodeposition period; SS = sandy shallow station, MS = muddy sallow station, MD = muddy deep station; CTRL = control site; FARM = scallop farming site; Shrimp ponds: T0 = before shrimp farming; Early = the early rearing stage, Late = the late rearing stage; NAT P = *Marsupenaeus japonicus* ponds; NIS P = *Litopenaeus vannamei* ponds

Scallop farms

Source	df	MS	P-F	P(MC)
Time	1	40.437	1.858	ns
Station	2	34.543	25.708	**
Site	1	6.9909	3.8255	*
TimexStation	2	21.753	16.189	***
TimexSite	1	10.518	5.911	*
StationxSite	2	1.8275	1.36	ns
TimexStationxSite	2	1.7792	1.324	ns
Res	24	1.3437		
Total	35			

Pairwise comparisons	t	P(MC)
SS: T0 vs T1	4.161	***
MS: T0 vs T1	4.161	***
MD: T0 vs T1	5.925	***
T0: SS vs MS	6.856	***
T0: SS vs MD	4.426	***
T0: MS vs MD	2.471	**
T1: SS vs MS	4.771	***
T1: SS vs MD	5.245	***
T1: MS vs MD	1.718	*

Shrimp ponds

Source	df	MS	P-F	P(MC)
Stage	2	40.098	32.641	**
Shrimp	1	30.671	24.387	**
Pond(Shrimp)	4	1.2577	0.77603	ns
StagexShrimp	2	6.4731	5.2691	**
StagexPond (Shrimp)	8	1.2285	0.75799	ns
Res	24	1.6207		
Total	41			

Pairwise comparisons	t	P(MC)
NAT: T0 vs Early	2.069	ns
NAT: Early vs Late	4.164	**
NIS: T0 vs Early	4.695	**
NIS: Early vs Late	1.453	ns
T0: NAT vs NIS	1.727	ns
Early: NAT vs NIS	2.92	**
Late: NAT vs NIS	3.902	***

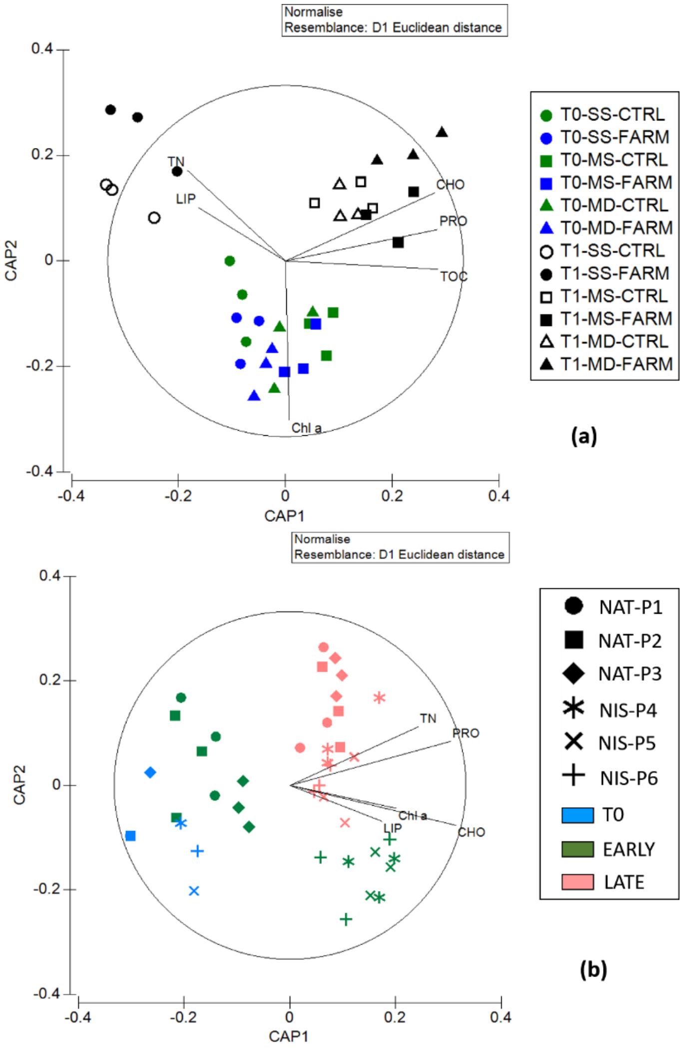

Figure 2.6. Bi-plot of CAP analysis based on bulk organic matter and biochemical parameters of the top 0-1 cm sediment in (a) scallop farming area in Laizhou Bay, China and (b) shrimp ponds in Bohai Bay coast, China. Vectors are proportional to the Pearson correlation coefficient of the identified variables with CAP axis. CTRL = control; NAT = *Marsupenaeus japonicus* ponds; NIS = *Litopenaeus vannamei* ponds; Chl *a* = Chlorophyll *a*; PRO = protein; CHO = carbohydrate; LIP = lipid; TOC = total organic carbon; TN = total nitrogen. Highly correlated variables (r > 0.9) were removed from the analysis.

2.4 Discussion

The sediment redox potential reflects the degree of anaerobic condition and it is widely used to assess the health of aquaculture environments (Anderson et al., 2005; Wilding, 2012; Wiyoto et al., 2017). In Laizhou bay, redox potential values in scallop farms during the high biodeposition season were positive in shallow stations (Stns SS and MS; 7 m of water depth) and dropped to a range of 0 to -150 mV in deep station (Stn MD; 12 m of water depth), indicating oxic and hypoxic benthic conditions respectively (Sutherland et al., 2007). For shrimp ponds, the average values of redox potential were always below -150 mV during the rearing stages, especially in the late stage that even showed more negative values, indicating highly reduced and anoxic conditions (Sutherland et al., 2007). The temporal changes in sedimentary Eh may attributed to the organic accumulation in the shrimp ponds (see further). However, this abiotic indicator was not sensitive enough to reflect the scallop farming (e.g. the muddy deep station). Our findings corroborate with other studies that suggested redox potential could indicate high organic loading such as in fish farming (Anderson et al., 2005; Hargrave et al., 1997), but was less efficient to detect biodeposition of bivalves in muddy areas (Callier et al., 2007; Crawford et al., 2003). On the other hand, only the Stn SS showed a lower (but still oxic) value in the farm than control site. This location specific response of Eh can associate with the habitat characteristics (e.g. the intensity of farming, types of sediment, water depth). The lack of response in Stn MS is likely attribute to the lower density of farmed scallops (50% less compared with Stns SS and MD). For Stn MS, the naturally reduced Eh and high contents of organic carbon (TOC and BPC) suggesting that the response to the relatively small enhancement of sedimentation can be obscured by background processes such as anaerobic biogeochemistry cycles of iron and sulfide (Hargrave et al., 2008). To further explain the mechanism, oxygen and sulfide concentrations are needed to unravel biogeochemistry cycles. It is also noteworthy that the redox potential in shrimp sediment during rearing stages reached a concerning threshold. It has been recommended to maintain the value above -206 mV in order to keep shrimp immunity response activated (Wiyoto et al., 2017). The highly reduced sediment can indicate the low oxygen concentrations and the generation of toxins (e.g. ammonium, sulfide hydrogen), which may inhibit the growth of shrimps (Avnimelech and Ritvo, 2003; Avnimelech et al., 2004).

The bulk organic matter (i.e. TOC and TN) was not indicative of mariculture effect if the effect was weak, e.g. the scallop farming activity. Due to the conservative nature of TOC and TN (Fabiano et al., 1995), they were less sensitive than the labile biochemical variables. It is possible that detritus from other sources (e.g. plankton, seagrass) may mask the biodeposition from cultured bivalves (Stenton-Dozey et al. 2001). Callier et al. (2008) also found bulk organic matter is only able to detect the biodeposition when the organic loading was heavy and thus has limitation when use in mariculture assessment. In the shrimp ponds, the feeding practices could be linked to the temporal pattern of TOC and TN, because their accumulations were synchronized with the timing of feed addition (NAT: start feeding in the late stage; NIS: start feeding in the early stage).

Chl *a* content relates to the abundance of microalgae (microphytobenthos or deposited phytoplankton) (Venturini et al., 2012). In the scallop farming area, Chl *a* in the sediment had a distinct temporal pattern due to seasonal variability. The observed pattern was consistent with Li et al. (2014) who found that the sedimentary Chl *a* was higher in spring and summer than those in the winter, which may be due to the lack of silicate in winter that inhibited the growth of diatoms (Zhao et al., 2004). Some researches on the mussel farming effect found a increase level of Chl *a* under the mussel farms (Mirto et al., 2000; Newell et al., 2002). They attributed this enhancement of microalge to either the settlement of phytopigments through the filtration-biodeposition of the bivalves (Navarro and Thompson, 1997) or an increase of the dissolved inorganic nitrogen (DIN) pool that stimulated the growth of microalgae (Newell et al., 2002). However, we did not find sedimentary Chl *a* indicative for the scallop farming. “Shading effect” (limited light penetration caused by the biodeposits and the shades of the raft) may be a reason that indirectly obscure the enhancement of Chl *a* (Franzo et al., 2014). In the shrimp ponds, the temporal pattern of Chl *a* content is similar for both *L. vannamei* and *M. japonicus* ponds. The elevated Chl *a* in the early stage might be due to the addition of Nitrogen-Phosphate-Potash (N-P-K) fertilizers right before the stocking that enhance the pond primary production (Rubright et al., 1981). After the early stage, Chl *a* content did not increase further. This was likely link to a less extent of light penetration in the late stage because of a heavier input of feeds that generate high turbidity (Alonso-Rodriguez and Páez-Osuna, 2003).

The biopolymeric fraction of sediment organic carbon (i.e. BPC) is the proportion of organic carbon potentially available to benthos (Bianchelli et al., 2008; Fabiano et al., 1995; Pusceddu et al., 2009). Due to the rapid changes in composition and quantity in early diagenesis, BPC responds more promptly to mariculture activities than bulk organic concentrations (Fabiano et al., 1995; Pusceddu et al., 2009). In this study, BPC managed to detect scallop biodeposition effect as well as the temporal changes of shrimp pond sediments during the rearing stages. We found higher BPC accumulation with higher production densities (FARM SS and MD) under scallop farms. This agrees with previous findings in mussel farms where high biodeposition caused a BPC enrichment on the farm sediments (Mirto et al., 2000). However, the response of BPC to farming bivalves is not entire universal. Another study in a high hydrodynamics regime reported no impact of mussel farming on sediment BPC, which attribute to the continuous resuspension and/or export of the biodeposits (Danovaro et al., 2004). Higher BPC levels commonly occur in sediments beneath fish cages, with a pronounced accumulation of proteins and lipids since the food pellets used in fish farming contain high proportions of proteins and lipids (Mazzola et al., 1999; Mirto et al., 2012; Pusceddu et al., 2007a). In our study, carbohydrates and proteins were preferentially accumulated under the scallop farms. This can be due to the biochemical composition of biodeposits with more abundant carbohydrates and proteins than lipids (Slater et al., 2009; Yuan et al., 2006). Even though the sedimentary biochemical fractions accumulated, according to thresholds for BPC concentrations as well as protein and carbohydrate concentrations, the scallop farming area still reflected a meso-oligotrophic condition, i.e., proteins < 1.5 mg g^{-1}, carbohydrates < 5 mg g^{-1} (Dell'Anno et al., 2002); BPC = 1-5 mg g^{-1} (Pusceddu et al., 2007b). This indicates a minimal impact of scallop farming on the benthic environment. In fact, in a meso-oligotrophic environment where the primary production is relatively low, the top-down control (food supply) is important to drive the benthic communities (Smetacek, 1984; Sanders et al., 1992; Vanreusel et al., 1995). Especially in the winter, the algae fractions to the BPC is relatively low than those in the summer, indicating a low level of food availability. With the enhancement of bioavailable organic carbon (BPC) may facilitate the benthos by providing extra food (Dell'Anno et al., 2002).

In the sediments of the tested shrimp ponds (especially in the *L. vannamei* ponds), the concentrations of biochemical variables were higher than those reported for other eutrophic coastal sediments (Dell'Anno et al., 2002; Pusceddu et al., 2009; Venturini et al., 2012). Moreover, in the early stage, the accumulation of proteins and lipids was noticeable for both types of ponds. This may be attributed to the growth of microphytobenthos or the deposition of phytoplankton, since several microalgae (e.g. diatoms) are important carriers of proteins and lipids to marine sediments (Danovaro et al., 1993; Baldi et al., 2001; Venturini et al. 2012). High BPC level but low algal fraction of BPC was observed in *L. vannamei* ponds in comparison to that of *P. japonicas*, suggesting that other than microalgae, additional organic sources (e.g. external shrimp feed: smashed soybean and frozen *Artemia*) also contribute to the BPC pool. In the late stage, *M. japonicus* pond continue to accumulate BPC which is likely due to the addition of trash fish, while BPC content in *L. vannamei* ponds remain unchanged, indicating a steady state in those ponds.

Interestingly, in the late stage when both types of pond faced heavy feed inputs, a lesser extent of labile organic accumulation (BPC and Chl *a*) was observed in the *M. japonicus* ponds than those in the *L. vannamei* ponds. This can be primarily attributed to the substantial difference in stocking density (*L. vannamei* density being 5-10 times higher than *M. japonicus*), since the quantity of generated wastes was found to be proportional to the stocking density (Martin et al., 1998). Water renewal strategy is also a factor affecting the pond environment (Pusceddu et al., 2011; Mohanty et al., 2015), because water exchange reduces turbidity, organic and nutrient loading and toxic metabolites, increases dissolved oxygen levels and promotes the growth of shrimps (Hopkins et al., 1993; Mohanty 2000). However, it should also be noticed that the discharge of waste pond water that contains high nutrient levels can pollute the adjacent estuarine (Hatje et al., 2016; Marins et al., 2011; Yang et al., 2017). The pond water may also include pathogens that cause shrimp diseases (Kausty et al., 2000). The pathogens may affect the wild shrimps when emitting them to the natural environment, and also spread among farms during intake of waters from adjacent estuarine/sea (Belak et al., 1999; Kausty et al., 2000; Smith, 1998). In addition, different bioturbation mechanisms of the *L. vannamei* and *M. japonicus* ponds might also contribute to the different extent of organic accumulation. As adult *L. vannamei* spends more time in the water column and does not create burrow (Briggs et al. 2004). Studies found that *L. vannamei* did not promote nitrogen dynamics at the sediment-water interface, but their faeces together with shrimp feed increased the rate of organic enrichment (Boyd 1995; Zhong et al., 2010). *M. japonicus* adults tend to be benthic oriented and has burrowing activity (Abe et al., 2007). Also, *Corophium* that transplanted in the *M. japonicus* ponds were also known as U-shaped structure burrowers that are important for the sediment reworking in the intertidal ecosystems (Mermillod-Blondin and Rosenberg, 2006; De Backer et al., 2011). Burrowing activity can increase the oxygen penetration which may lead to a more efficient sediment mineralization (Andersson et al. 1988, Kristensen, 2001). Thus, we hypothesize that the bioturbation effects are stronger in *M. japonicus* ponds than in *L. vannamei* ponds Further studies on the sediment oxygen profile and nutrient fluxes at water-sediment interface are required to verify the hypothesis.

Studies showed that several *Penaeus* species prefer to feed on natural productivity grown in the ponds than on the feed pellets (Burford et al., 2004; Nunes et al., 1997). Thus, the underestimation of natural productivity commonly leads to overfeeding issues especially for rearing *L. vannamei* which has relatively high tolerance to environmental changes (Briggs et al., 2004; Martinez-Cordova et al., 1998). In this study, the high BPC concentrations suggest that the sediments in both *M. japonicus* and *L. vannamei* ponds are had pronounced eutrophic sediments (especially in the late stage that both ponds were hypertrophic). Especially in *L. vannamei* ponds, the BPC concentrations were much higher than the threshold: 5 mg C g^{-1} (Pusceddu et al., 2007b). The concentrations of proteins and carbohydrates suggest that towards the end of the rearing season, both ponds were hypertrophic (Dell'Anno et al., 2002). This can be an indication of overfeeding. Moreover, the disposal of pond sediment is becoming a concern for the environmental management (Wu et al., 2014). As a matter of fact, those biopolymeric and organic enriched sediments can pollute the surrounding environment if they are disposed without any proper treatment. Further continuous monitoring should also emphasize the consequences of the biochemical characteristics for the adjacent costal ecosystem.

Sedimentary organic variables were analyzed in a multivariate approach in order to estimate the change of the sedimentary trophic state of the impacted sites (Fig.2.6). For the scallop farming case, the temporal and spatial variations were well-shown but the scallop farming effect cannot be distinguished. In the shrimp ponds, the sedimentary trophic status of both types of ponds changed significantly during the rearing stages. Although we cannot rule out the seasonal variability to this change, the high level of biochemical fractions, which were out of the natural ranges of coastal marine sediments (Pusceddu et al. 2007b), suggesting that shrimp farming may have played an important role in this alteration.

The protein to carbohydrate ratio (PRO: CHO) is recently used to indicate the benthic eutrophication status. The protein dominates over the other biopolymers and leads to PRO:CHO >1 in eutrophic ecosystem, including open-water fish farming areas (Dell'Anno et al., 2002; Pusceddu et al., 2009; Silva et al., 2017; Venturini et al., 2012). In our study, neither scallop farms nor shrimp ponds induced this condition, though the reasons were different. PRO:CHO < 1 obtained in the sediments of scallop farms actually indicates a meso-oligotrophic status that is supported by the protein, carbohydrate and BPC concentrations. However, low PRT: CHO values in the shrimp pond sediments is likely related to the activities of microbes. The microbial loop was found to be important in shrimp ponds (Moriarty, 1997). These microbes utilize proteins faster than carbohydrates (Joseph et al., 2008), and thus cause preferentially accumulation of carbohydrates. Our results agree with the finding of Pusceddu et al. (2011) and confirm that the use of the PRO: CHO ratio is less applicable in an artificial ecosystem like shrimp ponds with limited water exchange and intensive anthropogenic activities. Thus, shrimp farming activity, which face more serious benthic eutrophication than bivalve farming, does not share the same monitoring criteria with other open-water aquaculture such as bivalve or fish aquaculture.

2.5 Conclusions

Our study demonstrates that scallop farming in open water has generally no advance effect on the benthic environment in terms of physicochemical, bulk organic, and biochemical variables. On the other hand, the benthic environments of two different shrimp ponds were highly reduced, and switched to eutrophic/hypertrophic conditions. Furthermore, we found that the extent of benthic eutrophication was correlated to the farming practices used (e.g. feed inputs, water renewal, and stocking density) in shrimp ponds. Sediment biochemical analysis can reflect the trophic status in the scallop farms and shrimp ponds, and was capable to detect the scallop farming effect as well as the temporal changes throughout the shrimp rearing stages on the benthic environment. In comparison, bulk organic contents were less sensitive and thus less capable to indicate the scallop farming activity. The PRO:CHO ratio should be used with care as microbial activity can mask the initial concentrations of proteins and carbohydrates.

Chapter 3: Assessing environmental effects of the bay scallop *Argopecten irradians* farming in Laizhou Bay, China: using meiobenthos and harpacticoid copepods as bioindicators

Adapted from:

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Abstract

This study aims to identify the effect of biodeposition from a non-indigenous scallop (bay scallop *Argopecten irradians*) farm on meiobenthos in a semi-closed bay in the Bohai Sea, China. Sediment characteristics, the meiobenthic community and harpacticoid copepod assemblages were investigated before aquaculture and during the high biodeposition period at three scallop farms located in sandy-shallow (SS), muddy shallow (MS), and muddy deep (MD) areas, and their correspondent control sites. The accumulation of biopolymeric carbon (BPC) was enhanced by scallop farming activities, but such accumulation did not result in eutrophication. The meiobenthos and harpacticoid copepods as bioindicators responded location-specifically to scallop farming. Only in Farm SS the community of meiobenthos at higher-taxon level differed from the control site as there was a larger proportion of nematodes. The harpacticoid copepod assemblage was altered by scallop farming only in station MD, which had a higher diversity and number of copepod species. The overall results suggested that this non-indigenous scallop farming had minimal effect on the benthic environment in Laizhou Bay. Also, the BPC, the entire meiobenthic community and harpacticoid copepod assemblages can serve together as tools for assessing of the potential environmental impact of bivalve farming.

3.1 Introduction

The rapid expansion of marine aquaculture (a.k.a. mariculture) diminishes the tension between the demand and supply of seafood but also generates many environmental issues (FAO, 2016; Naylor et al., 2000). One of the most serious problems is the bottom-up effect on the surrounding ecosystem due to the release of abundant particulate or soluble organic materials to the sediment below (the so-called benthic environment). This can lead to organic accumulation on the sea bottom (Hargrave et al., 1997; Pearson and Black, 2000), affecting the cycles of sediment biogeochemistry (Chamberlain et al., 2001; Holmer et al., 2005), altering the diversity and structure of benthic communities (Mirto et al., 2000; Netto and Valgas, 2010), and eventually impacting the overall ecosystem functioning (Callier et al., 2013; Dubois et al., 2007). Although bivalve mariculture is considered to cause less environmental damage due to lack of feed loading, the high density of bivalve in mariculture farms can enhance the downward flow of organic matter (Crawford et al., 2003). Many studies have reported negative effects of bivalve farming on the benthic environment (Chamberlain et al., 2001; Mirto et al., 2000; Stenton-Dozey et al., 2001), while others found no detectable effects (Danovaro et al., 2004; Fabi et al., 2009; Han et al., 2013). So far, no general conclusion can be made and a better understanding of the environmental effects of bivalve farming is needed for its sustainable management.

China has the largest bivalve production worldwide (FAO 2016). Compared with other forms of bivalve mariculture, longline-suspended culture of scallops is relatively new and rapidly growing (Guo and Luo, 2016). The bay scallop (*Argopecten irradians* Lamarck) is a non-indigenous species (NIS) that was introduced from North America in the 1980s. Since then, scallop farming has been dominated by bay scallops, with a production of 0.817 million tonnes in 2012, which is still continuously expanding (Guo and Luo, 2016; MAC 2013). NIS are popular in aquaculture, generating high economic output because of their fast growth rates, wide range of diets, large environmental tolerance etc. (Ruesink et al., 2005; Silva et al., 2009). For example, bay scallop has a fast growth rate and reaches market size already within a year, which is twice as fast as native scallop species (Chinese scallop *Chlamys farreri*, Guo and Luo, 2016; Xiao et al., 2005). However, NIS aquaculture involves ecological risks as species growing outside their native ranges can potentially cause ecological and economic harm to the environment (Olenin et al., 2007). In particular, introduced ecosystem engineer like bivalves could have more dramatic impacts because their filter feeding and biodeposition activity can largely affect ecosystem structure and functioning (e.g. the biochemical fluxes and related organism communities, Sousa et al., 2009; Zaiko et al., 2009). Higher biodeposition rates of bay scallop were reported in comparison to the Chinese scallop (Li et al., 2009; Wang, 2015; Zhou et al., 2006), but little is known about its impacts on the native communities, particularly the ones occurring in the sediment.

In view of the contrast between the economical benefit provided by bivalve mariculture and its potential environmental impact, systematic monitoring of the receiving ecosystem is urgently needed (Fabi et al., 2009), especially for the cases of cultured non-indigenous bivalve species. Accordingly, identifying appropriate indicators of potential impact is one of the major tasks for monitoring bivalve mariculture (Cranford et al., 2006). Traditional parameters like sediment characteristics are sometimes not sensitive enough to identify the biodeposition effect (Callier et al., 2008). Meiobenthos is proposed as an integrative tool to monitor organic pollution and recognized as an informative bioindicator (Grego et al., 2009; Mazzola et al., 2000; Mirto et al., 2012) due to its fast response to environmental stressors, with meiobenthic community structure providing information that cannot be detected with macrobenthos (Giere, 2009; Kennedy and Jacoby, 1999; Semprucci et al., 2016). However, the sensitivity to environmental disturbance varies among meiobenthos taxa (Raffaelli and Mason, 1981; Warwick, 1988). Harpacticoid copepods (Crustacea, Copepoda), the second most abundant taxon within the meiobenthos, are sensitive to changing environmental conditions (De Troch et al., 2013; Giere, 2009; Wetzel et al., 2001). Also, they fulfill an important function in the energy transfer from primary producers to higher trophic levels (Cnudde et al., 2015; Hicks and Coull, 1983; Leduc et al., 2009). Consequently, a possible community alteration of harpacticoid copepods could have a cascading effect on the food web.

The aims of this study are to evaluate possible effects of non-indigenous scallop (*A. irradians*) mariculture on the meiobenthic community as well as to assess the sensitivity and efficiency of bioindicators responding to biodeposition in the receiving benthic environment. We hypothesize that: (1) the farming of *A. irradians* affects the structure of the meiobenthic community (higher-taxon level) and copepod assemblages (species-level); (2) meiobenthic community (rapid bioindicator) and copepod assemblages (lower-taxon bioindicator) provide different levels of information on the effect of bay scallop farming.

3.2 Material and Methods

3.2.1 Study area and field sampling design

The bay scallop farming area is located in Laizhou Bay (Bohai Sea, Shandong Province, China; Fig 3.1) and is one of the largest *A. irradians* mariculture areas in China since the 1980s, with a total area of 500 hectares and an annual production of 144470 tonnes in 2011 (Li, 2013). Bay scallops are typically cultivated with the suspended long-line method (See Chapter 1.5.1 for more details). Scallop farming activities are conducted from May to November each year without any provision of additional feed. Scallops are harvested when they reach a commercial size of 6 cm.

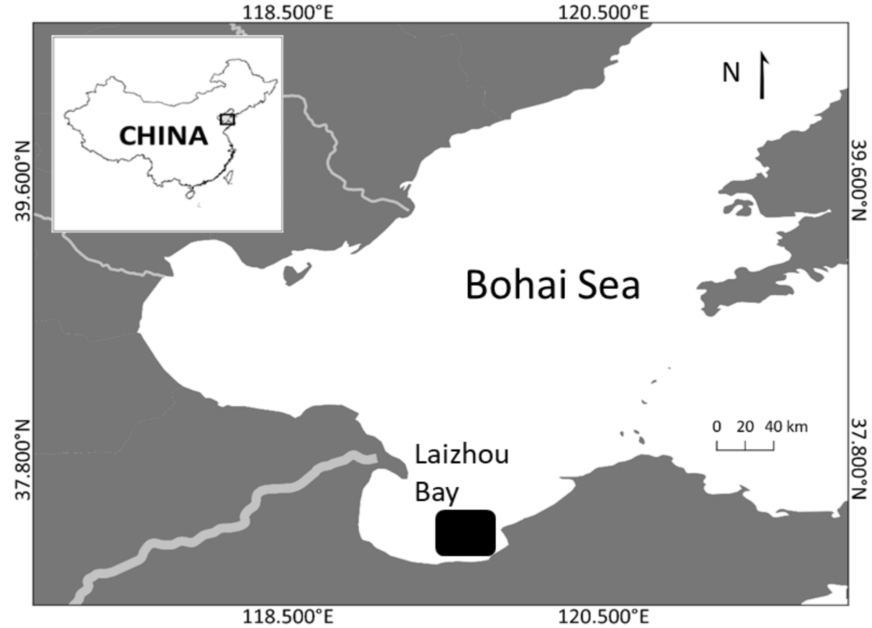

Figure 3.1 Map of the sampling area – Laizhou Bay, Bohai Sea, China

Three scallop farms, Farm SS (Sandy Shallow; with c.a. 100-120 lines c.a. 200 ind. m^{-2}), Farm MS (Muddy Shallow; with c.a. 60-80 lines and c.a. 100 ind. m^{-2}), and Farm MD (Muddy Deep; with c.a. 100-120 lines and c.a. 200 ind. m^{-2}), located about 5 km, 10 km, and 20 km offshore the Laizhou coast, were investigated in May (i.e. before the scallop nets are placed in this area) and in October-November 2016 (i.e. when the scallops reached heavy a biodeposition rate, Wang 2015). Correspondingly, three control sites (Ctrl SS, Ctrl MS, and Ctrl MD) were selected about 1.5-2.0 km away from each scallop farm to exclude any direct effect of farming. Farm & Ctrl SS (i.e. Station SS) were located at a depth of 5 m above a sandy bottom (2% clay, 24-28% silt, and 69-73% sand). Farm & Ctrl MS (i.e. Station MS) and Farm & Ctrl MD (i.e. Station MD) were both located above a muddy bottom (4-6% clay, 53-55% silt and 39-42% sand) at depths of 7 m and 12 m, respectively. A northeast oriented dominant current with a mean velocity of 20 cm s^{-1} runs perpendicularly to the shoreline (Zhao and Chen, 2001). Salinity and temperature of the water ranged between 30.7-31.1 and 20.5-21.7 °C in May 2016, and 29.8-30.1 and 11.4-12.2 °C in October 2016.

The sediment samples were collected by SCUBA diving using plexiglass cores (3.6 cm inner diameter, 10 cm^2 surface area) and PVC cores (15 cm inner diameter, c.a. 177 cm^2 surface area). Three deployments in each site were randomly chosen, and in each deployment three independent plexiglass cores were obtained for meiofauna analysis, chlorophyll *a* (Chl *a*) contents, and other environmental variables including total organic carbon (TOC), total nitrogen (TN), biochemical composition of organic matter (i.e. carbohydrates, proteins and lipids).

For Chl *a* and other biochemical variables, the top layer (0-1 cm) of the cores was sliced, homogenized and stored at -20 °C. For the meiofauna samples, the top layer (0-1 cm) was sliced and preserved in a 4% formaldehyde - filtered seawater solution. Sedimentary pH and Eh were measured from the top 1 cm layer of a PVC core for each deployment (See 2.2.2 from Chapter 2 for more details of the *in situ* measurements).

3.2.2 Sediment environmental variables

Chl *a* contents were measured in 2.5 g of wet sediments by fluorescence method after extraction with 90% acetone (Yentsch and Menzel, 1963). For the levels of TOC and TN, sediments were oven dried (60 °C), grounded, and treated with 1N HCl for 24 h to remove carbonates. TOC and TN were measured with an Element Analyser Flash 2000 (Thermo Fisher Scientific). The C/N ratio was calculated from the TOC and TN levels. Sediment granulometry was measured by laser diffraction with a Malvern Mastersizer 2000 particle analyzer (Malvern Instruments, UK).

The biochemical composition of the organic matter in the sediments (carbohydrates, proteins, and lipids) was measured photometrically following the procedure of Fabiano and Danovaro (1994). About 0.2-0.3 g of dried and grounded sediments were used as a replicate for each type of analysis, and pre-combusted sediments (450 °C, 4h) were used as blanks. Carbohydrate, protein and lipid contents were converted to the carbon equivalents by multiplying with the conversion factors of 0.40, 0.49, and 0.75 mg C mg^{-1}, respectively (Fabiano et al., 1995). Their sum was reported as biopolymeric carbon (BPC).

3.2.3 Meiobenthic community structure and copepod assemblages

Sediment samples preserved in 4% formaldehyde were rinsed with filtered freshwater through 500 μm and 38 μm sieves. The fractions retained on a 38 μm sieve were centrifuged three times with Ludox HS40 (density: 1.18 g cm^{-3}) and stained with 0.5 g L^{-1} of Bengal Rose. Meiobenthos was sorted to the higher taxon level. All the adult harpacticoid copepods in each sample were picked with a needle, preserved in 75% ethanol, and identified to species level. Since the purpose of this study was to estimate the community structure and diversity, we used the morphospecies (i.e. the different morphotypes belonging to each genus were indicated as sp1, sp2, etc.) for the copepod identification.

The ratio of nematodes to copepods (Ne/Co), total density and biodiversity indexes: number of species (S), Shannon-Wiener diversity index (*H*'), Peilou's evenness (*J*') and dominance (λ) were calculated.

3.2.4 Statistical data analyses

Three factors were considered in the sampling design: (1) Time - fixed factor with two levels: before farming (T0) and during heavy biodeposition stage (T1), (2) Station (Stn) - random factor with three levels: SS, MS, and MD, and (3) Site - fixed factor with two levels: farm and control.

Variation in sediment variables, Ne/Co and biodiversity indices were tested with permutational multivariate analysis of variance (PERMANOVA) across the three factors of Time, Station (Stn), and

Site, based on Euclidean distance matrices. The homogeneity of multivariate dispersion was checked with a distance-based test (PERMDISP). When significant differences were observed, post-hoc comparisons were performed to test for possible differences between farm and control at each sampling station using PERMANOVA pair-wise tests type III.

Differences in composition of biopolymeric carbon, meiobenthic community structure and harpacticoid copepod assemblages among the three factors were tested with a 3-way PERMANOVA (main test and pairwise test) based on the Bray-Curtis matrices of square-root transformed densities. The same matrices of PERMANOVA were used for principal coordinate analysis (PCO) to visualize the grouping of taxa or species among factors. Finally, similarity percentage (SIMPER) analyses were conducted to investigate which taxa or species contributed to the dissimilarity between groups. Links between faunal community structure and environmental variables were analysed using RELATE and BIO-ENV routine (Clarke and Ainsworth 1993). After removing strongly correlated environmental variables ($r > 0.9$), we included TN, TOC, Chl *a*, proteins, lipids, carbohydrates, pH and Eh to calculate the matrix and conduct the RELATE and BIO-ENV analysis.

PERMANOVA, PERMDISP, PCO, SIMPER, RELATE and BIO-ENV were performed with Primer V6 (Clarke and Gorley, 2006), using the PERMANOVA+ add-on package (Anderson et al., 2008).

3.3 Results

3.3.1 Meiobenthic community structure

In total, 11 taxa were found during the two sampling events in the study area. All stations were dominated by Nematoda. The second most abundant taxon was either Copepoda or Bivalvia, followed by Ostracoda, Oligochaeta, Polychaeta, and other taxa (Fig 3.2).

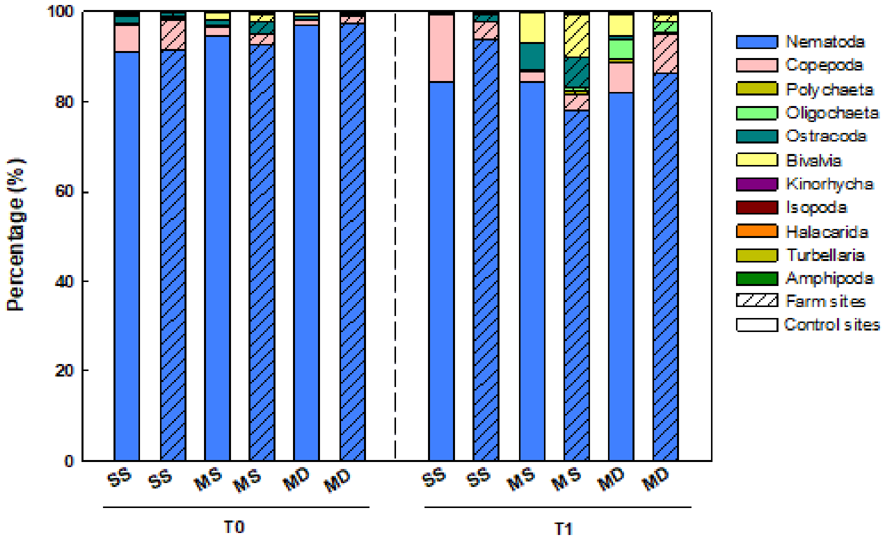

Figure 3.2 Relative (%) meiobenthic community composition in farm and control sites in three stations (SS, MS and MD)

The main tests of PERMANOVA showed Time × Station × Site to have a significant effect on the meiobenthic community structure (Table 3.1). The pair-wise tests between each farm and control site further indicated that the meiobenthic community structure only differed from the control site at Stn SS at T1 (Table 3.1: T1-SS $p < 0.05$).

This is in accordance with PCO that showed samples separated at T1 (Fig 3.3). The pattern indicates scallop farming had an effect on the meiobenthic composition at Stn SS.

Table 3.1 Results of factorial PERMANOVAs testing variations in the composition of (a) entire meiobenthic community and (b) harpacticoid copepod assemblages among the levels of the factors: Time (T0 vs T1), Site (Farm vs Control), and Station (Stn SS, MS, and MD). P-F = pseudo-F value; T0 = before farming; T1 = during heavy biodeposition; *= $p < 0.05$; **= $p < 0.001$; ***= $p < 0.001$

Source	df	MS	P-F	P (MC)
	(a) Meiofaunal community			
Time	1	4451.4	3.6957	ns
Site	1	324.14	1.9783	ns
Station	2	2357.8	26.38	***
Time ×Site	1	150.47	0.7005	ns
Time × Station	2	1204.5	13.476	***
Site × Station	2	163.85	1.8333	ns
Time × Station × Site	2	214.8	2.4034	*
Res	24	89.376		
pairwise test: FARM VS CTRL			t	P (MC)
T0-SS			0.950	ns
T0-MS			0.996	ns
T0-MD			0.306	ns
T1-SS			2.895	*
T1-MS			1.616	ns
T1-MD			1.584	ns
	(b) Copepod assemblages			
Time	1	12678	1.5519	ns
Site	1	18815	20.093	ns
Station	2	1281.8	0.70041	***
Time × Site	1	1377	0.78827	ns
Time × Station	2	8169.2	8.7244	***
Site × Station	2	1830	1.9544	*
Time × Station × Site	2	1746.9	1.8656	*
Res	24	936.36		

pairwise test: FARM VS CTRL	t	P (MC)
T0-SS	1.665	ns
T0-MS	0.781	ns
T0-MD	1.192	ns
T1-SS	1.148	ns
T1-MS	0.529	ns
T1-MD	1.891	*

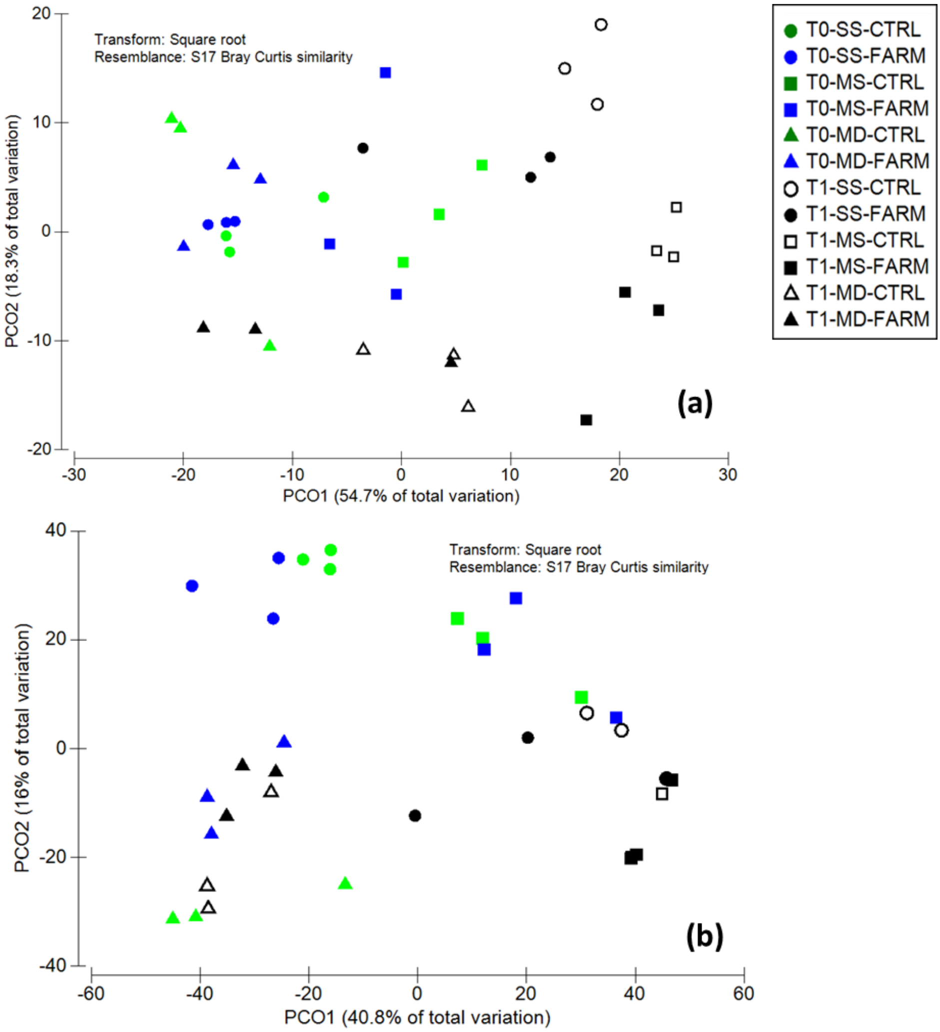

Figure 3.3 Principal coordinate analysis (PCO) based on (a) meiobenthos at higher-taxon level and (b) copepod species in the sampled sediments. The sample grouping was based on Bray-Curtis clustering from square-root transformed abundance data. T0 = before farming; T1 = during heavy biodeposition; CTRL = control site; FARM = farming site.

The main taxa responsible for this separation in Stn SS were Nematoda (SIMPER: 50.37% of dissimilarity; Fig 3.4) that occurred at higher densities under the farm. Copepoda contributed 3.7% of dissimilarity as the density decreased with the presence of the farm (Pairwise comparison: $p < 0.05$). Ostracoda and Bivalvia contributed to the dissimilarity as well and occurred in higher densities at the farm sites.

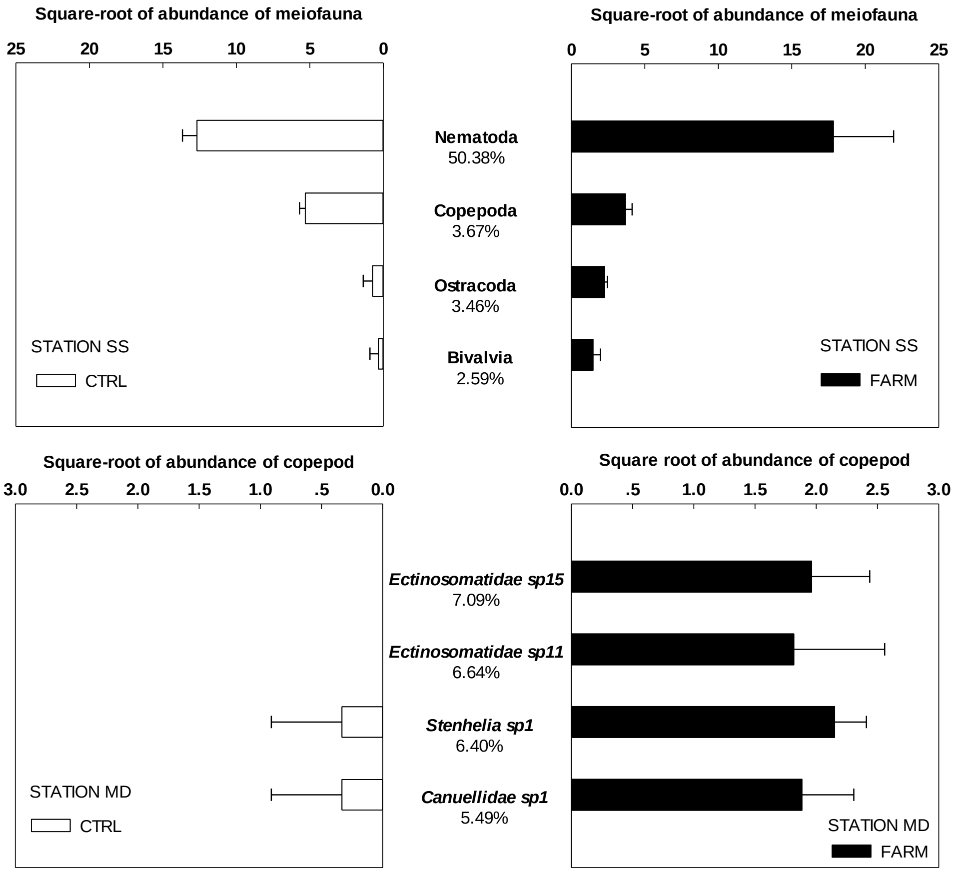

Figure 3.4 SIMPER analysis of meiobenthos and copepod abundance (top 4 taxa or species contributing to differences) between farm and control sites at Stns SS and MD in T1 (during heavy biodeposition). SIMPER analysis was based on the Bray-Curtis dissimilarity of square-root transformed data. Percentage dissimilarity between farm and control sites is indicated. T0 = before farming; T1 = during heavy biodeposition; CTRL = control site; FARM = farming site.

The Ne/Co index did not differ at T0 between farm and control sites for all stations. At T1 only the farm site in Station SS showed a higher Ne/Co (24.4±9.6) than that of control (5.8±1.3; Pairwise comparison: *p* = 0.029; Fig 3.5; Table S2).

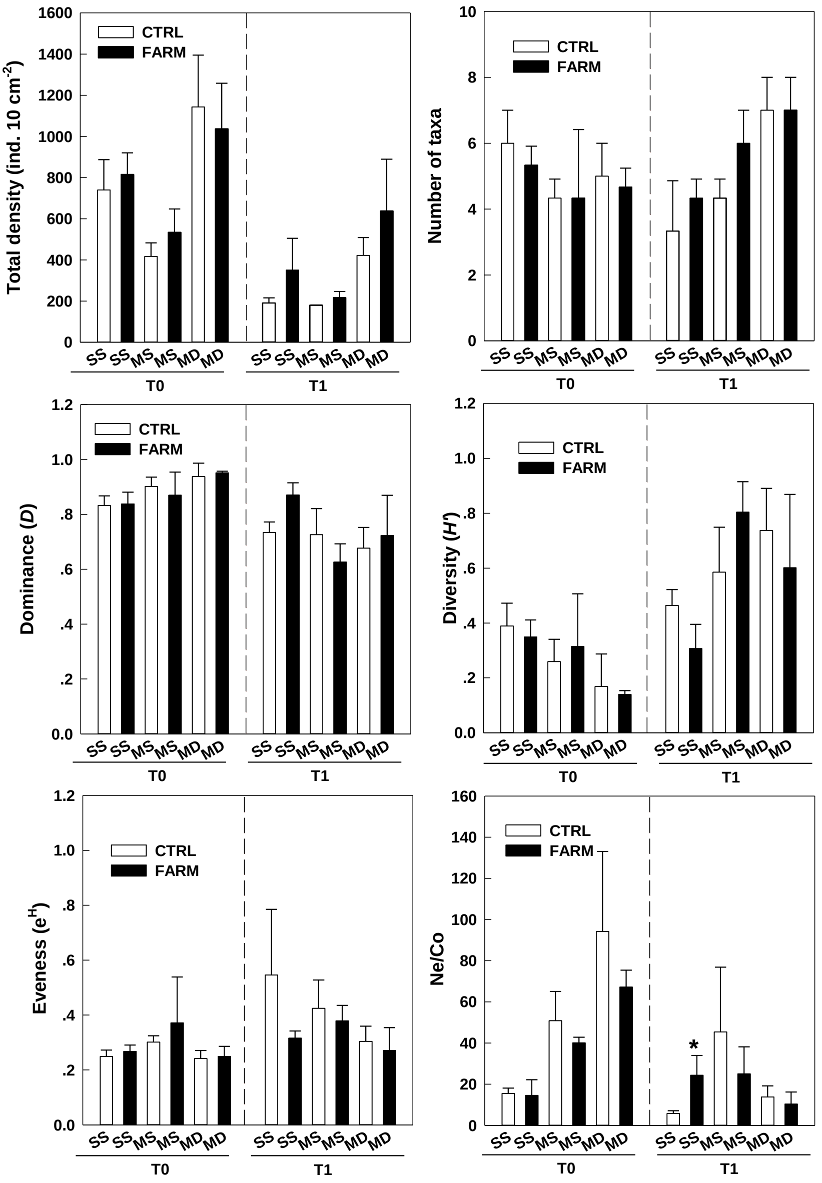

Figure 3.5 Meiobenthic diversity indices and the ratio of nematodes to copepods (Ne/Co) in farm and control sites in three stations (SS, MS, and MD). Error bars indicate standard deviations among replicates. Significant differences (pairwise comparison: $p < 0.05$) are indicated by an asterisk. T0 = before farming; T1 = during heavy biodeposition; CTRL = control site; FARM = farming site.

No significant differences were found for the biodiversity indices between each pair of farm and control site (pairwise comparisons: $p < 0.05$).

3.3.2 Harpacticoid copepod assemblages

The PERMANOVA results revealed that copepod assemblages were affected by the interaction effect of Time × Station × Site (Table 3.1). Copepod assemblages only differed between farm and control at Stn MD (Table 3.1 Pairwise comparison: T1-MD $p < 0.05$). SIMPER analysis showed two species of Ectinosomatidae, a species of Miraciidae (belonging to the genus *Stenhelia*), and a species of Canuellidae to occur either exclusively or with higher density at Farm MD compared to its control site (Fig 3.4).

Furthermore, in Station MD at T1, scallop farming resulted in higher total density and higher species richness of copepods (Fig 3.6; Appendix II Table S3).

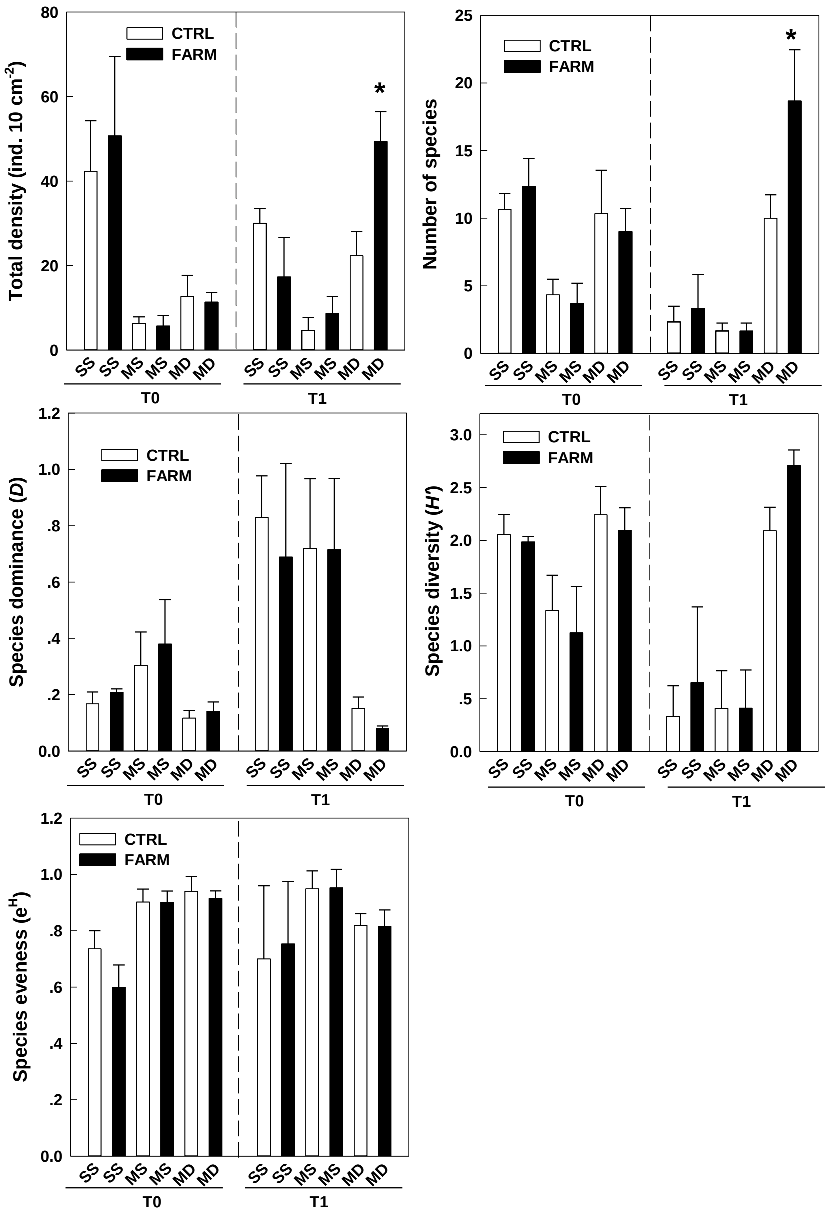

Figure 3.6 Copepod diversity indices in farm and control sites in three stations (SS, MS, and MD). Error bars indicate standard deviations among replicates. Significant differences (pairwise comparison; $p < 0.05$) are indicated by an asterisk. T0 = before farming; T1 = during heavy biodeposition; CTRL = control site; FARM = farming site.

3.3.3 Environmental properties and the relationships with meiobenthos

We analyzed several physical/chemical properties (pH, Eh, total organic carbon and total nitrogen, chlorophyll *a*, and biopolymeric carbon: proteins, carbohydrates, and lipids) of the top 0-1 cm sediment layer at farm and control sites from three stations during T0 and T1 (See Chapter 2 sections 2.3.1, 2.3.2 and 2.3.3). Biopolymeric carbon (BPC), which reflects the labile fractions of sedimentary organic matter, could indicate scallop farming effects on the sediment. Compared with control sites, the farm sites in Stns SS and MD had a higher BPC content during the scallop farming (Fig. 3.7). Alterations to the biochemical composition of the farmed sediments in Stns SS and MD were also observed (Table S4 and Fig 3.7).

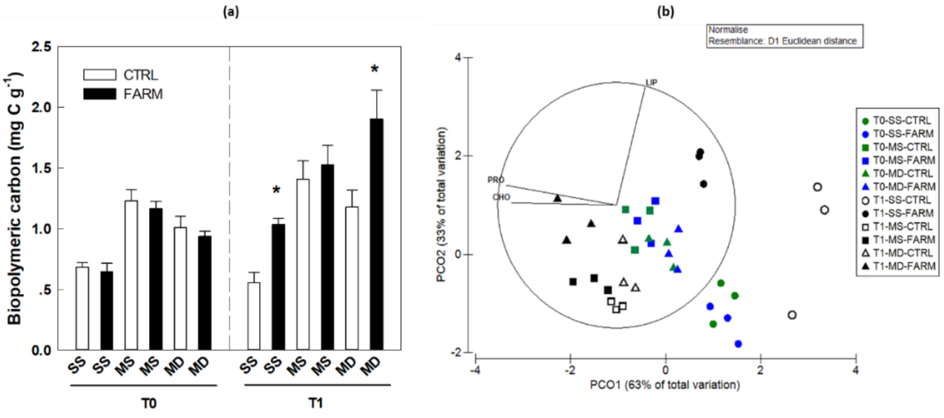

Figure 3.7 Organic matter in the top 1 cm sediment layer from scallop farm and control sites at three stations (Stations SS, MS, and MD) in Laizhou Bay, China. (a) Sediment content of biopolymeric carbon (BPC). (b) Variations in the biochemical composition of the sedimentary organic matter (biplot after Principal coordinate - PCO). Error bars indicate standard deviations among replicates. T0 = before farming; T1 = during heavy biodeposition; CTRL = control site; FARM = farming site.

To link the environmental variables and the faunal community, RELATE analysis showed both meiobenthos community structure and copepods assemblage had low correlation coefficient with the environmental variables (Meiobenthos: RELATE - Spearman's rho = 0.316; Copepods: RELATE – Spearman's rho = 0.163; Also see Table S5 for univariate RELATE analysis). Sedimentary proteins and carbohydrates were shown optimally explain the meiobenthos structure (rho = 0.453), while proteins and Chl *a* best explained the copepod assemblages (rho = 0.223).

3.4 Discussion

3.4.1 Effects of bay scallop farming on sediment trophic status

Our study found an impact of scallop farming activities on the sediment trophic status. In Stns SS and MD where the production density was c.a. twice as high as in Stn MS, the sediments under the farms were characterized by an increase in BPC levels, indicating an increase in the digestible organic carbon fraction that is available to the benthos (Fabiano et al., 1995). Specifically, proteins and carbohydrates were found to accumulate in the sediment under the scallop farms, which is likely related to the scallops' nutrient-enriched biodeposits (Kautsky and Evans, 1987; Miller et al., 2002). However, the Chl *a*, protein, carbohydrate and BPC contents in the farming area were all within the range of a meso-oligotrophic status as proposed by Dell'Anno et al. (2002) and Pusceddu and Danovaro (2007). In such an environment with low primary production, the increased amount of bioavailable organic matter can be a potential extra food supply to benthos (Dell'Anno et al., 2002).

3.4.2 Effects of bay scallop farming on the meiobenthic community

Scallop farming only affected the meiobenthic community under the scallop farm at Stn SS (the sandy bottom station), mainly because of an increase in nematode density. This was not observed at the muddy stations. This location-specific response is mostly due to the granulometry, which is an important factor that governs the biogeochemical reactions and community structure of the benthic infauna (Giere, 2009; Martinez-Garcia et al., 2015). The biodeposition from bivalve farms can decrease the grain size of the sediments and turn fine sand bottoms into a muddy deposit (Netto and Valgas, 2010). This facilitates nematodes, which reach higher densities in sediments with lower grain size (Boucher and Lambshead, 1995; Heip et al., 1985). As for total meiobenthic densities, a negative impact of mariculture is commonly observed due to organic loading (Grego et al., 2009; La Rosa et al., 2001; Mirto et al., 2000; Mirto et al., 2012), but no such change was observed under the scallop farms in our study.

The Ne/Co ratio is associated with the benthic impact of organic pollution, which leads to an increase of the ratio (Raffaelli, 1981; Shiells and Anderson, 1985). We found the Ne/Co ratio to follow a similar trend as the meiobenthic community structure. At the sandy bottom station, the Ne/Co ratio was four times higher in sediments beneath the farms, indicating a response to the biodeposition. However, as suggested by Raffaelli and Manson (1981), the sediments in our study sites had not reached the threshold of organic pollution. However, the Ne/Co ratio should be used as an indicative proxy rather than an exact measurement of biodeposition, since it is affected by several environmental parameters, e.g. grain size, temperature (Lee et al., 2001; Giere, 2009). Moreover, the ratio does not differentiate between epibenthic and interstitial copepods, the latter being more sensitive to oxygen depletion.

The increased nematode densities at the sandy scallop farm have been linked to organic matter loading (Mirto et al., 2014; Mirto et al., 2010). Yet many studies showed a converse pattern of nematodes densities at rather high OM loading in a shallow coastal environment (Duplisea and Hargrave, 1996; Mirto et al., 2002; Sutherland et al., 2007). Nevertheless, opportunistic nematode species can always benefit from the organic input in both scenarios (Mirto et al., 2014; Netto and Valgas, 2010). In fact, the cryptobioturbation and bioirrigation related to nematodes might promote small-scale but important ecosystem functioning through biogeochemical interactions e.g. developing the bacterial population, accelerating organic matter remineralization, and thereby supplying the inorganic nutrient supply (Danovaro et al., 2008; Pike et al., 2001).

3.4.2 Effects of bay scallop farming on copepod assemblages

We found a location-specific response in the copepod assemblages. In the station located at the muddy deep area, the copepod community showed a higher density and number of species under the farm than at the control site. This pattern agrees with the concept of Pearson and Rosenberg (1987) and Rosenberg (2001) that food availability is an important factor to structure benthic communities, i.e. their number of species, density and biomass are higher when the food is abundant. Along with the higher amount of bioavailable organic matter under the scallop farm, the increased copepod densities were likely related to additional food supply (i.e. the scallop biodeposits). In meso-oligotrophic ecosystems with low-quality food sources (Venturini et al., 2012), biodeposits with a low C/N ratio, high proportion of labile organic matter, rebuilt microbial protein and attached microorganisms (Kautsky and Evans 1987, Wotton and Malmqvist 2001, Miller et al. 2002) can add nutritional value to the benthic environment, and also serve as an alternative food source to harpacticoid copepods (Huang et al., 2018). In addition, we found the change in copepod assemblages to be underpinned by Ectinosomatidae, Canuellidae, and Miraciidae (genus Stenhelia), whose morphology and feeding behavior are well-adapted to biodeposition environments. The hairy antenna of the filter-feeding Canuellidae and the sweeper-like maxilla of the scrapper Stenhelia can attract more particles (Boxshall and Halsey, 2004; Mu and Huys, 2004). The increased number of microorganisms (e.g. bacteria, ciliates and dinoflagellates) resulting from organic loading facilitates the carnivorous Ectinosomatidae (Bongiorni et al., 2005; Commito and Tita, 2002; Coull and Dudley, 1976). However, in case of a constant accumulation of large amounts of organic matter (e.g., a fish farm, resulting in anoxic sediment), the density of copepods can ultimately be impaired (Grego et al., 2009).

In the sandy station where a species of the family Canuellidae dominated in both farm and control site, the total copepod density was slightly reduced under the scallop farm, yet the copepod community structure did not change. This suggests a minor effect of the scallop farm on the copepod community in the sandy bottom. Although the bioavailable carbon was observed to be higher under this farm as well, the decreased density of Canuellidae was likely a consequence of intraspecific competition which is more intense than interspecific competition (Westoby, 1984).

The harpacticoid copepod community responded differently than the entire meiobenthic community. In contrast to some other studies (Daudi et al., 2012; Kennedy and Jacoby, 1999), higher-taxon surrogacy was not sufficient to identify the effect of organic enrichment since higher meiobenthos taxa did not show the same pattern as the lower taxon level in our study. Although higher-taxon surrogacy can be a fast and reliable way to evaluate eutrophic ecosystems with high organic loading such as fin-fish farming areas and shrimp ponds (Aryuthaka and Kito, 2002; Grego et al., 2009; Mirto et al., 2012), our result suggests that under moderate organic loading into a meso-oligotrophic system, combining information from the higher- and lower-taxon level (e.g. at copepod-species level) together can provide a clearer insight of assessing mariculture activity.

3.4.3 Overall effect of bay scallop farming

Other studies focusing on the pelagic community found that the bay scallop farming in Laizhou Bay lowered chlorophyll *a* levels in the water column but did not have a strong effect on nutrient levels (Liu et al., 2004; Wang, 2015). Another research found no significant changes in the macrofauna community in the bay scallop farming area in Laizhou Bay (Zhou, pers. comm.). Together with our results, we can suggest that the NIS bay scallop farming in Laizhou Bay has minimal adverse effects on the local ecosystem.

Even though the biodeposition rate of NIS bay scallop is quite high (10 times higher than native scallop *Chlamys farreri*, Wang, 2015) and this study area supports a high density of mariculture, no negative effect was observed. This was attributed to the meso-oligotrophic nature of the recipient environment (Liu et al., 2004; Zhuang et al., 2014; this study). Also, the relatively high hydrological dynamic reduces the potential impact of organic loading (Fabi et al., 2009), because more frequent resuspension decreases organic matter accumulation and enhances oxygen penetration and biogeochemical reactions e.g. mineralization (Mirto et al., 2000). Here, we observed a relatively high water flow velocity (mean velocity 20 cm s^{-1}), compared to bivalve farms that had negative impacts on the benthic fauna (2.7 cm s^{-1} in Netto and Valgas (2010); 5 cm s^{-1} in Callier et al. (2008); 3.16-10.21 cm s^{-1} in Hartstein and Rowden (2004)). This suggests that the high-water velocity at our sampling site prevents the organic matter accumulation due to either direct dispersal or via enhanced mineralization of biodeposits.

It should be noticed that at Stn MS, no localized response of the benthic community to the scallop farm was found. This can be due to the lower densities of cultured scallops compared to the other stations.

3.5. Conclusions

The reported results can serve as a reference for a better monitoring of the NIS bay scallop farming in a bay with a meso-oligotrophic benthic environment. In general, this mariculture activity had minimal effects in terms of the meiobenthic community structure and harpacticoid copepod assemblages. The effects of this scallop farming on local assemblages varied among different benthic components, depending on the sediment type as well as on the intensity of the mariculture (i.e. densities of cultivated species). Furthermore, in the condition of moderate organic loading in meso-oligotrophic benthic environments, sedimentary BPC as well as the meiobenthos and harpacticoid copepod community can be useful tools to assess the environmental impact of mariculture.

Acknowledgements

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Chapter 4: Impact of farming non-indigenous scallop *Argopecten irradians* on the benthic ecosystem functioning: a case-study in Laizhou Bay, China

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Abstract

The farming of the non-indigenous bay scallop *Argopecten irradians* in coastal waters generates large amounts of biodeposits that potentially change the trophic pathways and quality of the benthic food web at lower trophic levels such as meiobenthos. To understand the trophic link between faecal pellets of bay scallop and meiobenthos in the aquaculture area, we investigated the resource use of harpacticoid copepods and nematodes inside and outside of 3 bay scallop farms in Laizhou Bay (Bohai Sea, China), using natural abundance of stable carbon and nitrogen isotopes together with fatty acid profiling. Faeces were found to be enriched in ^{15}N compared to all other food sources, which made faecal matter traceable. The enriched ^{15}N in several meiobenthos at the farms together with the mixing model results indicated that faeces could be a new food source for most of harpacticoid copepods and some nematodes. The quantities and the pathways of assimilation differed between the copepod families depending on their feeding behaviors and the receiving environment. Furthermore, due to the presence of higher levels of polyunsaturated fatty acids, in particular docosahexaenoic acid, the dominant copepod family Canuellidae that abundantly consumed scallop faeces showed enhanced nutritional quality compared with those in the control sites. Thus, aquaculture of non-indigenous bay scallops provided a food source that was directly and indirectly consumed by meiobenthos underneath the scallop farms and improved the quality of lower level consumers as a food item in the benthic food web.

4.1 Introduction

Coastal marine aquaculture is growing steadily worldwide as a means of food production but has been criticized on environmental grounds (Pillay 2008). One of the most contentious issues is the possible impact of aquaculture effluents accumulating on the seabed on community structure and ecosystem functioning of the benthic ecosystem (Kalantzi and Karakassis 2006, Dubois et al., 2007). Compared to finfish farming, bivalve aquaculture is considered to cause less damage to the environment as there is no addition of feed (Crawford et al., 2003). However, bivalve species are recognized as keystone species that exert a bottom-up effect on marine ecosystems through biodeposition (i.e. the deposition of faeces and pseudofaeces), transferring materials and nutrients to the benthic environment (Newell 2004). This process can affect ecosystem functioning by serving as additional food sources to the benthos and can thus potentially change the benthic energy flow (Peterson and Heck 1999, Callier et al., 2008). Although many studies have recorded the influence of biodeposition on benthic assemblages (Hartstein and Rowden 2004, Callier et al., 2008), little is known about the fate of biodeposits and their functional effect on the benthic ecosystem, e. g. through the assimilation of these deposits by benthic organisms. While biodeposits have a good nutritional value (McKindsey et al., 2011), it is unclear whether the benthos can benefit from incorporating these deposits into their diet.

China is the biggest bivalve aquaculture producer worldwide and scallop farming using longlines in coastal marine waters is a major part of its aquaculture industry (FAO 2016). Among the cultured scallop species, the non-indigenous species (NIS) *Argopecten irradians* (a.k.a.

bay scallop), introduced from North America, has dominated the Chinese scallop production over the past 30 yr (Guo and Luo 2016). In terms of economic output, *A. irradians* has an advantage over the native scallop *Chlamys farreri* due to the faster growth rate (Guo and Luo 2016). However, the high biodeposition rate of *A. irradians* (almost 10 times higher than *C. farreri*) also poses a threat to the benthic ecosystem (Zhou et al., 2006, Li et al., 2009, Wang 2015). However, little is known about whether and how the biodeposition of non-indigenous *A. irradians* can potentially affect organisms in the sediment and the overall functioning of the benthic ecosystem.

Ecosystem functioning integrates the energy flux within a system (Power 1992). The process of resource utilization by benthic consumers, especially meiobenthos (the smaller fraction of metazoans passing through a 1 mm sieve but being retained on a 38 µm sieve), is crucial to understand the energy flux of an ecosystem, because: (1) meiobenthos are highly abundant and form a link between primary producers and higher trophic levels (TLs) (Leduc et al., 2009); and (2) due to their small size, short generation times and close associations with sediments, they are sensitive to stressors and respond functionally to them (Kennedy and Jacoby 1999). However, there is little information on functional responses (in particular, resource utilization) of meiobenthos to aquaculture biodeposition. Since the effects of aquaculture waste are known to differ among trophic guilds and feeding behaviors of animals (Wai et al., 2011), it is important to incorporate the knowledge of functional responses of different taxonomic groups in order to understand the impact on ecosystem functioning. Furthermore, harpacticoid copepods are important food sources and providers of fatty acids (FAs), especially highly polyunsaturated fatty acids (PUFAs), to higher marine consumers (de Lima et al., 2013). Their FA profiles depend on their food sources and environmental conditions (Nanton and Castell 1999, De Troch et al., 2012, de Lima et al., 2013). Determining the presence of quality-indicator FAs, such as PUFA, 20:5ω3 (eicosapentaenoic acid; EPA) and 22:6ω3 (docosahexaenoic acid; DHA), in harpacticoid copepods will contribute to the assessment of the functional impacts of biodeposition by bay scallop in aquaculture areas.

The analysis of the natural abundance of stable isotopes together with FA profiles is an efficient tool to investigate the diet of meiobenthos (Leduc and Probert 2009, Cnudde et al., 2015). Stable carbon ratios reflect the food sources of consumers, and nitrogen ratios are used to determine their trophic positions (DeNiro and Epstein 1978, Minagawa and Wada 1984). Moreover, stable isotopes help to trace the fate of aquaculture waste in different communities (Gondwe et al., 2012, Vizzini and Mazzola 2012, Sanz-Lázaro and Sanchez-Jerez 2017). FA profiles provide information on food sources such as diatoms and bacteria (Kelly and Scheibling 2012).

The aim of this study was to evaluate the impact of aquaculture of the NIS bay scallop *A. irradians* on benthic ecosystem functioning. Specifically, we used stable isotopes and FAs to test the following hypotheses: (1) the presence of a scallop farm affects the isotopic values of primary organic sources in the sediment; (2) scallop faeces are consumed by meiobenthos in the receiving sediment; (3) the quantity of faeces consumption differs among taxa/families of meiobenthos; and (4) the presence of a scallop farm changes the FA profile of the harpacticoid copepods occurring in the sediment.

4.2 Materials and Methods

4.2.1 Study area

Samples were collected in scallop farms located in the eastern part of the Laizhou Bay (37°00’-38°30’ N, 118°45’-120°30’ E). Laizhou Bay is located in the south Bohai Sea, Shandong Province, on the north coast of China. It has a mean depth of 9 m (maximum ~18 m), a coastline of 320 km and a total area of ~700000 ha (Zhuang et al., 2014), and a mean water semi-exchange period of 85 days (Zhao and Chen, 2001). The area is an important spawning and breeding ground for many fishes but is currently under pressure due to human activities such as intensive mariculture (Jin et al., 2013).

The bay scallop *Argopecten irradians* is a major mariculture species, which has been cultivated using the suspended-longline method in this area for 30 yr. Bay scallop mariculture in Laizhou Bay is one of the most important sources of scallop production in China, with a total area of 500 ha and a density of 200 ind. m^{-2} yielding a production of around 20000 t yr^{-1} (Liu et al., 2004). Bay scallops are cultured from May to November each year without adding any feed. The rest of the year, the area is devoid of any aquaculture activities.

4.2.2 Sampling design

We selected 3 large scallop farms and 3 corresponding control sites. To exclude any direct effect of aquaculture on the control sediment, each control site was located 2 km to one side of the farm, at the same depth as the farm, so that it was exposed laterally to the predominant current that also flowed through the farm. Each pair of sites, ‘farm’ and ‘control’, was considered as a single station, and the 3 stations were characterized by sediment type as sandy-shallow (Stn SS) (2% clay, 24–28% silt, 69–73% sand; water depth 5 m), muddy-shallow (Stn MS) and muddy-deep (Stn MD) (4–6% clay, 53–55% silt, 39–42% sand; water depth 7.5 and 12 m respectively). Previous observations in Laizhou Bay revealed that biodeposition rates of bay scallops peaked in November, and rates of larger individuals were higher than those of smaller ones (Wang 2015). We thus conducted sampling for stable isotopes and FA analysis in October−November 2015 and 2016, before the harvest of scallops, in order to cover the period of high biodeposition rates in this area. For FA samples, an additional sampling campaign was conducted in May 2016 before the scallop lantern nets were placed in this area.

4.2.3 Sampling procedure

We considered that meiobenthos from each farm and control site were exposed to the following common primary organic sources: (1) phytoplankton from the water column, (2) microphytobenthos (MPB) in the sediment surface, and (3) fragments of seagrass *Zostera marina* leaves in the sediment (senescent fragments of *Z. marina* verified by microscopical observation). Bay scallop faeces were only considered as a potential food source at the farm sites. Phytoplankton was considered to be the major component in the pre-filtered particulate organic matter (POM) because the weight-to-weight ratio of particulate organic carbon to chlorophyll *a* (POC/chl *a*) ranged from 19 to 37 in October-November in our sampling area (Wang 2015). This is within the known range of 2 to 200 for POC/chl *a* in algae (Cifuentes et al., 1988). POM samples were obtained at each control site in order to avoid the potential addition of aquaculture-derived organic matter. About 500 ml seawater was filtered through a 58 μm net to remove zooplankton and large detritus, and subsequently filtered on pre-combusted (450°C, 4 h) 0.7 μm Whatman GF/F glass fiber filters. MPB samples were collected at each farm and control sites in 2016. Due to the insufficient MPB biomass for reliable stable isotope analysis, we pooled farm and control together for each station, which is justified by the similar $\delta^{13}C$ and $\delta^{15}N$ values of the surface sediment at each farm and control site (see below). MPB were separated from the sediment surface using a modified method of Doi et al. (2003): the top 1 cm sediment collected by a Van Veen grab was covered by a 2 mm layer of quartz sands (25 to 65 μm diameter, pre-combusted at 500°C for 2 h), a nylon net (75 μm), and another layer of 2 mm pre-combusted quartz sand. The dishes were illuminated for 24 h, while moisture was maintained with continuous spraying of filtered seawater on the sand. After illumination, the upper sand layer was scraped off and resuspended in filtered seawater. The supernatants were filtered on pre-combusted Whatman GF/F filters. We also collected sediment surface organic matter (SSOM) to evaluate the impact on the organic pool of surface sediments. SSOM was collected at each farm and control site by scraping the top layer (0 to 1 cm) of sediment from the Van Veen grab. To collect the bay scallop faeces, 20 individuals of scallop (average body length of 5.5 cm) were placed in the containers with filtered seawater overnight and faeces were obtained by filtering the water from the containers on pre-combusted GF/F filters. Triplicate samples were taken for POM, MPB, SSOM and scallop faeces.

Meiobenthos for stable isotopes and FAs were collected qualitatively by scraping the top 1 cm layer of the sediment from 3 randomly selected patches (approx. 1 × 1 m) that were at least 1 m apart at the farm and control sites.

4.2.4 Stable isotope analysis

The POM, MPB, and faeces samples were oven-dried at 60°C to a constant weight and divided into 2 subsamples. One set was treated with hydrochloric acid (HCl) fume to remove carbonates for $\delta^{13}C$, and the other, without acid treatment, was used for $\delta^{15}N$ analysis. All samples were put into tin capsules (Elemental Microanalysis, 8 × 5 mm) and pinched closed.

30 to 60 mg of sediment were acidified in silver capsules (Elemental Microanalysis, 8 × 5 mm) with diluted HCl to remove carbonates and washed with distilled water. The capsules were dried and pinched closed afterwards.

Sediments were sieved with filtered seawater through a 500 μm and a 150 μm sieve. The fraction retained on the 150 μm sieve was frozen and transported to the lab. The frozen samples were thawed and meiobenthos were handpicked with a needle under a stereomicroscope. Meiobenthos were sorted into nematodes (150 to 300 ind. per sample) and copepods. For the samples collected in 2015, copepods were pooled together (80 to 100 ind. per sample) while copepod samples in 2016 were sorted to family level (80 to 120 ind. per sample). Meiobenthos were rinsed with MilliQ water twice before being transferred to tin capsules, oven-dried overnight at 60°C and pinched closed. All samples were stored in a desiccator prior to further analysis.

C and N stable isotopes were measured with an isotope ratio mass spectrometer (type Europa Integra) at UC Davis Stable Isotope Facility

(University of California). Isotope values are expressed as $\delta^{13}C$ and $\delta^{15}N$ (‰) determined by the following equation:

$$\delta^{13}\mathrm{C}\ \text{or}\ {}^{15}\mathrm{N}=([\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}}]-1)\times 10^{3} \quad (1)$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Reference standards were PDB for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$.

4.2.5 FA profiling

FA samples included only specimens of the harpacticoid copepod family Canuellidae collected at Stn SS. The abundances of the other copepods at the other sites were too low to obtain enough biomass for a reliable FA analysis. Canuellidae were extracted alive from sediments following the method of Svensson et al. (2010) and stored at room temperature overnight to clear their gut content. The next day, 100 to 150 individuals were picked, washed with filtered seawater, and transferred to glass tubes for storage at –80°C prior to FA extraction.

Lipid extraction, methylation to fatty acid methyl esters (FAMEs), and FAME analysis followed the procedure of De Troch et al. (2012). FAME of 19:0 (Fluka 74208) was added as internal standard. The FAMEs were analyzed with a gas chromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973). FAMEs were identified by comparing the retention time and mass spectra with authentic standards and mass spectral libraries (WILEY, NITS05) and then analyzed with the software MSD ChemStation (Agilent Technologies). Individual FAMEs were quantified by using a component FAME and BAME mix (Supelco #47885 and #47080 respectively, Sigma-Aldrich) and additional standards (Larodan). Shorthand FA notations were expressed as A: BωX, where A gives the number of carbon atoms, B represents the number of double bonds and X is the position of the first double bond closest to the terminal methyl group.

4.2.6 Data analysis

Variations in $\delta^{13}C$ and $\delta^{15}N$ values of potential food sources, SSOM, and meiobenthos were tested using analysis of variance (ANOVA) followed by Student-Newman-Keuls pairwise comparisons. Prior to ANOVA, the assumption of homogeneity of variances were diagnosed with Kolmogorov-Smirnov tests and Levene's tests, respectively. Log transformations were used to meet this assumption if necessary. Non-parametric Kruskal-Wallis tests were conducted when homogeneity of variation could not be reached. Differences between farm and control at each station for $\delta^{13}C$ and $\delta^{15}N$ values of SSOM, TL, and FA indicators were assessed using *t*-tests. ANOVA and *t*-tests were conducted with the software SPSS 20.0.

To identify the difference in isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of meiobenthos between farm and control, the $\delta^{13}C$ and $\delta^{15}N$ ratios of taxon or family co-occurring in farm and control sites were plotted in the same biplot (i.e. $\delta^{13}C$ control/$\delta^{13}C$ farm and $\delta^{15}N$ control/$\delta^{15}N$ farm). Taxa or families were considered to have similar $\delta^{13}C$ or $\delta^{15}N$ values if the ratio fell within the 95% confidence interval (CI) encompassing the 1:1 correlation between farm and control isotopic values.

The TL of meiobenthos was estimated based on $\delta^{15}N$ values:

$TL_{consumer} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{base}) / \Delta^{15}N$ (2)

where $\delta^{15}N_{base}$ is the $\delta^{15}N$ of primary consumer i.e. the family of harpacticoid copepods that has the lowest $\delta^{15}N$ in each site. The $\Delta^{15}N$

of 2.3‰ for marine organisms was adopted (Zanden & Rasmussen 2001).

A mixing model MixSIR applied Bayesian method was used to calculate the potential food sources of meiobenthos (Moore & Semmens 2008). Trophic enrichment factors (TEF) of 0.3 ± 1.3‰ $\delta^{13}C$ and 2.3 ± 1.8‰ $\delta^{15}N$ were adopted for herbivores and omnivores, i.e. individuals with TL <3 (Zanden & Rasmussen 2001). Trophic enrichment factors (TEF) of 0.3 ± 1.3‰ $\delta^{13}C$ and 2.3 ±1.8‰ $\delta^{15}N$ were adopted for each trophic step (Zanden & Rasmussen 2001). The isotopic values of the seagrass *Z. marina* were adopted from Hoshika et al. (2006). We calculated isotopic data only for the year 2016 because MPB was not collected in 2015. MPB and POM were pooled as a logical group representing the microalgae-derived organic matter (Phillips et al., 2005).

A 2-way permutational multivariate analysis of variance (PERMANOVA, main test and pairwise test) and analysis of similarity (ANOSIM) were run on the relative FA profiles of Canuellidae in Stn SS in 2016. Time ('Before vs. During', where 'Before' refers to the period prior to the start of seasonal aquaculture production) and site ('Farm vs. Control') were orthogonal and fixed factors. A distance-based test for homogeneity of multivariate dispersions (PERMDISP) was used to test the homogeneity of multivariate dispersion (Anderson 2006). To visualize the degree of dissimilarity of FA composition between groups, principal coordinates analysis (PCO) was conducted based on a Bray-Curtis resemblance matrix of untransformed relative FA profiles. The contribution of individual FA to these clusters was tested by similarity percentages (SIMPER) analysis. All multivariate analyses were performed with Primer V6 (Clarke & Gorley 2006), using the PERMANOVA+ add-on package (Anderson et al., 2008).

4.3 Results

4.3.1 Stable isotopes of food sources

Scallop faeces showed enriched δ^{15}N values (9.40 ± 1.03 ‰ in 2015 and 9.44 ± 0.93‰ in 2016, Fig 4.1), which were higher than those of any other potential food source (ANOVA for 2016: $F_{6,13} = 20.999$, $p < 0.001$; 2015: $F_{3,11} = 8.666$, $p = 0.003$). For the δ^{13}C values, significant differences were found, with decreasing signatures from seagrass (*Zostera marina*) to scallop faeces to POM and MPB (Fig 4.1A). Isotopic composition of POM and MPB varied among stations in terms of δ^{15}N values in 2016 (ANOVA, POM in 2016: $F_{2,6} = 6.806$, $p = 0.029$; MPB in 2016: $F_{2,5} = 13.900$, $p = 0.009$) (Fig 4.1B). Within each station, only Stn MD and Stn SS showed significant differences in isotopic signatures. At Stn MD the MPB had more depleted δ^{15}N values compared with POM, while there were no differences at SS and MS (*t*-test, SS: $t_{1.074} = 6.157$, $p = 0.091$; MS: $t_4 = 1.622$, $p = 0.180$; MD: $t_4 = 3.715$, $p = 0.021$). At Stn SS δ^{13}C values of POM were more depleted than those of MPB, while there were no differences at MS and MD (*t*-test, SS: $t_3 = 9.203$, $p = 0.003$; MS: $t_4 = 6.908$, $p = 0.415$; MD: $t_4 = 1.050$, $p = 0.353$). The δ^{15}N values of POM at Stns SS and MS were lower in 2015 compared with those in 2016 (Fig 4.1B), and Stns SS and MD in

2015 displayed more depleted δ^{15}N than in 2016 ($F_{5,14}$ = 3.477, p = 0.030; post hoc test α = 0.014 for Stn SS and α = 0.023 for Stn MS).

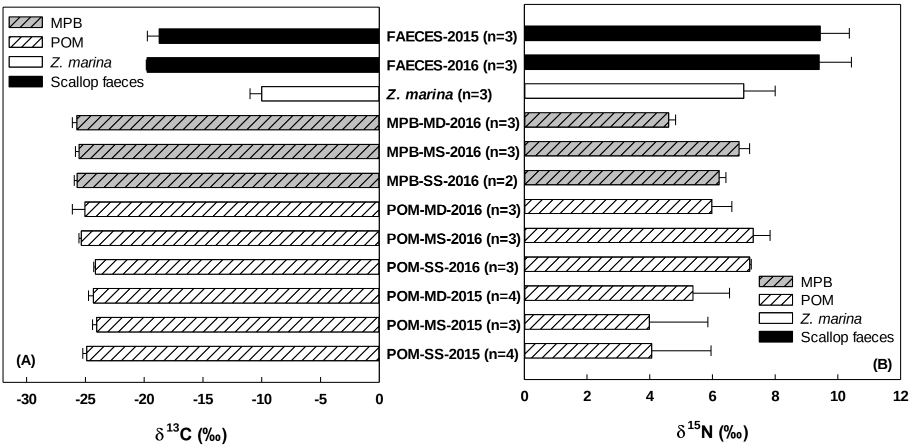

Figure 4.1. Average stable (A) carbon and (B) nitrogen isotope signatures (mean ± SD for n replicates) of primary organic food sources of meiobenthos taxa collected from 'farm' and 'control' sites at 3 stations at scallop farms in Laizhou Bay, China in 2015 and 2016. Food sources analysed were scallop faeces, seagrass *Zostera marina* (isotopic values adopted from Hoshika et al., 2006), particulate organic matter (POM) and microphytobenthos (MPB). Stations were characterized by sediment type as sandy-shallow (SS), muddy-shallow (MS) and muddy-deep (MD)

4.3.2 Stable isotopes of SSOM

Results of *t*-tests showed there was no significant difference for either δ^{13}C or δ^{15}N of SSOM between farm and control sites at each station (Fig 4.2; see also Table S1 in the Appendix at test: p > 0.05). The ANOVA test for the δ^{15}N values of SSOM at 3 stations in 2015 showed a spatial variation (ANOVA: $F_{5,12}$ = 33.338, p < 0.001) with Stn SS having slightly lower δ^{15}N values (Fig 4.2).

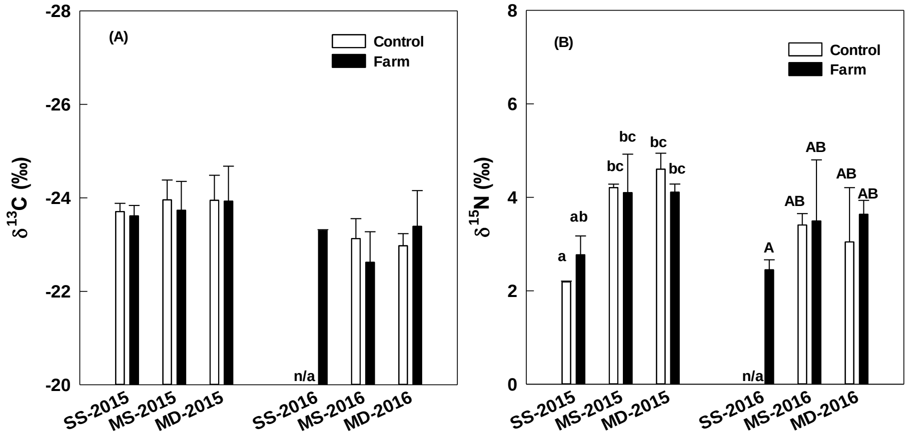

Figure 4.2 (A) δ^{13}C and (B) δ^{15}N values (mean ± SD, n = 3) of sediment surface organic matter collected from 'farm' and 'control' sites at 3 stations (SS, MS and MD) at scallop farms in Laizhou Bay, China in 2015 and 2016. See Fig 4.1 legend for abbreviations of stations. Significant differences ($p < 0.05$) among groups identified by Student-Newman-Keuls pairwise comparisons are indicated by different letters above the bars. n/d: no data (sampling failed due to practical reasons)

4.3.3 Stable isotopes and trophic level of meiobenthos

Copepods and nematodes were the major meiobenthos taxa in all stations, accounting from 8 to 15% and 45 to 92% for meiobenthos biomass respectively (authors' unpubl. data). For copepods, in total 4 families were identified: Canuellidae, Laophontidae, Ectinosomatidae, and Miraciidae. At Stns SS and MS, Canuellidae was the dominant copepod family at both control and farm sites. At Stn MD, copepods were more diverse at the farm site, where Laophontidae, Ectinosomatidae, Miraciidae, and Canuellidae were abundant; while at the control site only Laophontidae and Ectinosomatidae were abundant. C and N isotopic values varied spatially and were not always the same in the 2 sampling events, ranging from −23.02 ± 0.76 ‰ to −19.16 ±

0.20 ‰ for δ^{13}C and 8.13 ± 0.13 ‰ to 14.37 ± 0.17 ‰ for δ^{15}N (Table 4.1). Carbon and nitrogen isotopic values varied among meiobenthos taxa (in 2015, δ^{13}C: $F_{12, 31} = 31.126$, $p < 0.001$; δ^{15}N: $F_{12, 31} = 9.832$, $p < 0.001$; in 2016, δ^{13}C: $F_{13, 31} = 30.035$, $p < 0.001$; δ^{15}N: $F_{13, 31} = 8.089$, $p < 0.001$). In general, nematodes had higher δ^{15}N values compared with copepods from the same site, except for the nematodes from 2015 Stn SS that showed a slightly lower value at the farm and a similar value at the control site (Table 4.1). For the common copepod family Canuellidae, isotopic values displayed high variation (mean values ranged from −21.27 to −19.16 ‰ and from 8.13 to 12.55 ‰ for δ^{13}C and δ^{15}N respectively). Family Ectinosomatidae showed the highest δ^{15}N among copepods at the farm at Stn MD in 2016.

With regard to TL, nematodes at Stns MD and MS showed higher values than any other meiobenthos, ranging from 2.9 to 3.7 (ANOVA: F19, 47 = 6.024, $p < 0.001$). Compared with nematodes from the same site, the TL values of copepods were lower and varied among families and sites. Canuellidae always displayed the lowest TL value at each site, except for Stn SS in 2015 when nematodes had the lowest TL (Table 4.1). Laophontidae and Miraciidae showed intermediate TL corresponding to an omnivore signal (Post 2002, Maria et al., 2012). Only Ectinosomatidae from the farm site at Stn MD in 2016 showed a car-nivorous signal (TL = 3.2); the was significantly higher than at the control site at the same station (TL control = 2.4; $p = 0.004$; Table 4.1).

Table 4.1 δ^{13}C and δ^{15}N values (mean ± SD, with number of replicates shown in parentheses) and trophic levels (TL, mean) of harpacticoid copepods and nematodes from scallop farms in Laizhou Bay, China in 2015 and 2016. Samples were taken from ‘control’ and ‘farm’ sites at 3 stations, characterized by sediment type as sandy-shallow (SS), muddy-shallow (MS) and muddy-deep (MD). Results of t-tests of TL in taxa co-occurring in farm and control sites are shown. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; NS = non-significant; n/a: not applicable

			Control			**Farm**			***t*–test for TL**	
Year	**Station**	**Taxon**	**^{13}C**	**^{15}N**	**TL**	**^{13}C**	**^{15}N**	**TL**	***t* (df)**	**p-**
2016	SS	Canuellidae	–20.32 ± 0.08 (3)	11.63 ± 0.08 (3)	1.9	–20.40 ± 0.01 (4)	12.55 ± 0.03 (4)	2	0.818	0.451^{NS}
	MS	Canuellidae	–19.28 ± 0.08 (3)	8.52 ± 0.05 (3)	2	–19.50 ± 0.07 (3)	8.13 ± 0.13 (3)	2	0.088	0.943^{NS}
		Nematoda	–19.68 ± 0.12 (3)	12.04 ± 0.29 (3)	3.5	–20.21 ± 0.16 (3)	12.07 ± 0.20 (3)	3.7	1.922	0.117^{NS}
	MD	Laophontidae	–23.02 ± 0.76 (3)	8.71 ± 0.97 (3)	2	–20.98 ± 0.18 (3)	9.61 ± 0.19 (3)	2.4	1.705	0.163^{NS}
		Ectinosomatidae	–20.39 ± 0.11 (3)	9.68 ± 0.19 (3)	2.4	–19.51 ± 0.26 (3)	11.41 ± 0.48 (3)	3.2	6.014	0.004*
		Miraciidae	n/a	n/a	n/a	–19.72 ± 0.31 (3)	9.67 ± 0.25 (3)	2.4	n/a	n/a
		Canuellidae	n/a	n/a	n/a	–19.16 ± 0.20 (3)	8.63 ± 0.28 (3)	2	n/a	n/a
		Nematoda	–21.36 ± 0.21 (3)	11.83 ± 0.26 (3)	3.4	–19.88 ± 0.32 (4)	12.04 ± 0.42 (4)	3.5	0.987	0.369^{NS}
2015	SS	Canuellidae^{a}	–20.35 ± 0.42 (4)	8.98 ± 0.22 (4)	2	–20.58 ± 0.33 (4)	10.66 ± 0.11 (4)	2	1.731	0.134^{NS}
		Nematoda	–19.54 ± 0.65 (3)	8.25 ± 0.51 (3)	1.7	–19.67 ± 0.72 (4)	10.89 ± 0.31 (4)	2.1	3.820	0.012*
	MS	Canuellidae^{a}	–21.27 ± 0.83 (4)	10.36 ± 0.38 (4)	2	–20.95 ± 0.07 (4)	11.55 ± 0.11 (4)	2	0.986	0.362^{NS}
		Nematoda	–20.83 ± 0.19 (3)	13.28 ± 0.21 (3)	3.3	–20.38 ± 0.12 (3)	13.97 ± 0.09 (3)	3	3.960	0.017*
	MD	Bulk copepoda	–21.08 (1)	11.66 (1)	2	–21.45 ± 0.04 (2)	12 ± 0.4 (2)	2	n/a	n/a
		Nematoda	–20.88 ± 0.29 (2)	13.65 ± 0.01 (2)	2.9	–20.22 ± 0.07 (2)	14.37 ± 0.17 (2)	3	n/a	n/a

*Family Canuellidae represented >70% of sampled organisms per replicate

The $\delta^{13}C$ and $\delta^{15}N$ ratios of co-occurring species at control and farm sites from each station are presented in Fig 4.3. For $\delta^{13}C$, most taxa showed similar values at farm and control sites since they fell within the 1:1 correlation 95% CI. For $\delta^{15}N$, nematodes at Stns MS and MD had similar values between farm and control sites, but nematodes at the farm site at Stn SS showed a more enriched $\delta^{15}N$ compared to the control site. All copepods in 2015 and all the abundant families in 2016 (except family Canuellidae at Stn MS) showed more enriched $\delta^{15}N$ at farm sites com-pared to control sites.

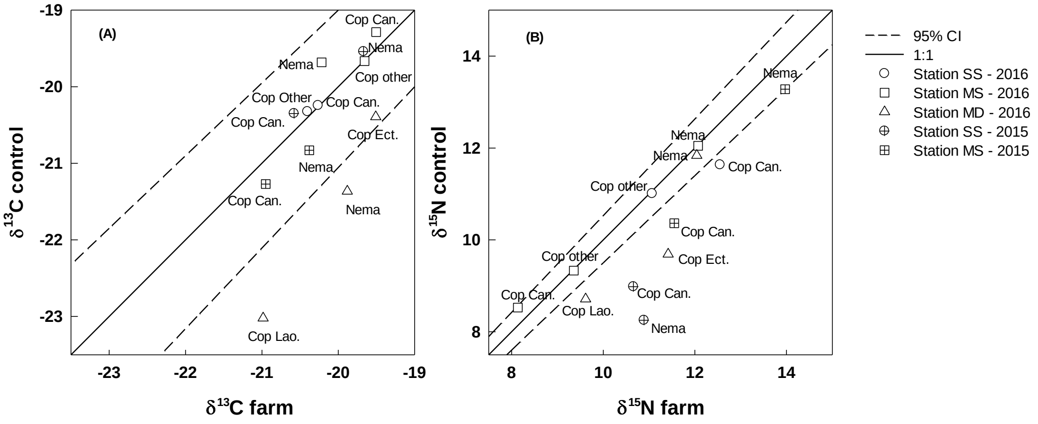

Figure 4.3 Comparison of (A) $\delta^{13}C$ and (B) $\delta^{15}N$ values for harpacticoid copepod and nematode taxa between 'farm' and 'control' sites at 3 stations (SS, MS and MD) at scallop farms in Laizhou Bay, China in 2015 and 2016. See Fig 4.1 legend for abbreviations of stations. In each panel, the solid line represents a 1:1 correlation between $\delta^{13}C$ or $\delta^{15}N$ values at farm and control sites, and the 2 dashed lines show the 95% CI. Values outside the 95% CI are significantly different. Nema: nematodes; Cop Can.: cope-pod family Canuellidae; Cop Ect.: copepod family Ectinosomatidae; Cop Lao.: copepod family Laophontidae; Cop other: rest of the pooled copepods. In 2015, all copepods are pooled but still represented by the dominant family: Canuellidae, representing > 70% per replicate

4.3.4 Mixing model estimation of utilization by meiobenthos

In general, faeces-derived materials were utilized by many copepods under the farms, contributing 12 to 61%, 13 to 60%, 44 to 83%, and 49 to 83% to the diets of Miraciidae, Laophontidae, and Ectinosomatidae at Stn MD, and Canuellidae at Stn SS, respectively (Table 4.2). However, the quantities of faeces consumed by copepods differed among the stations. For Canuellidae, scallop faeces were predominantly consumed at the farm site at Stn SS (contributing at least almost half of their diets), while faeces were less important at the farm sites at Stns MS and MD (Table 4.2, Fig 4.4). By contrast, nematodes at Stns MS and MD consumed very little faeces-derived material (<1%).

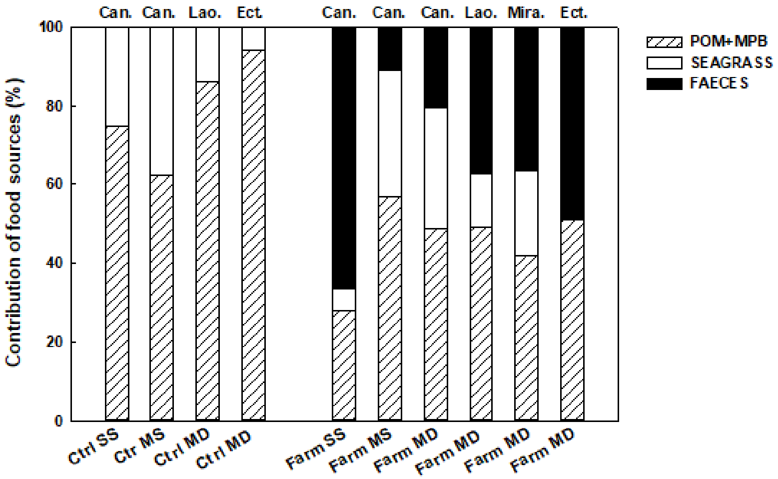

Figure 4.4 Proportion of mean contributions of food sources to different families of harpacticoid copepods collected from 'farm' and 'control' sites at 3 stations (SS, MS and MD) at scallop farms in Laizhou Bay, China in 2016. See Fig 4.1 legend for abbreviations of stations. Food sources analysed were scallop faeces, seagrass *Zostera marina*, and particulate organic matter plus microphytobenthos (POM+MPB). Can.: Canuellidae, Lao.: Laophontidae, Mira.: Miraciidae, Ect.: Ectinosomatidae

Table 4.2 Contribution (mean, with 95% CIs in parentheses) of particulate organic matter plus microphytobenthos (POM+MPB), seagrass and faeces in the diet of meiobenthos taxa collected from 'farm' and 'control' sites at 3 stations at scallop farms in Laizhou Bay, China in 2015 and 2016. See Table 4.1 legend for abbreviations of stations. The values were calculated using the Bayesian stable isotope mixing model (MixSIR). n/a: not applicable

Station	Site	Taxon	POM+MPB	Seagrass	Faeces
SS	Control	Canuellidae	0.75 (0.68–0.82)	0.25 (0.17–0.32)	n/a
	Control	Other Copepoda	0.73 (0.64–0.81)	0.27 (0.19–0.36)	n/a
	Farm	Canuellidae	0.28 (0.14–0.42)	0.05 (0–0.12)	0.66 (0.49–0.83)
	Farm	Other Copepoda	0.41 (0.22–0.62)	0.12 (0.02–0.22)	0.45 (0.16–0.71)
MS	Control	Canuellidae	0.62 (0.57–0.67)	0.38 (0.32–0.43)	n/a
	Control	Other Copepoda	0.64 (0.59–0.70)	0.35 (0.29–0.40)	n/a
	Control	Nematoda	1 (0.99–1)	0 (0–0.01)	n/a
	Farm	Canuellidae	0.57 (0.44–0.66)	0.32 (0.24–0.39)	0.11 (0.01–0.3)
	Farm	Other Copepoda	0.51 (0.33–0.64)	0.27 (0.16–0.36)	0.21 (0.02–0.48)
	Farm	Nematoda	1 (0.99–1)	0 (0–0.01)	0 (0–0.01)
MD	Control	Laophontidae	0.86 (0.79–0.92)	0.14 (0.07–0.20)	n/a
	Control	Ectinosomatidae	0.69 (0.62–0.75)	0.31 (0.24–0.37)	n/a
	Control	Nematoda	1 (0.98–1)	0 (0–0.01)	n/a
	Farm	Laophontidae	0.49 (0.33–0.64)	0.13 (0.03–0.23)	0.37 (0.13–0.60)
	Farm	Ectinosomatidae	0.23 (0.09–0.36)	0.13 (0.03–0.22)	0.64 (0.44–0.83)
	Farm	Miraciidae	0.42 (0.25–0.57)	0.22 (0.10–0.31)	0.36 (0.12–0.61)
	Farm	Canuellidae	0.49 (0.34–0.60)	0.31 (0.21–0.39)	0.20 (0.02–0.42)
	Farm	Nematoda	0.98 (0.95–1)	0 (0–0.01)	0.01 (0–0.04)

4.3.5 Fatty acid profiles of the copepod family Canuellidae

There was a significant difference in FA profiles over time (PERMANOVA, $p < 0.01$; ANOSIM, $R > 0.75$; Table 4.4), indicating that the FA profile of Canuellidae changed in time. In line with this, the PCO showed a grouping for samples before aquaculture activity (T0) and the ones collected during aquaculture activity (T1) (Fig 4.5). Based on the results of SIMPER, DHA contributed the most to this dissimilarity (29.12%). Before aquaculture activity, 16:0 was the major component of the total FA, contributing 46.1 and 47.0% of the total FA of family Canuellidae at farm and control sites respectively, while DHA was the most important FA during farming activities, contributing 39.01 and 29.38% at farm and control sites respectively (Table 4.3).

Table 4.3 Relative fatty acid composition of the harpacticoid copepod family Canuellidae from 'farm' and 'control' sites at Stn SS (see Table 4.1 legend for abbreviation) at a scallop farm in Laizhou Bay, China before and during seasonal aquaculture in 2016. Values for 'during aquaculture' are mean ± SD, with the number of replicates given in parentheses. ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; -: below detection limit.

	Before aquaculture (T0)		**During aquaculture (T1)**	
Fatty acid	**farm**	**control**	**farm**	**control**
14:0	6.61 (1)	7.52 (1)	1.03±0.15 (4)	1.63±0.59 (3)
15:0	5.95 (1)	6.67 (1)	0.88±0.12 (4)	1.18±0.19 (3)
16:0	46.1 (1)	47.0 (1)	14.67±1.19 (4)	20.54±3.80 (3)
16:1ω7	-	-	3.34±0.55 (4)	3.91±0.73 (3)
17:0	7.09 (1)	7.4 (1)	3.41±0.14 (4)	3.00±0.13 (3)
17:1ω7	-	-	-	0.06±0.11 (3)
18:0	27 (1)	25.6 (1)	12.47±0.43 (4)	17.60±1.09 (3)
18:1ω9t	-	-	2.62±0.83 (4)	2.79±0.92 (3)
18:1ω9c	-	-	4.90±0.14 (4)	4.25±0.75 (3)
18:2ω6c	-	-	1.03±0.69 (4)	1.30±0.13 (3)
20:0	1.53 (1)	1.01 (1)	0.50±0.08 (4)	0.50±0.04 (3)
18:3ω3 ALA	-	-	0.77±0.08 (4)	0.74±0.07 (3)
20:1ω9	-	-	2.05±0.19 (4)	1.30±0.02 (3)
20:2ω6	-	-	0.38±0.02 (4)	-
22:0	2.01 (1)	1.49 (1)	0.83±0.01 (4)	0.56±0.04 (3)
20:3ω6	-	-	-	0.16±0.28 (3)
22:1ω9	-	-	0.13±0.26 (4)	-
23:0	0.96 (1)	0.74 (1)	0.61±0.02 (4)	0.25±0.22 (3)
20:5ω3 EPA	-	0.45 (1)	9.82±0.64 (4)	9.77±1.45 (3)
24:0	2.23 (1)	1.62 (1)	0.93±0.03 (4)	0.60±0.02 (3)
24:1ω6	-	-	0.51±0.03 (4)	0.38±0.36 (3)
22:6ω3 DHA	-	-	39.01±1.84 (4)	29.38±3.59 (3)

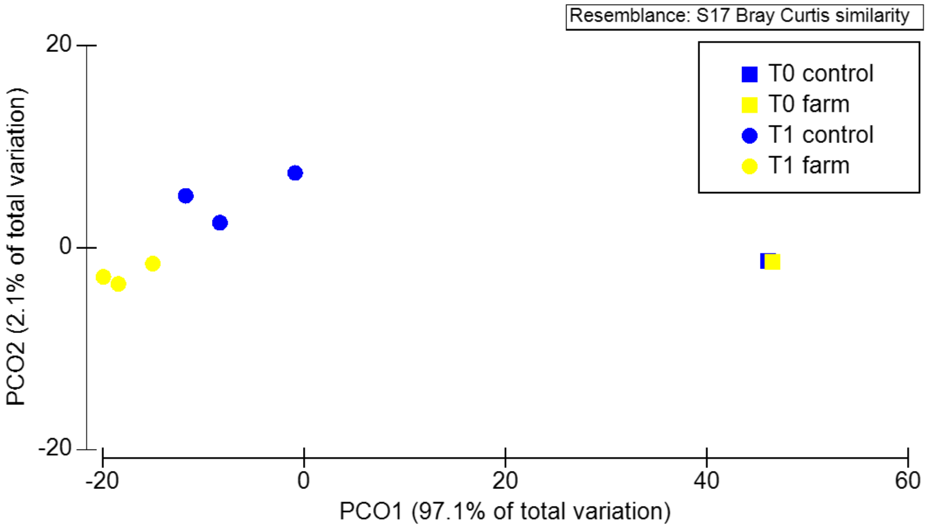

Figure 4.5 Principal Coordinates Analysis (PCO) of the relative fatty acid composition of the harpacticoid copepod family Canuellidae in 'farm' and 'control' sites at Stn SS (see Table 4.1 legend for abbreviation) at a scallop farm in Laizhou Bay, China before (T0) and during (T1) seasonal aquaculture in 2016.

In addition, a seasonal effect contributed to this pattern: significant differences were found between before and during aquaculture ('Before vs. During') within control and farm sites (see pair-wise PERMANOVA tests in Table 4.4).

Table 4.4 Effect of time (‘Before vs. During’ seasonal farming production) and site (‘Farm vs. Control’) on fatty acid profiles of Canuellidae at scallop farms in Laizhou Bay, China, sampled at Stn SS (see Table 4.1 legend for abbreviation) in 2016. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; NS = not significant. –: no testing possible due to insufficient number of replicates

Main test	**PERMANOVA**				**ANOSI**
source of variation	**d**	**MS**	**F(p)**	**p**	**R**
Time: Before vs	1	0.365	206.9	0.008**	1^{*}
Site: Farm vs Ctrl	1	0.006	3.51	0.094^{NS}	0.926^{*}
Time*site	1	0.006	3.569	0.086^{NS}	
Residuals	5	0.002			

Pairwise test	Group	*t*	p (perm)
Within "Ctrl"	Before vs	6.445	0.018^{*}
Within "Farm"	Before vs During	18.205	$<0.001^{***}$
Within "Before"	Farm vs Ctrl	-	-
Within "During"	Farm vs Ctrl	3.894	$<0.001^{***}$

More importantly, the difference ‘Before vs During’ was more significant within ‘Control’ than within ‘Farm’ ($p < 0.05$ and $p < 0.001$ respectively; Table 4.4), demonstrating that aquaculture made a crucial contribution to the FA profile of Canuellidae at Stn SS. Pairwise PERMANOVA also showed that farm and control sites strongly differed during the aquaculture stage (‘Farm vs. Control’ with ‘During’: $p < 0.001$; Table 4.4), though more replicates were needed to confirm this pattern in a pairwise test between farm and control within ‘Before’.

When excluding the seasonal effect, SIMPER showed that DHA contributed 33.71% to the difference between farm and control sites during farming. During aquaculture at Stn SS, Canuellidae at the farm site had higher amounts of DHA and PUFAs than at the control site (Fig 4.6, Table 4.3, Table S2 in the Appendix). 20:1ω9 and PUFA/SFA, considered as the indicators of carnivorous diet (Stevens et al., 2004), showed higher values in Canuellidae at farm sites during aquaculture at Stn SS. The trophic marker of diatoms/dinoflagellates, i.e. EPA/DHA (Cripps and Atkinson 2000), was lower at farm sites, indicating the different diets of Canuellidae at farm sites compared to those at control sites. Other trophic FA biomarkers showed no difference between farm and control sites (Fig 4.6; see also Table S2; $p > 0.05$).

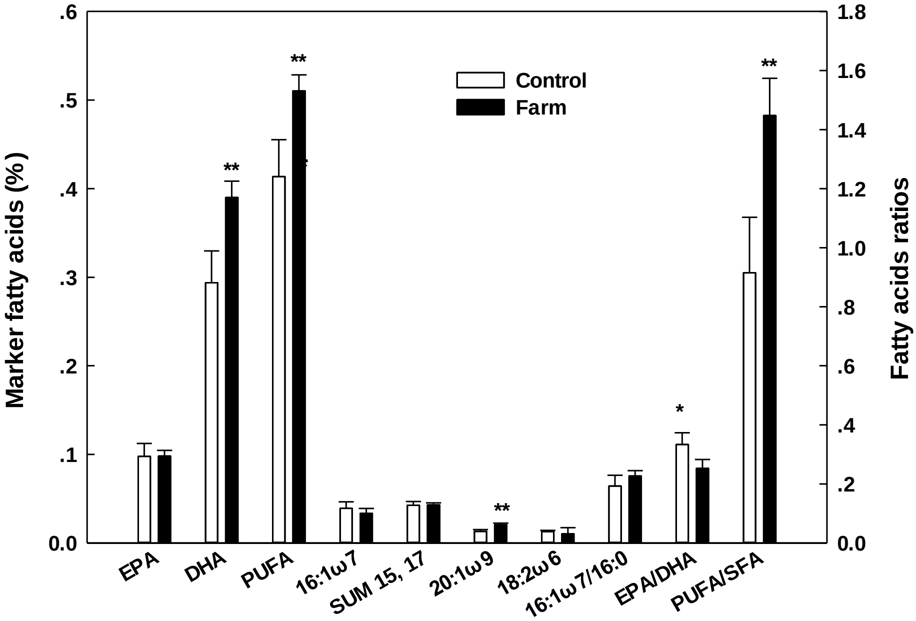

Figure 4.6 Marker fatty acids (FA), with values shown for relative FA composition and FA ratios, in the harpacticoid copepod family Canuellidae at 'farm' and 'control' sites at Stn SS (see Table 4.1 legend for abbreviation), during seasonal aquaculture at a scallop farm in Laizhou Bay, China in 2016. Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and polyunsaturated fatty acids (PUFA) are indicative of food quality. Other biomarkers shown are: 16:1ω7 and 16:1ω7/16:0 (indicative of diatoms in the diet); EPA/DHA (diatoms/dinoflagellates); SUM 15,17 (i.e. the sum values of 15:0, 17:0, 17:1ω7 in this study; indicating bacterial FAs); 18:2ω6 (terrestrial detritus or green algae); 20:1ω9 and PUFA/SFA ratio (indicative of carnivory). Asterisks show significant differences between values for 'farm' and 'control' based on the results of t-tests: **$p < 0.01$; *$p < 0.05$

4.4 Discussion

4.4.1 The fate of bay scallop biodeposits in aquaculture area

Stable isotopes analysis has been applied to trace the fate of aquaculture waste in several studies (Kon et al., 2009, Gondwe et al., 2012, Callier et al., 2013), since the aquaculture-derived waste has distinctive isotopic values. In our study, the enriched $\delta^{15}N$ of bay scallop faeces compared to other organic sources was a prerequisite in order to be able to trace its fate. Contrary to some studies that demonstrate the accumulation of aquaculture waste in the sediment organic matter (OM) pool, SSOM in our study did not show the enrichment of ^{15}N by scallop farming activity. This could be explained by 2 hypotheses. First, the aquaculture-derived OM was diluted in the water column and thus did not sink to the sea floor (Vizzini and Mazzola 2012). Second, the local consumers rapidly incorporated the aquaculture-derived matter (Kon et al., 2009). As we found more enriched $\delta^{15}N$ values of most copepods and some nematodes in the sediment under the farms (see 'Effect of bay scallop aquaculture on the diets of meiobenthos'), the second explanation is more plausible. Callier et al. (2013) reported a similar outcome; i.e. isotopic values shifted in invertebrates rather than in SSOM. Taken together with the results of our study, this suggests that analyzing the response of the benthos (i.e. a biotic response) to evaluate the effect of aquaculture will provide a more comprehensive view than investigating only the response of the sediment (i.e. an abiotic response).

4.4.2 Biodeposits of bay scallop as food sources to meiobenthos

The resource utilization of meiobenthos appears to be a promising tool to evaluate the effect of aquaculture effluents on ecosystem functioning in terms of energy flow (Kennedy and Jacoby 1999, De Troch et al., 2013). The incorporation of scallop faeces into the diets of most harpacticoid copepods at all stations and nematodes in the sandy station under the farms demonstrates that bay scallop farming changed the energy flow in the basal part of the food web. This evidence agrees with findings of other studies that aquaculture effluents serve as alternative food sources for the benthos (Dubois et al., 2007, Callier et al., 2013).

The flux of OM is usually enhanced in the bivalve mariculture area (Newell 2004, McKindsey et al., 2011); consequently, the food availability for benthic organisms increases. Moreover, in terms of food source profitability, biodeposits are considered to be of good nutritional value because of their high carbon and nitrogen contents, large proportions of labile OM, and the low C/N ratios (Kautsky and Evans 1987, Miller et al., 2002). Biodeposits are mucus-enriched and function as good substrates for bacteria (Hargrave 1976). Thus, abundant microorganisms colonize on the pellet particles during gut passage (Werry and Lee 2005, Cnudde et al., 2011) and after defecation (Fabiano et al., 1994), and efficiently rework the labile components within hours to days (Carlsson et al., 2010). They break down the refractory OM from the faecal pellets and also produce microbial nutrients (e.g. extracellular protein and exudates) that make them available to other benthic organisms (Kautsky and Evans 1987, Wotton and Malmqvist 2001).

Furthermore, increasing densities of microorganisms (e.g. bacteria, ciliates and dinoflagellates) themselves provide more foods for meiobenthos (Epstein 1997, Moens and Vincx 1997). Therefore, biodeposit-derived materials can easily be consumed by meiobenthos and enter the basal food web.

4.4.3 Effect of bay scallop aquaculture on the diets of meiobenthos

While aquaculture-derived OM is a nutritional food source (McKindsey et al., 2011, Callier et al., 2013), the quantities and pathways of consumption by benthos vary among trophic groups and feeding modes (Dubois et al., 2007, Wai et al., 2011). In accordance with the large trophic diversity of meiobenthos in coastal areas (Hicks and Coull 1983, Jensen 1987), we found that the quantities and pathways of faeces consumption differed according to the feeding behaviors of the meiobenthos. This was clearly illustrated by the harpacticoid copepod families. Substrate browsers, like Laophontidae and Miraciidae, possibly took faecal OM through scraping or sweeping off the attached bacteria (Hicks and Coull, 1983, Cnudde et al., 2013, Mascart et al., 2013). For Canuellidae, faecal OM were presumably assimilated by filtering-feeding the small particles suspended in the water column (Cnudde et al., 2015). With the provision of bacteria and protists on the biodeposits (Wotton and Malmqvist 2001, Bongiorni et al., 2005), Ectinosomatidae showed an increased TL, suggesting that the feeding tendency of this taxon changed from omnivorous to carnivorous. Carnivory predation on protists (e.g. ciliates) has been recorded in family Ectinosomatidae (Coull and Dudley, 1976, Seifried and

Dürbaum, 2000). It is possible that the feeding behavior of Ectinosomatidae had been modified by scallop farming, since the protists could be facilitating by biodeposits (Mirto et al., 2000, La Rosa et al., 2001, Bongiorni et al., 2005).

In addition, the copepod family Canuellidae demonstrated that the effect of bay scallop biodeposition depends on the receiving environment. With a broad range of niche breadth (De Troch et al., 2003), Canuellidae could switch their diets to more nutritious organic sources such as scallop biodeposits if other sources were insufficient, for instance in a sandy bottom with low chlorophyll levels and poor organic resources (Cartaxana et al., 2006). At muddy stations, the small contributions of biodeposits to Canuellidae may be explained by the greater availability of autotrophic production.

As for nematodes, their TLs indicate that the communities were different according to the sediment type. Nematodes at muddy stations were carnivores and did not incorporate faeces-derived materials into their diets. The data suggest that even when biodeposits were present, they did not modify their feeding mode i.e. they were either consuming bacteria derived from MPB extracellular polymeric substrate (EPS) or predating on small nematodes that fed on EPS of MPB (Moens et al., 2005, Rzeznik-Orignac et al., 2008, Majdi et al., 2012). It is also possible that this group of nematodes resided deeper and could not access the biodeposits, which had either already been consumed by surface locating copepods or were not buried deep enough into the sediments. In this case, bay scallop farming has no impact on the resource utilization by nematodes that are trophic specialists. In contrast, nematodes at the sandy station incorporated biodeposits under the scallop farm. Microvory of nematodes has been reported to be dominant below a mussel farm (Netto and Valgas 2010), where they presumably benefit from higher microbial densities by organic loading (Mirto et al., 2000). In our study, the feeding group of nematodes was likely changed by bay scallop farming.

4.4.4 Effect of bay scallop aquaculture on the quality of harpacticoid copepods for higher trophic levels

PUFA concentrations, and especially those of DHA increased in Canuellidae (the most abundant harpacticoid copepods) as they consumed certain amounts of scallop faeces at farm sites during the high biodeposition stage. PUFAs are important compositions of cell membranes and are needed in animals at all taxonomic levels, including copepods (Ederington et al., 1995, Hartwich et al., 2013). Especially at low temperature, PUFAs promote membrane fluidity (Farkas 1979, Stillwell and Wassall 2003). Also, with higher levels of PUFA, copepods have higher reproduction ability in terms of eggs production (Ederington et al., 1995). In our study, as the winter had just started during our sampling season, we hypothesize that the higher contents of PUFA provided Canuellidae with better opportunities to reproduce and deal with the low temperature and food-stress periods.

Furthermore, PUFAs, EPA and DHA have been recognized as good biomarkers to describe the quality of food (Boon and Duineveld 1996, Goedkoop et al., 2000). As higher-level consumers in marine ecosystems cannot synthesize highly unsaturated fatty acids (HUFAs) such as DHA, their HUFA levels are derived entirely from their food sources (Iverson 2009, Hartwich et al., 2013). As harpacticoid copepods are important food items for epibenthic organ-isms such as shrimps and juvenile fishes (Coull et al., 1995), their quality in terms of PUFA or HUFA levels becomes an important factor influencing the quality of higher consumers as a food source and thus enriching the entire food web. Thus, the fact that Canuellidae consuming biodeposits of bay scallops contained more PUFAs (especially DHA) implies that they constituted a more nutritious food item for higher level consumers.

However, the mechanisms behind the increasing levels of PUFA (especially DHA) are not clear yet because the pathways to accumulate DHA are complicated and depend on several factors (Bell and Tocher 2009, Werbrouck et al., 2016). Copepods may gain DHA directly from the food or synthesize DHA from shorter chain of PUFAs (Schlechtriem et al., 2006, De Troch et al., 2012). It has been suggested that organisms using 'foreign foods' (i.e. foods not originating from their habitat) were not accustomed to these, and this might have stimulated the compensatory biochemical pathways (Iverson 2009). It is possible that loading of biodeposits induces the pathway to accumulate PUFAs and DHA in Canuellidae. To further elucidate the role of faeces in the eco system functioning, there are many options for future investigation for example to determine whether, how, and to what extent aquaculture-derived PUFAs is transferable through the food chain.

4.4.5 Bay scallop as a non-indigenous species for local environment: a positive perspective

It is always a concern that culturing NIS, especially non-indigenous bivalves, in coastal marine ecosystem, includes ecological risks (Newell 2004, Shelton and Rothbard 2006, Minchin et al., 2009). Our study showed that, to some extent, farming the non-indigenous bay scallop provides an extra food source for the benthos and consequently improves the quality of the local benthic environment and the basal food web. This suggests that the farming bay scallop has a positive effect on ecosystem functioning. To make a more complete assessment of NIS aquaculture, further research is needed to compare the effects with those of culturing native species.

Our observations may also apply to other bivalve farms, but additional factors should be considered, such as aquaculture characteristics (e.g. cultured species, stocking densities, etc.), and the hydrodynamics and sediment type of the receiving environment, because these can also affect the activities of the benthos (Chamberlain et al., 2001, Giles et al., 2006).

4.5 Conclusions

Our study showed that stable isotopes, especially $\delta^{15}N$, are a powerful tool to trace possible functional changes due to aquaculture activities. We also showed that the biodeposits were directly/indirectly consumed by meiobenthos, and accordingly, harpacticoid copepods residing under the scallop farms improved their quality as food items for the next trophic level. Therefore, we demonstrate a positive effect of intensive farming of bay scallop *Argopecten irradians* on ecosystem functioning.

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Chapter 5: The role of meiobenthos in the functioning of *Marsupenaeus japonicus* and *Litopenaeus vannamei* earthen ponds: Benthic assemblages and food webs

Abstract

Shrimp farming in earthen pond is by far one of the largest aquaculture industries among coastal mariculture, but has faced environmental issues in recent years, e.g. overloading of supplementary feed cause deterioration of pond environments. In order to assess the impact of pond shrimp farming on the benthic environment, we investigated meiobenthic community structure before aquaculture as well as in the early and late rearing stages in two types of earthen ponds - *Marsupenaeus japonicus* (native species) ponds and *Litopenaeus vannamei* ponds. We found meiobenthic community structure to change over the different rearing stages due to an increase in nematode densities, which were closely associated with shrimp farming activity in both types of ponds. We found nematode densities to be closely associated with shrimp culture in both ponds, which led to different meiobenthic community structure through time. We also investigated the functional roles of natural productivity (i.e. microalgae and meiobenthos) and supplementary feed by studying the benthic food webs in both types of shrimp ponds in the early and late rearing stages, with special emphasis on meiobenthos. Natural productivity contributed abundantly to *M. japonicus* and *L. vannamei*' diets in different forms between stages. Meiobenthos in the early stage, shared similar diets with shrimps (with both feeding on primary organic sources), potentially competing for resources. Due to the ontogenetic diet shift in shrimp diet, at late rearing stages, meiobenthos represented however constituted a functional link between primary producers and shrimps, serving as nutritional food

source providing high amounts of polyunsaturated fatty acids (PUFA) to shrimps. Supplementary feed was not consumed abundantly by both shrimp species, but instead fueled the benthic food web through other pathways and consequently promoted secondary productivity.

5.1 Introduction

To meet the demands for shrimp consumption worldwide, farmed shrimp production has globally increased by 33% from 2010 to 2015 (FAO, 2016). China is the world leader in shrimp farming industry, accounting for more than 40% of the world production in 2015 (FAO 2016). In China and worldwide, shrimp farming is largely conducted directly on the natural basin (the so-called earthen pond), a farming technique which is characterized by the high abundances of natural established communities (i.e. natural productivity), with periodic use of fertilizers and various types of supplementary feed (Gamboa-Delgado, 2014; Xie and Yu, 2007).

The expansion of shrimp farming has been linked to considerable environmental concerns (Bondad-Reantaso et al., 2005; Naylor et al., 1998; Yang et al., 2017). One of the major problems is the accumulation of nutrients, which derived from the high density of the farmed shrimps and excessive feed input (Martinez-Cordova et al., 1998; To, 2016). The unconsumed feed and the animal wastes with high concentrations of organic carbon and nitrogen remain in the pond water and sediment, initiating anoxic metabolic pathways that affect the reproduction, survival, growth and finally the production of the shrimps (Boyd, 2015; Boyd and Clay, 1998).

As the environment of the pond bottom (i.e. the benthic environment) is important to the benthic life habit of shrimps (Boyd and Clay, 1998; Pine et al., 2018), assessment of the state and changes in the benthic environment is crucial to shrimp farming management. The benthic organisms, such as meiobenthos (benthic invertebrates that pass through a 38 µm sieve but are retained on a 0.5 mm sieve), are efficient indicators of changes in the benthic environment, because of their sensitive response to environmental disturbance (Kennedy and Jacoby, 1999; Moreno et al., 2008). Meiobenthic community structure at higher taxon level have been widely applied in assessing the impact of open-water aquaculture, e.g. finfish and bivalve farming (Grego et al., 2009; Mazzola et al., 2000; Mirto et al., 2012), but fewer studies have examined the response of meiobenthos to shrimp farming (Hena et al., 2011; Patrona et al., 2012).

Another key issue in shrimp farming is the optimization of food and feeding strategies (Casillas-Hernández et al., 2007), since overfeeding has been demonstrated to be economically inefficient and has also been shown to degrade the environmental quality of shrimp ponds (Burford et al., 2004; De Silva and Hasan, 2007; Martinez-Cordova et al., 1998; Reymond and Lagardère, 1990). One way to improve the feeding strategy and avoid overfeeding is to take into account the feeding ecology of shrimps within the benthic food web (Burford et al., 2004; Rothlisberg, 1998). While earthen ponds largely depend on the addition of supplementary feed, the role of natural productivity is often underestimated (Martinez-Cordova et al., 1998; To, 2016). Many shrimp species have been reported to preferentially feed on natural food sources, e.g. microalgae and (meio)benthos, rather than on supplementary feed (Burford et al., 2004; Rothlisberg, 1998). In particular, meiobenthos represents a nutritional food source for higher consumers, providing nutrients which are essential to the growth of shrimps, such as polyunsaturated fatty acids (PUFAs, Brett and Muller-Navarra, 1997; Lavens and Sorgeloos, 2000). Conversely, shrimps may compete with macrobenthos that shares a similar feeding strategy, i.e. grazing on primary/secondary producers (Gee, 1989; Leduc and Probert, 2009). Thus, the clarifying the functional role of meiobenthos in earthen pond food webs can provide crucial information to improve the feeding strategy in shrimp farming.

The trophic interactions in earthen ponds are complex, relating to the species cultured, stocking densities, culture stage etc. (Gamboa-Delgado, 2014; Moriarty, 1997), which represents a challenge for assessing the role of natural productivity and supplementary feed in shrimp farming. The application of up-to-date biomarkers such as stable isotopes and fatty acids can help to unravel complicated trophic interactions, especially among organisms of small size (Cnudde et al., 2015), and such biomarkers are being gradually incorporated into aquaculture studies (Gamboa-Delgado et al., 2013; Gatune et al., 2012; White et al., 2017).

Here, we studied the functional role of meiobenthos in two popular types of earthen shrimp ponds (the native shrimp *M. japonicus* and the non-indigenous shrimp *L. vannamei*) in Dongying, along the Bohai Bay, China. We aimed to: (1) evaluate the influence of shrimp farming on the benthic environment by analyzing the structural response of meiobenthos at higher taxon level, (2) investigate if the functional response of meiobenthos to shrimp farming differed depending on the culture periods and (3) identify the trophic relationships among shrimp feeds, primary/secondary producers, meiobenthos and shrimp, to unravel the benthic food web structure in shrimp ponds. We hypothesized that (1) different patterns of temporal variability in meiobenthic community structure can be observed in the two types of shrimp ponds; (2) the functional role of meiobenthos depends on the rearing stages in the shrimp ponds; (3) natural productivity (i.e. microalgae and meiobenthos) plays a more important dietary role for shrimps than supplementary feeds.

5.2 Materials and methods

5.2.1 Study sites and sample collection

The study was conducted in the Yellow River delta, at the south-west coast of the Bohai Sea, Dongying, Shandong Province, China (38°00’26.81 N, 118°33’57.61 E). Farming of *Marsupenaeus japonicus* (native species) and *Litopenaeus vannamei* (non-indigenous species) in earthen pond has been practiced in the area for at least 5-10 years and at least 10 years, respectively. Prior to the culture activity in April, ponds are dried out, sterilized with calcium oxide and prepared by rotenone to kill the predators of shrimps. Commercial hatcheries rear shrimps to post-larvae stage (mean total length 18 mm) and transfer them into ponds at a density of 3-5 shrimps m^{-2} for *M. japonicus* and 15-30 shrimps m^{-2} for *L. vannamei*. Both species are cultured from May to August. Another culture crop is usually applied after August until November. After that, the ponds stay empty until next April. More details about the *M. japonicus* and *L. vannamei* farming can be found in

Chapter 1 Section 1.5.2.

M. japonicus and *L. vannamei* ponds are characterized by different feeding practices. In *M. japonicus* ponds, commercially raised amphipods (*Corophium*) are added to the ponds during the first 30 days (i.e. the early stage). Once established (i.e. release and breeding successfully) in the ponds, the amphipods are expected to serve as a food source to adult *M. japonicus*. The addition of wet feed (small fishes and mollusks) occurs when the shrimps reach a size of 40-50 mm (i.e. late stage). In the early stage of *L. vannamei* ponds, juvenile shrimps are typically fed with smashed soy-bean and wet feed of frozen *Artemia*, whilst in the later rearing stages the diet is shifted to formulated pellet feed.

We collected two types of samples: First, to identify the structural response of meiobenthos to the shrimp farming, triplicate sediment cores (3.6 cm inner diameter, 10 cm^{-2} surface area) were taken by a hand-operated core sampler in three *M. japonicus* ponds (NAT P1, NAT P2 and NAT P3) and three *L. vannamei* ponds (NIS P4, NIS P5 and NIS P6) at three occasions: before aquaculture - January 2016 (sampling for NAT P3 and NIS P6 was failed), early stage - May 2016; shrimp body length between 15 and 30 mm), and late stage - August 2016; shrimp body length between 70 and 80 mm). Second, to investigate the benthic food web in the two types of ponds, the following samples were collected for stable isotopes analysis: middle-layer pond waters for the particulate organic matter (POM); the top sediment layer (0-1 cm) for the microphytobenthos (MPB), meiobenthos, and *Corophium*; feeds of shrimps, and shrimps. Fatty acid samples (see 5.2.3) were obtained from the meiofauna collected at 0-1 cm sediment depth. The samples to analyse the benthic food web were collected at the sampling sites NAT P1, NAT P2, NIS P1 and NIS P2 during the early and late stages of the aquaculture activity.

The basic background information, amount of feeding input and environmental parameters in the water column and sediment of our sampled ponds are listed in Chapter 2 (Table 2.1, Table 2.2 and Appendix I Table S2). There was no and at most once water renewal in the early stage for *M. japonicus* and *L. vannamei* ponds, respectively. In the late stage, at most 30% of the water was exchanged once a month for *M. japonicus* ponds and 10-20% of the water was exchanged 2-4 times per month for *L. vannamei* ponds.

5.2.2 Sample treatment

The top layer of the sediment cores (0-1 cm) from each pond was sliced off to study the structure of the meiobenthic community. The top sediments were preserved in a 4% formaldehyde – filtered tap water solution and washed with filtered tap water through 500 μm and 38 μm sieves in the lab. The fractions retained on the 38 μm sieves were centrifuged three times in Ludox HS40 with a density of 1.18 g cm^{-3} and stained with Bengal Rose (density: 0.5 g L^{-1}). Meiofauna was identified under a binocular microscope at higher taxon level.

About 350-500 mL pond water for POM was filtered onto pre-combusted (450 °C, 4h) 0.7 μm Whatman GF/F glass fiber filters. Microphytobenthos (MPB) samples were separated from the sediment surface using a modified method after Doi et al. (2003) and transferred onto pre-combusted Whatman GF/F filters (See Huang et al. 2018 for more details). The filters were oven-dried at 60 °C to a constant weight and divided in two subsamples, one of which was treated with hydrochloric acid fume to remove carbonates for $\delta^{13}C$, the other was prepared without acid treatment for $\delta^{15}N$. Shrimp feeds were dried (60 °C) and grounded. All the above samples were put into tin capsules (Elemental Microanalysis, 8×5 mm). To separate the meiobenthos (including the live feed *Corophium*) from the sediments, sediments were sieved with filtered pondwater through 500 µm and 150 µm sieves. The fractions retained on the 150 µm sieves were frozen and transported to the lab. The frozen samples were thawed and nematodes (150-300 individuals per sample; n=2-4), copepods (80-120 individuals per sample; n = 2-4) and *Corophium* (9-12 individuals per sample; n = 3) were handpicked with an eye-shaped needle under a binocular microscope. The above organisms were rinsed with MilliQ water twice before transfer to the tin capsules and oven dried overnight at 60 °C, and pinched closed. The samples were stored in desiccator prior to further stable isotope analysis.

Meiofauna (including *Corophium*) for fatty acid analysis was extracted alive from sediments. The extraction of nematodes and copepods followed the method of Mangubhai and Greenwood (2004) and Svensson et al. (2010), respectively. *Corophium* were directly hand-picked using an eye-shaped needle under a binocular microscope. The above organisms were stored at room temperature overnight to clear the gut contents. The following day, nematodes (200-300 individuals per replicate), copepods (100-150 individuals per replicate), and *Corophium* (30-40 individuals per replicate) were picked with an eye-shaped needle under a stereomicroscope, washed in filtered pondwater, and transferred to glass tubes for storage at -80 °C until further fatty acids analysis. *Corophium* for stable isotopes and fatty acids were only extracted in the late stage, since *Corophium* were just added in the pond during our sampling in the early stage and were not considered as a food source to shrimp yet.

5.2.3 Stable isotopes and fatty acids analysis

C and N stable isotopes were measured with an isotope ratio mass spectrometer (type Europa Integra) at UC Davis Stable Isotope Facility (University of California, USA). Isotope values are expressed as $\delta^{13}C$ and $\delta^{15}N$ (with units of ‰) determined by the following equation: $\delta^{13}C \text{ or } {}^{15}N=([\frac{R_{sample}}{R_{standard}}]-1)\times10^{3}$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Reference standards are Pee Dee Belemnite and atmospheric nitrogen.

Lipid extraction, methylation to fatty acid methyl esters (FAMEs), and FAME analysis followed the procedure of De Troch (2012). FAME composition of each sample was determined by gas chromatography analysis (HP 6890N) coupled to a mass spectrometer (HP 5973). 19:0 (Fluka 74208) was added as internal standard. The FAMEs were identified by comparing the retention time and mass spectra with authentic standards and mass spectral libraries (WILEY, NITS05) and then analyzed with the software MSD ChemStation (Agilent Technologies). Individual FAMEs were quantified by using a component FAME and BAME mix (Supelco #47885 and #47080 respectively, Sigma-Aldrich) and additional standards (Larodan). Each fatty acid was calculated as a proportion of the total identified fatty acids (% TFA). Shorthand FA notations were expressed as A: BωX, where A gives the number of carbon atoms, B represents the number of double bonds and X is the position of the first double bond closest to the terminal methyl group. Five groups of fatty acids trophic markers (FATMs) were selected as dietary tracers for meiobenthos: the sum of FA with 15 and 17 carbons (∑15, ∑17), the sum of branched short-chained FA (∑i-FA+ai-FA), and 18:1ω7 to indicate bacterial feeding (Budge and Parrish, 1998; Stevens et al., 2004; Viso and Marty, 1993), 16:1ω7 and EPA as diatom markers (Dalsgaard et al., 2003; Meziane and Tsuchiya, 2000; Reuss and Poulsen, 2002), DHA, DHA/EPA, the sum of PUFAs with 18 carbons (18 PUFA), 18:4ω3 and 18:1ω9 as markers for dinoflagellates (Dalsgaard et al., 2003; Kelly and Scheibling, 2012; Mansour et al., 1999), 18.2ω6 and 18.3ω3 for the Chlorophyta (Graeve et al., 2002; Kelly and Scheibling, 2012), and 18:1ω9/18:1ω7 to point at carnivorous feeding (Nyssen et al., 2005).

5.2.4 Data analysis

To analyse the meiobenthos community structure, three factors were considered: (1) Stage – fixed factor with three levels: before aquaculture (T0), early stage, and late stage, (2) Shrimp – fixed factor with two levels: *M. japonicus* and *L. vannamei*, and (3) Pond – random factor and nested in shrimp species: NAT P1, NAT P2 and NAT P3; and NIS P4, NIS P5 and NIS P6. Differences in meiobenthos community structure among factors were tested with PERMANOVA based on the Bray-Curtis similarities of square-root transformed densities. Prior to PERMANOVA, the homogeneity of multivariate dispersion was checked with homogeneity of dispersion (PERMDISP). The same matrices of PERMANOVA were used to conduct principal coordination analysis (PCO) to observe the grouping of taxa among factors. Similarity percentage (SIMPER) analysis was used to investigate the taxa that contribute to the dissimilarity between groups.

Variations in stable isotopes values of POM, MPB, and meiobenthos were tested using PERMANOVA tests followed by post-hoc pairwise contrasts between Stages, Shrimp and Pond. The trophic levels (TL) of consumers were estimated by the equation: $TL_{consumer}=2+(\delta^{15}N_{consumer}-\delta^{15}N_{base})/\Delta^{15}N$, in which $\delta^{15}N_{base}$ is the $\delta^{15}N$ of primary consumer i.e. the consumer taxon that had the lowest $\delta^{15}N$ in each pond. The $\Delta^{15}N$ of 2.3‰ for marine organisms was adopted (Zanden and Rasmussen, 2001).

Since the biomass of MPB in NIS P4 and NIS P5 in the early stage was not sufficient enough for reliable stable isotope analysis, we used the top 0.5 cm sediments (grounded, 60 °C oven-dried, HCl acidified samples for $\delta^{13}C$, and non-acidified subsamples for $\delta^{15}N$) as substitutions. The isotopic values of fishmeal were adopted from Yokoyama et al. (2006). The enrichment factors: 0.3±1.3‰ for $\delta^{13}C$ and 2.3±1.8‰ for $\delta^{15}N$ were applied for each trophic level (Zanden and Rasmussen, 2001). To facilitate the comparison of pattern between rearing stages and types of ponds, the isotopic data of the same item from two ponds were pooled together based on the similarity to isotopic values (tested by PERMANOVA: except for the *M. japonicus* ponds in the late stage, there was no significant difference in isotopic values of primary sources between two ponds from the same treatment, for instance, the early stage - NIS P4 & P5). To further interpret the pattern of shrimp's diets, the POM and MPB were combined as 'Primary organic source', and copepods and nematodes were combined as

'Meiobenthos' in a *posteriori* approach from the mixing model outputs (Parnell et al., 2010).

For the comparison of the FATMs variations in meiobenthos between the aquaculture stages and the meiobenthos taxa, PERMANOVAs based on Bray-Curtis resemblance matrix were applied for *M. japonicus* and *L. vannamei* ponds, separately. Principal coordination analyses (PCO) on the same resemblance matrices were performed with vectors showing FATMs correlating at least 50% with one of the first 2 PCO-axes. Non-parametric Kruskal-Wallis tests were used to compare the relative contribution of selected FATM between stages in meiobenthos belonging to the same taxon.

Kruskal-Wallis tests were conducted by SPSS 20.0. Multi-variate analyses were conducted by PRIMER V6 using the PERMANOVA+ add-on package (Anderson et al., 2008; Clarke and Gorley, 2006).

5.3 Results

5.3.1 Community structure of meiobenthos

The *L. vannamei* (NAT) and *L. vannamei* (NIS) ponds harbored different meiobenthos communities (Fig 5.1; Table 5.1: Shrimp $p < 0.05$; Fig 5.2a). In the *M. japonicus* ponds, Nematoda were the main taxon (mean: 85%, range: 65-98%), followed by Copepoda (mean: 8%, range: 0-15%). Oligochaeta, Ostracoda, Turbellaria and Amphipoda were also represented with mean proportions >1%. In the *L. vannamei* ponds, the meiobenthic diversity was lower than *M. japonicus* ponds (See Shannon *H'* in Appendix IV Table S1; Fig. 5.2), with Nematoda dominating the community, accounting always for > 98% of meiobenthic abundance at all stages.

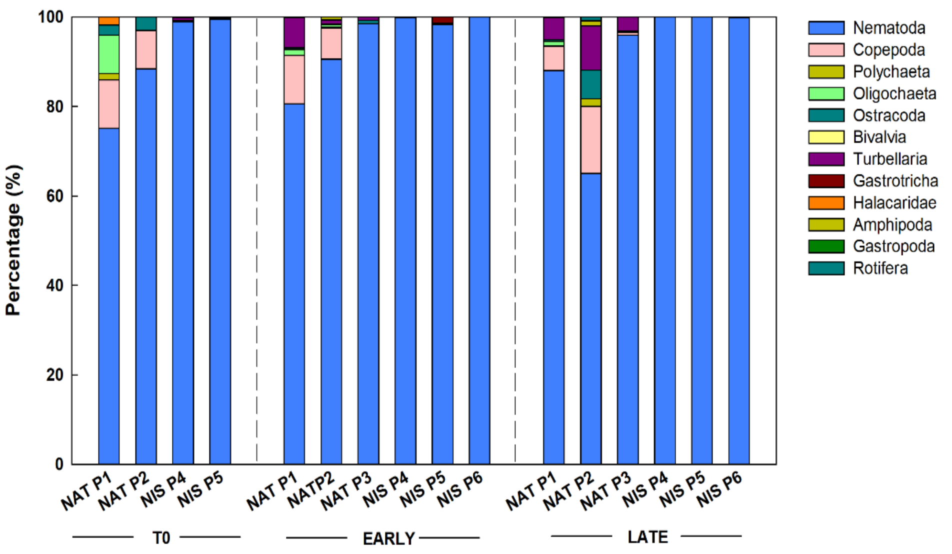

Figure 5.1 Relative (%) meiobenthos communities in native shrimp (*Marsupenaeus japonicus* - NAT) and non-indigenous shrimp (*Litopenaeus vannamei* - NIS) ponds at T0 (before aquaculture) and in the early and late rearing stage. NAT P = *M. japonicus* pond, NIS P = *L. vannamei* pond, T0 = before aquaculture, Early = early rearing stage, Late = late rearing stage.

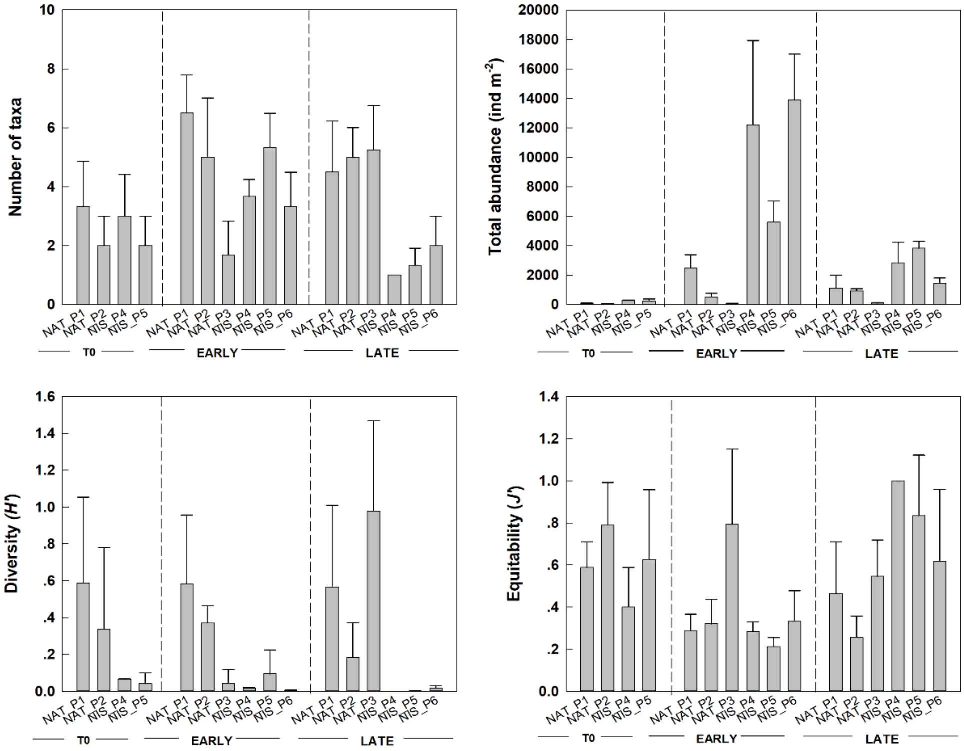

Figure 5.2 Number of taxa, total abundance, diversity (Shannon *H'*) and equitability (*J'*, Pielou's evenness) of meiobenthic community *in Marsupenaeus japonicus* (native) and *Litopenaeus vannamei* (NIS) ponds. NAT = *M. japonicus*, NIS = *L. vannamei*, T0 = before aquaculture, Early = early rearing stage, Late = late rearing stage.

Interaction effects of Stage × Shrimp and Stage × Pond (Shrimp) were found on the pond meiobenthic communities (Table 5.1), showing temporal variations in each types of ponds (see further). The PERMANOVA pairwise comparisons showed that in *M. japonicus* ponds, meiobenthic communities only differed between T0 and the early stage, due to the increasing density of Nematoda (SIMPER: 42% dissimilarity), Turbellaria (SIMPER: 19% dissimilarity) and Copepoda (SIMPER:18% dissimilarity). Due to the variation among ponds, the

PCO ordination did not show any grouping by Stage in the *M. japonicus* ponds (Fig 5.3b). The total abundance of meiobenthos showed a significantly increase from T0 to the early stage and then remained stable in the late stage (Table S1; Fig 5.2). However, in *L. vannamei* ponds, meiobenthic communities were different among T0, the early and the late stage (Table 5.1), with distinct grouping at the different stages in the PCO plot (Fig 5.3 c). SIMPER revealed that the influential taxon on the grouping was Nematoda, whose abundance sharply increased from 151-392 ind. m^{-2} at T0 to 5132-18640 ind. m^{-2} at the early stage, then dropped to 1147-4419 ind. m^{-2} in the late stage (Fig 5.2). The diversity of meiobenthos showed a significant decrease from the early to the late stage (Table S1; Fig 5.2).

Table 5.1 Permutational analysis of variance (PERMANOVA) tests the effect of three factors. The pairwise tests of the factors stage, type of ponds and ponds on meiobenthic communitie structure showed differences within the factors. The tests were based on Bray-Curtis clustering from fourth-root transformed abundance data. Significance level: $p < 0.05$; *$p < 0.01$; **$p < 0.001$. NAT = *M. japonicus*, NIS = *L. vannamei*. T0 = before aquaculture, Early = early rearing stage, Late = late rearing stage. -: no testing due to the the lack of T0 samples for NAT P3 and NIS P6.

Source	df	SS	MS	Pseudo-	P (MC)
Stage	2	12198	6098.9	7.29	0.002**
Shrimp	1	12064	12064	5.41	0.0157*
Pond (Shrimp)	4	9684.4	2421.1	7.15	0.0001***
Stage × Shrimp	2	6612.4	3306.2	3.95	0.0202*
Stage × Pond (Shrimp)	6	5107.7	851.29	2.52	0.0043**
Res	35	11844	338.39		
Total	50	57561			

Pairwise test

Stage × Shrimp	NAT		NIS	
	t	P (MC)	t	P (MC)
T0 vs Early	4.37	0.018*	3.48	0.048*
Early vs Late	0.79	0.629^{NS}	4.18	0.010*
T0 vs Late	2.61	0.067^{NS}	4.83	0.020*

Pairwise test						
Stage × Shrimp	T0		Early		Late	
	t	P (MC)	t	P (MC)	t	P (MC)
NAT vs NIS	3.51	0.008**	1.7	0.086^{NS}	3.34	0.009**
Stage × Pond (Shrimp)	NAT-P1		NAT-P2		NAT-P3	
	t	P (MC)	t	P (MC)	t	P (MC)
T0 vs Early	2.57	0.017*	2.27	0.041*	-	-
Early vs Late	1.43	0.147^{NS}	1	0.412^{NS}	2.77	0.021*
T0 vs Late	2.16	0.026**	2.8	0.013**	-	-
Stage ×Pond (Shrimp)	NIS-P4		NIS-P5		NIS-P6	
	t	P (MC)	t	P (MC)	t	P (MC)
T0 vs Early	3.27	0.012*	3.47	0.007**	-	-
Early vs Late	4.88	0.001**	3.62	0.005**	5.01	0.001**
T0 vs Late	2.83	0.016**	2.99	0.014**	-	-

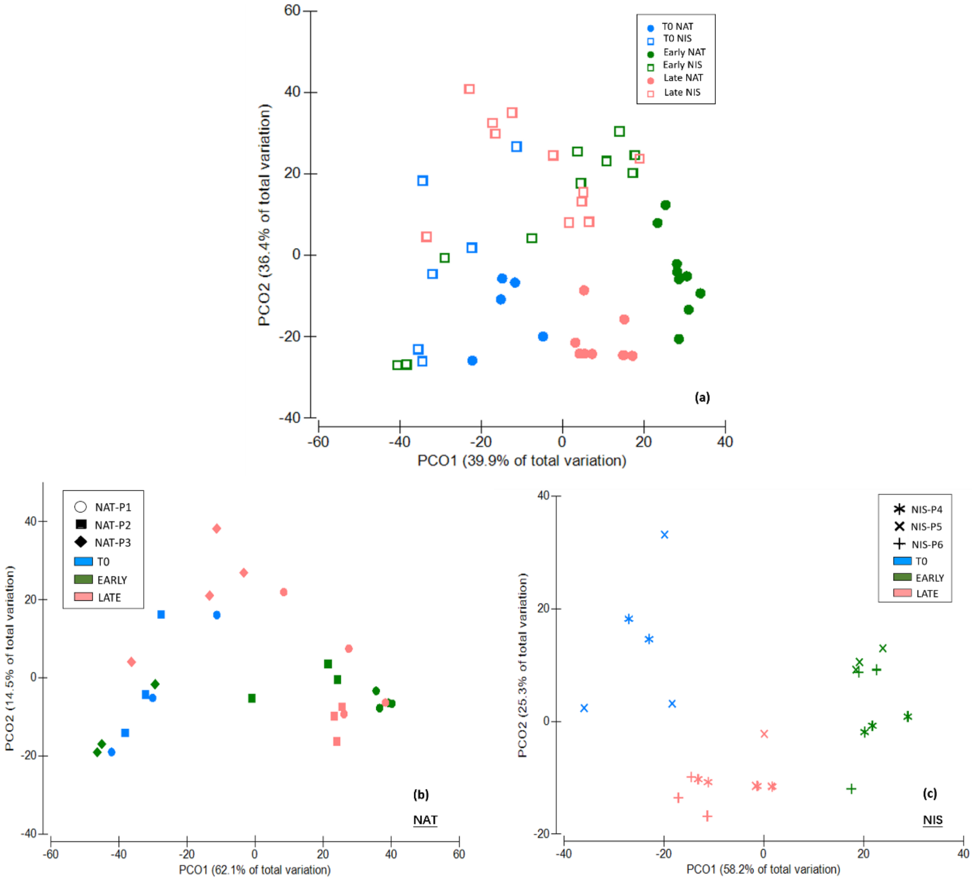

Figure 5.3 Principal coordinate analysis (PCO) plot based on meiofauna higher taxa in (a) *Marsupenaeus japonicus* and *Litopenaeus vannamei* ponds, (b) *Marsupenaeus japonicus* ponds (NAT ponds: NAT P1, P2, and P3), and (c) *Litopenaeus vannamei* ponds (NIS ponds: NIS P4, P5, and P6). T0 = before aquaculture, Early = early rearing stage, Late = late rearing stage. The sample grouping was based on Bray-Curtis clustering from fourth-root transformed abundance data.

5.3.2 Food source utilization of meiobenthos: fatty acids biomarkers

The FATMs of copepods and nematodes in *M. japonicus* ponds varied with time (Table 5.2a: Stage × Meiobenthos taxa $p < 0.05$). Both copepods and nematodes in the early stage of *M. japonicus* ponds showed high proportions of the diatom FA 16:1ω7 (Table 5.3), which is 5 and 14 times higher respectively than in the late stage. In the late stage, copepods showed a flagellates-based diet, characterized by a ratio DHA/EPA > 1 and high DHA (20.7±3.8%) (Fig 5.4a). Transplanted *Corophium* in the late stage clustered together with copepods (Fig 5.4a), indicating a similar flagellates-based diet. Nematodes showed a high ratio of carnivore marker 18:1ω9/18:1ω7 with the mean value above 1 (Table 5.3).

Table 5.2 Permutational analysis of variance (PERMANOVA) tests of the effect of different sampling time and meiobenthos taxa on fatty acids trophic markers in (a) the *Marsupenaeus japonicus* and (b) *Litopenaeus vannamei* ponds. The tests were based on Bray-Curtis clustering from fatty acids trophic markers calculated by proportional fatty acids. Significance level: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

(a) Main test: *M. japonicus* ponds

Source	df	SS	MS	Pseudo-F	P(MC)
Stage	1	7913.3	7913.3	18.36	**0.0001*****
Meiobenthos taxa	2	6182.7	3091.4	7.1725	**0.0006*****
Stage×Meiobenthos taxa	1	5487	5487	12.731	**0.0002*****
Res	18	7758	431		
Total	22	35084			

(b) Main test: *L. vannamei* ponds

Source	df	SS	MS	Pseudo-F	P(MC)
Stage	1	1246.5	1246.5	7.3644	**0.0174***
Res	4	677.07	169.27		
Total	5	1923.6			

For *L. vannamei* ponds, shrimp farming stages also affected the FATMs of nematodes, which dominated the meiobenthic community (Table 5.2b Stage < 0.05). In a PCO plot, nematodes clustered separately between stages, though samples showed high variability within the late stage (Fig 5.5b). More specifically, in the early stage, the diatom FA 16:1ω7 in nematodes were almost 6 times higher than those of late stage (Table 5.3). In the late stage, nematodes rather showed a flagellate-based diet (DHA/EPA > 1).

All benthos (except for nematodes in the late stage of *M. japonicus* ponds) showed high proportions of bacterial FA (11-37%). Also, except nematodes in *M. japonicus* ponds, meiobenthos from both ponds and transplanted *Corophium* in the late stage contained higher levels of PUFAs (in particular, DHA, EPA, and ω3 FA) compared with those in the early stage (Appendix IV Table S2) and their DHA/EPA ratio were all increased to above 1 (Fig 5.4).

Table 5.3 Selected fatty acids trophic markers (FATM) (mean % ± SD in relative abundance) of meiobenthos in *Marsupenaeus japonicus* and *Litopenaeus vannamei* ponds in the early and late rearing stage. EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, COP = copepods, NEMA = nematodes, COR = transplanted *Corophium*. * indicates significant differences ($P < 0.05$) in the meiobenthos between early and late stage.

FA marker	FAs	*M. japonicus* ponds					*L. vannamei* ponds	
		Early		Late			Early	Late
		COP	NEMA	COP	COR	NEMA	NEMA	NEMA
Bacteria	Σ 15, Σ 17	9.1±1.9	6.0±0.4*	6.6±4.1	3.2±0.6	2.7±1.0	24.8±4.3*	4.5±0.8
	Σi-FA+ai-FA	2.2±1.0*	2.7±0.5*	0.5±0.0	1.3±0.5	0.5±0.5	6.6±5.0	1.6±0.5
	18:1ω7	-	9.2±0.8*	3.4±0.3*	6.5±0.0	3.6±1.2	5.5±1.3	8.0±1.7
Diatoms	16:1ω7	4.2±0.8*	5.8±0.8*	0.8±0.7	0.3±0.1	0.4±0.5	5.3±1.3*	0.9±0.1
	EPA	4.4±1.1	14.5±1.6*	10.2±0.8*	14.5±3.5	4.4±1.8	8.3±2.1	6.5±1.0
Flagellates	DHA/EPA	0.3±0.1	0.5±0.0	2.0±0.5	1.74±0.6	0.5±0.5	0.6±0.0	1.2±0.1
	18 PUFA	1.2±0.6	6.3±0.5*	3.4±1.6*	11.8±4.8	3.5±0.8	3.5±3.3	2.9±0.5
	18:4ω3	-	0.9±0.5	0.9±0.6	5.7±2.0	0.3±0.5	-	0.2±0.4
	18:1ω9	-	5.4±1.3	1.7±0.8	4.2±1.1	5.1±4.0	4.5±1.9	6.8±0.9
	DHA	1.3±0.7	8.4±1.0*	20.7±3.8*	24.2±2.8	2.6±2.2	5.5±1.5	8.4±1.7
Chlorophyte	18.2ω6	-	2.3±0.2	1.0±0.2	2.9±1.1	2.3±1.5	2.9±3.8	1.7±0.2
	18.3ω3	1.2±0.6	3.0±0.3*	1.4±0.8	3.1±1.6	0.9±0.8	0.5±0.6	0.9±0.2
Carnivore	18:1ω9/18:1ω7	-	0.5±0.0	0.5±0.2	0.6±0.1	1.2±0.9	0.9±0.6	0.8±0.0

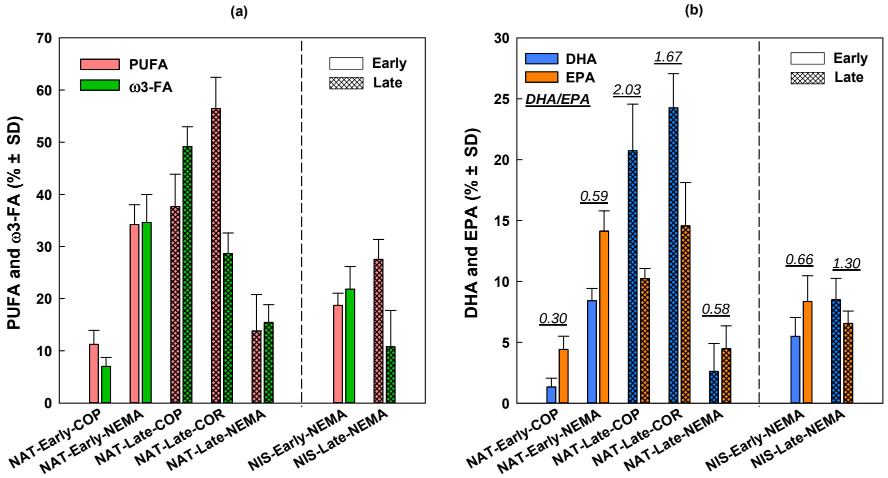

Figure 5.4 selected fatty acids (FA) and DHA to EPA ratio of meiobenthos in *Marsupenaeus japonicus* (NAT) and *Litopenaeus vannamei* (NIS) ponds in the early and late rearing stages. DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, PUFAs = polyunsaturated fatty acids, DHA/EPA = DHA to EPA ratio. COP = copepods, NEMA = nematodes, COR = transplanted *Corophium*

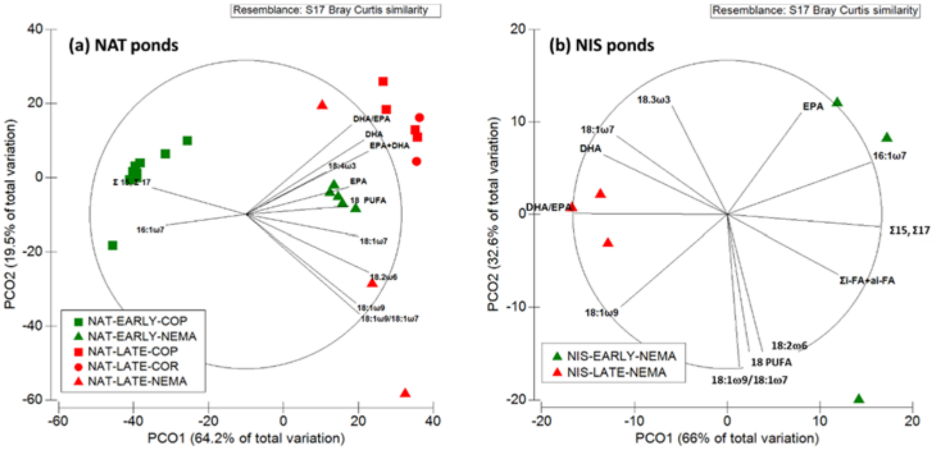

Figure 5.5. Principal coordinates plots of selected fatty acid trophic markers (FATMs) in meiobenthos in (a) *Marsupenaeus japonicus* ponds (NAT) and (b) *Litopenaeus vannamei* ponds (NIS). Vectors represent specific FATMs correlating >

***Litopenaeus vannamei* ponds (NIS).** Vectors represent specific FATMs correlating > 50% with the first 2 PCO axes. EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; COP = copepods; NEMA = nematodes; COR = *Corophium*.

5.3.3 Stable isotopes

The stable isotopes of the primary organic sources, i.e. POM and MPB, were different depending on the types of shrimp pond and the sampled stages (Appendix Table S3), but generally had relatively low $\delta^{15}N$ values compared to consumers from the same environment (Fig 5.6). Both the primary organic sources and the shrimp feeds can be distinguished from each other in the same environment in terms of stable isotope values (Fig 5.6).

In *M. japonicus* ponds, nematodes showed the most enriched $\delta^{15}N$ values among consumers except for the shrimps (Fig 5.6) and displayed high trophic levels with the means > 3 (carnivores). In the *L. vannamei* ponds, $\delta^{15}N$ values and the trophic level of the nematodes were significantly higher at the early stage ($\delta^{15}N$: 6.32±0.44‰ $H = 8.265$, $p < 0.01$; TL: 3.0 $H = 3.433$, $p < 0.05$) than those measured at the late stage ($\delta^{15}N$: 2.68±0.58‰; TL: 2.0). The $\delta^{13}C$ values of all the nematodes ranged between -16.57‰ to -20.92‰ in both stages.

Copepods, appearing only in *M. japonicus* ponds, showed slight differences between the early and the late stages, but similar $\delta^{13}C$ values, ranging from -18.02‰ to -18.95‰, and displayed the lowest $\delta^{15}N$ as well as the lowest trophic level among the consumers in the same environment ($\delta^{15}N$: 4.46-5.37; TL: 1.9-2.1).

The $\delta^{15}N$ value and the trophic level of *M. japonicus* were higher in the late stage than those of early stage ($\delta^{15}N$ $H = 3.857$, $p < 0.01$; TL H

= 3.000, $p = 0.056$). The $\delta^{15}N$ values of *L. vannamei* were rather constant, ranging from 4.07‰ to 4.59‰. At the late stage, both *M. japonicus* and *L. vannamei* had a higher $\delta^{13}C$ content than at the early stage (*M. japonicus* $H = 3.857$, $p < 0.05$; *L. vannamei* $H = 5.400$, $p < 0.05$).

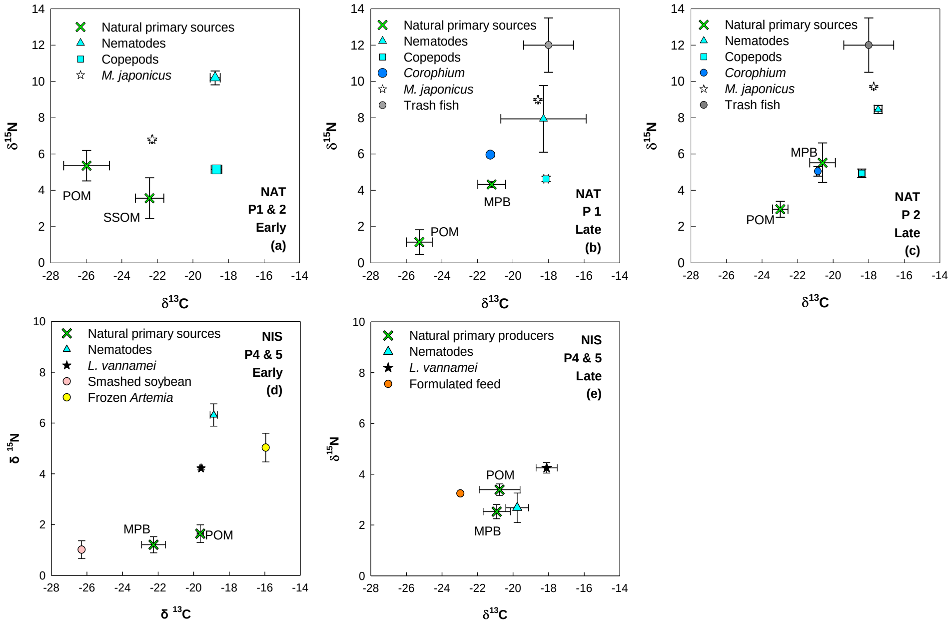

Figure 5.6 Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) bi-plots of primary producers and consumers in *Marsupenaeus japonicus* ponds (NAT) in the (a) early and (b) (c) late rearing stage, *Litopenaeus vannamei* ponds (NIS) in the (d) early and (e) late rearing stage. Except at the late stage of NAT ponds, the isotopic data between ponds were pooled. POM = particulate organic matter; MPB = microphytobenthos; SSOM = surface sediment organic matter; NAT P = *M. japonicus* pond; NIS P = *L. vannamei* pond.

5.3.4 The diets of shrimps as unraveled by a mixing model

In the early stage, primary organic food sources were important for juvenile *M. japonicus* and *L. vannamei,* contributing 43.5-83.3% (mean: 64.2%) and 68.9-91% (mean: 80.9%) to their diets, respectively. Meiobenthos were also consumed substantially by juvenile *M. japonicus* (16.7-56.5% of their diet).

In the late stage, *M. japonicus* (adult) became more carnivorous, characterized by high δ^{15}N and TL, thus POM and MPB were not counted as direct food sources to adult *M. japonicus*. Naturally occurring meiobenthos (copepods in particular) represented the major food sources for adult *M. japonicus* (34.8-77.2%), followed by transplanted *Corophium*, whereas trash fish contributed the least to the diet of *M. japonicus* (Table 5.4; Fig 5.7). However, adult *L. vannamei*, characterized by a constant TL compared to juvenile stages, still incorporated POM+MPB (17.7-76.2% of the diet). Nematodes, the dominant meiobenthos taxon, became a more important food item for adult *L. vannamei* (11.3-73.3% of the diet).

Interestingly, according to the stable isotope signatures and the mixing model, neither the wet feed (processed *Artemia* and fishmeal) nor pellet feed (smashed soybean and formulated feed) were abundantly consumed by either of the two shrimp species when these feed types were provided.

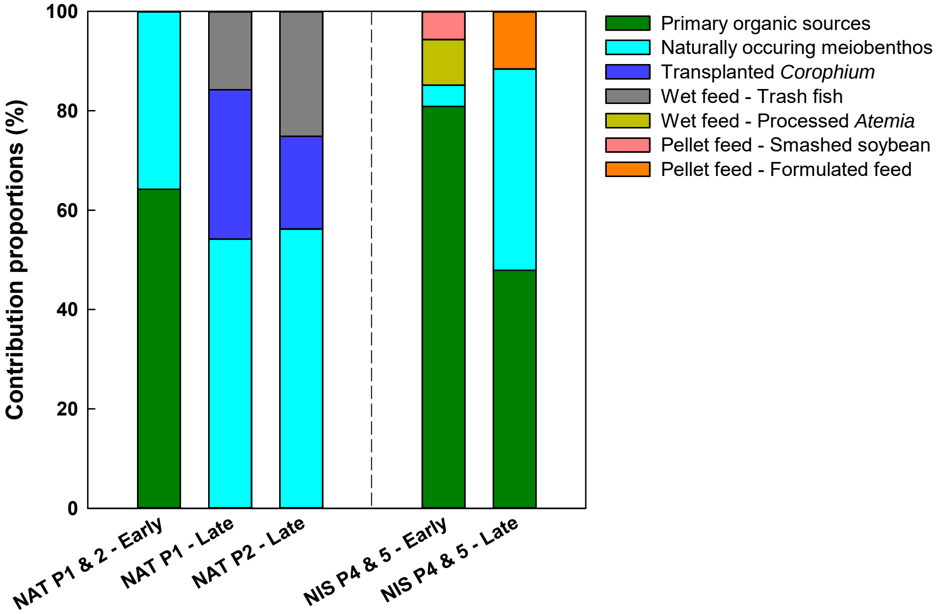

Figure 5.7 Mean contribution proportions (%) of the food sources to *Marsupenaeus japonicus* (NAT) and *Litopenaeus vannamei* (NIS). Primary organic sources = POM+MPB; meiobenthos = copepods + nematodes (NAT ponds) / nematodes (NIS ponds).

Table 5.4 Mean contributions and 95% credible interval of each potential food source to the diets of *Marsupenaeus japonicus* and *Litopenaeus vannamei* in the early and late rearing stages, estimated by SIAR. Wet feed was trash fish and processed *Artemia* for late stages of native shrimp (NAT) ponds and early stages of non-indigenous shrimp (NIS) ponds, respectively. Pellet feed consisted of smashed soybeans and formulated feed for early and late stages of NIS ponds, respectively.

	***M. japonicus* (NAT) ponds**			***L. vannamei* (NIS) ponds**	
Sources	**Early (NAT P1 & NAT P2)**	**Late (NAT P1)**	**Late (NAT P2)**	**Early (NIS P4 & NIS P5)**	**Late (NIS P4 & NIS P5)**
POM	33.6 (12.4-51.4)	-	-	66.3 (47.4-82.2)	17.4 (1.1-41.5)
MPB	30.6 (6.9-54.1)	-	-	14.6 (1.5-31.9)	30.5 (4.4-57.9)
Nematodes	14.2 (1.3-33.7)	14.4 (1.5-33.6)	22.2 (3.3-42.3)	4.3 (0.3-10.8)	40.5 (11.3-73.3)
Copepods	21.6 (2.8-41.8)	39.8 (21.9-55.2)	34.0 (11.1-57.6)	-	-
Corophium	-	30.0 (15.1-43.7)	18.7 (2.3-37.2)	-	-
Wet feed	-	15.8 (5.3-25.6)	25.1 (10.8-38.4)	9.2 (1.2-18.6)	-
Pellet feed	-	-	-	5.7 (0.6-12.4)	11.60 (0.7-32.1)

5.4 Discussion

5.4.1 Meiobenthos: temporal changes during the shrimp farming

Our results showed the temporal changes in meiobenthic community structures. Specifically, in the early stage (spring), this change was mostly associated with increased densities of nematodes. It is known that nematode density commonly peaks in spring in the intertidal and subtidal environment because of the rising temperatures and increasing food availability (Harris, 1972; Scholz and Liebezeit, 2012; Smol et al., 1994). Such increasing food availability could be enhanced by the addition of feeds in shrimp ponds, and also, the growth of microphytobenthos (De Pauw et al., 1984; Chapter 2), which leaded to an increase in nematode densities. In the late stage, the meiobenthos assemblage remained similar in *M. japonicus* ponds, while it shifted again in *L. vannamei* ponds as the density of nematodes decreased. We assume that the decline in nematodes in *L. vannamei* ponds could be due to the predation by *L. vannamei* (see further). Also, the organically enriched sediment with reduced redox potential at the shrimp pond bottom (Chapter 2) can generate chemical stressors such as hydrogen sulfide and ammonia (Holmer et al., 2005), which affect the living conditions for nematodes (Armenteros et al., 2010; Losi et al., 2013). We found meiobenthic diversity to be reduced in the late stage of *L. vannamei* ponds, which at the end of the shrimp rearing harbored a high-density but low-diversity meiobenthic community typical of disturbed environments (Kennedy and Jacoby, 1999; Lee et al., 2001). The lack of a clear response of meiobenthos in *M. japonicus* ponds in the late stage might due to a lesser extent of organic accumulation, as a result of the potentially stronger bioturbation activities as well as a larger water exchange frequency in *M. japonicus* ponds (Chapter 2).

5.4.2 Meiobenthos: diets and functional response to shrimp farming

The FATMs analysis showed that bacterial biomarkers were abundant in the benthos collected in the sediment of both *M. japonicus* and *L. vannamei* ponds. Although not many FA data of copepods and nematodes from the field are available for comparison, the concentration of bacterial FAs in the present study is higher than the ones reported for other intertidal areas worldwide (Cnudde et al., 2015; Huang et al., 2018; Leduc and Probert, 2009; Mascart et al., 2013), indicating a high consumption of bacteria in the two types of shrimp ponds. The addition of animal-origin feed (*Corophium* and trash fish in *M. japonicus* ponds, and frozen *Artemia* in *L. vannamei* ponds in our study) might stimulate bacteria growth (Høj et al., 2009; Thompson et al., 1999). Also, the unconsumed feed pellets can provide a base for bacteria and promote their growth (Moriarty, 1997). Our results highlight the importance of the bacterial loop in the basal energy pathway of shrimp ponds, which is possibly enhanced by the feed addition (Moriarty, 1986; Nevejan et al., 2018; Qin et al., 2016).

Nematodes and copepods in both types of ponds also showed higher levels of 16:1ω7 (i.e. a diatom marker) in the early stage compared to the late stage, indicating a higher assimilation of diatoms at the start of the rearing activity. Meiobenthos in the late stage (except for nematodes from *M. japonicus* ponds), had a DHA/EPA ratio > 1, combined with a high level of either 18 PUFA or 18:1ω9, suggesting a flagellate-based diet. This temporal diet shift has previously been reported for intertidal nematodes and copepods, and has been related to the change of food availability (Cnudde et al., 2015; Lebreton et al., 2012). In the shrimp ponds, diatoms and flagellates are important natural primary producers and their abundance fluctuated throughout the rearing stage (Cardozo et al., 2011; Porchas-Cornejo et al., 2011; Thompson et al., 2002). Diatoms grew more slowly after the initial stage, possibly due to a depletion of silicate (Burford and Pearson, 1998). The higher levels of suspended solids blocking the light penetration also likely reduced diatom growth (Alonso-Rodriguez and Páez-Osuna, 2003) In contrast, flagellates usually reached higher densities at the late stage (Cardozo et al., 2011; Fernandes Da Silva et al., 2008), because the gradually accumulated floc-derived matter on the pond bottom stimulates their growth (Burford et al., 2003).

Only the nematodes from the *M. japonicus* ponds had an 18:1ω9/18:1ω7 ratio >1 in the late stage, which is often interpreted as carnivorous feeding (Legeżyńska et al., 2014; Nelson et al., 2001). The trophic level deduced from the stable isotope results also showed nematodes to be omnivores/carnivores. The shift from a bacteria-microalgae-based diet to a rather carnivorous diet might relate to the appearance of algivorous and omnivorous ciliates in the second half of the shrimp rearing period (Decamp et al., 2007). It should be noticed that the low 18:1ω9/18:1ω7 ratio in those nematodes from the early stage is in contrast with the high values of $\delta^{15}N$ and the deduced high trophic level. However, their high level of bacterial FAs underpins bacterivory of nematodes which can lead to high $\delta^{15}N$ values (Majdi et al., 2012; Moens et al., 2005; Rzeznik-Orignac et al., 2008). Feeding experiments in the lab are needed to further reveal more detailed trophic information. It should however be considered that in the late stage of *M. japonicus* ponds, 20% of *Corophium* have a body length larger than 500 μm (Zhong and Ma, 2012) and were thus not retained in our sampling (maximum sieve mesh size 500 μm), which could have led to an underestimation of the FA content of *Corophium*.

We found higher PUFA levels in meiobenthos in the late stage (except for the predatory nematodes in *M. japonicus* ponds). Correspondingly, meiobenthos' ω3 FAs were relatively high in the late stage. This can probably be explained by the high ω3-FA contents of both the formulated feed and marine-derived living feed (Lytle et al., 1990; Ouraji et al., 2011). High PUFAs, especially high ω3 levels, can stimulate the maturation of penaeid shrimp (Lytle et al., 1990). Also, at the late stage, copepods and *Corophium* from *M. japonicus* ponds and nematodes from *L. vannamei* ponds had a high DHA:EPA ratio. A high DHA:EPA ratio is recognized as a response to living food items in aquaculture (Bell et al., 2003; Nanton and Castell, 1999). Increasing the DHA:EPA ratio from 0.1 to 0.5 in the diet improved survival of turbot (Bell et al., 1985). Thus, our results support the suggestion that meiobenthos can serve as a nutritious prey for shrimps (Ballester et al., 2007; Hena et al., 2011).

5.4.3 The diets of *M. japonicus* and *L. vannamei*

The most important food items for both *M. japonicus* and *L. vannamei*, estimated by the mixing model, were derived from naturally occurring food sources, implying the importance of promoting a pond's natural productivity in terms of the efficiency of energy flow. Also, the enhancement of natural productivity can improve the water quality, promote the nutritional condition of cultured shrimp, and reduce feeding costs (Porchas-Cornejo et al., 2011; Tacon et al., 2002). The drivers of natural productivity however changed depending on the rearing stage, with primary producers (i.e. POM and MPB) contributing abundantly in the early stage, and meiobenthos becoming more important in the late stage. This is in accordance with the observations of an ontogenetic shift in penaeid shrimps from planktonic and omnivore diets to more benthic and carnivorous feeding habits in natural and pond environments (Bojórquez-Mascareño and Soto-Jiménez, 2013; Nunes et al., 1997; Schwamborn and Criales, 2000). In addition, the higher trophic level of *M. japonicus* indicates a more carnivorous feeding behavior when compared with *L. vannamei*, which is supported by a pronounced ontogenetic shift in *M. japonicus* with higher assimilation of meiobenthos and transplanted *Corophium* in the late stage.

Laboratory studies on shrimp feeding showed that *L. vannamei* could abundantly assimilate artificial pellet feeds, such as soymeal (Gamboa-Delgado and Le Vay, 2009; Yang et al., 2015) and formulated feed (Bojórquez-Mascareño and Soto-Jiménez, 2013). In our study, the smashed soybean and formulated feed were found to contribute little to the diet of juvenile and adult *L. vannamei*, respectively. This finding can be due to the selective feeding behavior of shrimps (Dall et al., 1991). When the natural productivity was sufficient, shrimps showed a high preference for natural food over the formulated feed (Gamboa-Delgado et al., 2003; Nunes et al., 1997; Porchas-Cornejo et al., 2012). But as shrimps can adapt their diet to food availability (Forster, 1976), artificial feeds may also be consumed largely when natural food is lacking or grazing pressure becomes intense (Cam et al., 1991; Porchas-Cornejo et al., 2012). A large portion of artificial feeds was not directly consumed by shrimps, but might have indirectly contribute to diverse pathways of the benthic food web (Anderson et al., 1987), e.g. by fueling the bacterial loop, thereby increasing the quality of meiobenthos, which is being consumed by shrimps. Also, the input of artificial feed can play an essential role in aquaculture by providing the nutrients, e.g. vitamins, minerals, pigments, that natural food cannot supply (Gamboa-Delgado, 2014; Porchas-Cornejo et al., 2012).

Transplanting benthos into shrimp ponds has been proposed as a promising method that provides extra food items to shrimps (Deng et al., 2007; Zhou, 1994). In our study, the amphipod *Corophium* was introduced once into the *M. japonicus* ponds in the early stage (at 50-100 kg per pond; c.a. 100-200 ind. m^{-2}). We still observed their presence in the late stage (the density of 38-500 μm individuals in the top 1 cm sediment: 460±570 ind m^{-2}), indicating the successful establishment of *Corophium* in the ponds. The mixing model showed that the transplanted *Corophium* was largely assimilated by adult *M. japonicus*, which agrees with the findings of Zhong & Ma (2012) who observed a significant decline in *Corophium* density in the late stage of *M. japonicus* ponds. Although some studies showed adult *M. japonicus* to prefer larger and elongated benthos (Reymond and Lagardère, 1990; Zupo et al., 1998), our study did not support this feeding preference (i.e. *Corophium*) over copepods and nematodes, probably because the density of *Corophium* was not high enough. *Corophium* has several reproduction peaks in their lifespan (Nair and Anger, 1979). It has been suggested to synchronize one of those reproduction peaks of *Corophium* with the feeding behavior of *M. japonicus* (Zhong and Ma, 2012). The reproduction peaks of *Corophium* can vary according to species, temperature, etc. (Nair and Anger, 1979; Wang et al., 2009). Further research and field trials are needed to trace the density of male and female *Corophium* in a full sediment profile (e.g. top 0-1, 1-5, 5-10 cm) and at a finer time scale (e.g. weekly basis), in order to manipulate the time to introduce proper amounts of *Corophium* into the ponds.

As for the animal-originated wet feed, i.e. frozen *Artemia* for juvenile *L. vannamei* and fishmeal for adult *M. japonicus*, we found both types of feeds to contribute little to the shrimps' diet. Although the addition of wet feed might also enhance the microbial loop in aquaculture ponds (Moriarty, 1997), wet feed can easily deteriorate water quality (Cobo et al., 2015). In addition to the sustainability issue, *Artemia* and fishmeal are also costly from an economical point of view, and thus the replacement of wet feed is suggested (Bulbul et al., 2016; Bulbul et al., 2013).

5.4.4 Benthic food webs in *M. japonicus* and *L. vannamei* ponds

The likely pathways of benthic energy flow can be summarized in a conceptual diagram (Fig 5.8). In *M. japonicus* ponds, there are complex trophic interactions involved with more components of primary consumers, e.g. copepods and transplanted *Corophium*. *L. vannamei* ponds have a rather simple trophic structure, mainly because the meiobenthic community is dominated by nematodes. In general, meiobenthos functioned differently between rearing stages in both ponds. Since juvenile shrimps derived large proportions of their energy from primary organic sources (Dall et al. 1991; this study), meiobenthos as primary consumers can be potential competitors in the early rearing stage (Fig 6.5 a, c). In the late stage, meiobenthos (copepods in the *M. japonicus* ponds and nematodes in the *L. vannamei* ponds specifically) rather served as nutritious food sources to shrimps, transferring energy from primary producers to the higher consumers (Fig 6.5 b, d). It should be noticed that adult *L. vannamei* still derived a part of their food intake from herbivorous feeding on primary producers (Maldonado et al., 2009), thus, nematodes might as well become competitive with shrimps if the food resource is limited (Fig 6.5 d). The supplemental foods, fishmeal in *M. japonicus* ponds, processed *Artemia*, soymeal and formulated feed in NIS ponds, did not represent a major direct contribution to the shrimps' diet. However, the possibility of an indirect contribution, particularly through stimulating the bacterial loop, cannot be ruled out (Anderson et al., 1987; Burford et al., 2004).

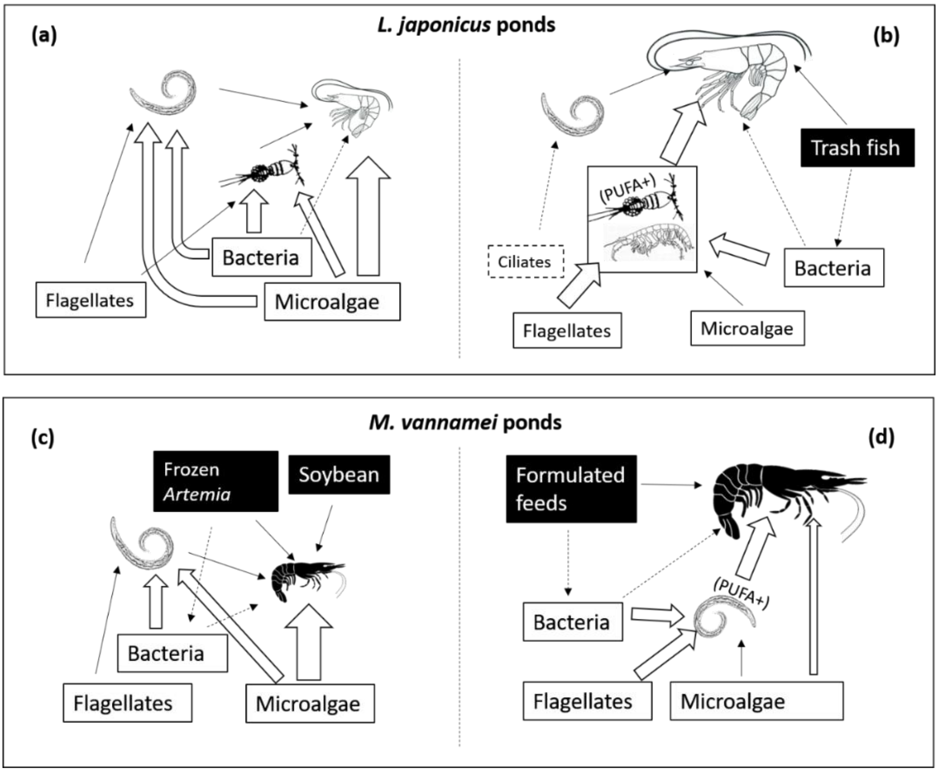

Figure 5.8 Conceptual diagram of the benthic energy flow in *Marsupenaeus japonicus* and *Litopenaeus vannamei* ponds in the early and late stage. The size of the arrows indicates the proportion of contributions. The dashed lines indicate uncertain contributions to benthic energy flow.

5.5 Conclusion

Based on the above analysis of the meiobenthic communities and food web in *M. japonicus* and *L. vannamei* ponds, the following key findings were obtained:

(1) Meiobenthos assemblages were affected by shrimp aquaculture activities, and responded differently in *M. japonicus* and *L. vannamei* ponds. Nematode density was the main driver of changes in meiobenthic community structure. *L. vannamei* ponds formed a less stable benthic

environment, characterized by high-density but low-diversity meiobenthic community structure.

(2) Bacteria were an important food source for meiobenthos in both ponds. The contribution of diatoms and flagellates varied in between rearing stages and was related to food availability. Most naturally occurring meiobenthos and the transplanted *Corophium* contained high levels of PUFA in the late rearing stages.

(3) Natural productivity played an important dietary role for shrimps for the entire rearing period. Due to the ontogenetic shift of shrimps, primary organic matter was predominantly consumed by juvenile shrimps, while meiobenthos was an important food source for adults. The supplementary feeds were only consumed at a low extent by shrimps in both ponds, but might fuel the benthic food web through secondary production (e.g. increase the quality of meiobenthos).

(4) In terms of the entire food web, meiobenthos had the potential to compete for resources with juvenile shrimps, but became an important functional link between primary producers and shrimps in the late stage the of pond ecosystem.

Chapter 6: Overall discussion

Mariculture activities generate environmental issues regarding the health of coastal marine ecosystems that receive much concern (Black, 2001; Pillay, 2008). One of them is the impact of mariculture on the benthic ecosystem because of nutrient loading (Holmer et al., 2005). If not managed adequately (e.g. stocking density, proper amount of feed input and water exchange), such changes can be irreversible beyond the resilience of the recipient ecosystem (Pillay, 2008). The cultivation of non-indigenous species (NIS) forms a particular case that is also of high concern, since NIS have the potential to generate adverse impacts on local ecosystems (Newell, 2004).

In this thesis, the effects of mariculture were analysed in terms of organic waste loading on the sedimentary characteristics, the benthic trophic status, meiobenthic community structure and the trophic interactions between mariculture-derived sources (animal faeces and/or feeds) and benthic organisms. Two mariculture cases in the Bohai Sea, China, i.e. open-water suspension farming of *A. irradians* (non-indigenous) (Chapter 2, 3, 4) as well as coastal shrimp farming of *L. vannamei* (non-indigenous) and *M. japonicus* (native) (Chapter 2, 5), were used as case-studies to analyse the potential effects and also to unravel the implications of the use of NIS in mariculture. In this chapter, we integrate the most important findings from previous chapters to describe the effects of mariculture of NIS at the abiotic, biotic and ecosystem functional levels. Based on those effects, the efficiency of abiotic and biotic indicators as well as using biomarkers (stable isotopes and fatty acids) to assess the effect of mariculture activities are compared and discussed. This chapter is concluded by some recommendations for management of mariculture, especially culturing

NIS.

6.1 Effects of mariculture on benthic ecosystems: an integrated overview

6.1.1 Case 1: open-water farming of the bay scallop *A. irradians*

Bivalve mariculture can potentially influence coastal marine ecosystems through filtering-biodeposition processes (Dame, 1996; Newell, 2004). In fact, the amount of farmed bay scallops (c.a. 1.07×10^9 ind.) at our study area, i.e. Laizhou Bay, potentially generates 1140 tonnes of organic particulate matter each day (Wang et al., 2018). We here argue that whether this downward organic flux of biodeposits affects the recipient benthic ecosystem in terms of the abiotic and biotic elements (Chapter 2, 3, 4).

One of the major concerns for environments receiving high amounts of organic deposits is that the resulting large oxygen consumption initiates anaerobic metabolic pathways, producing reduced sediments, decreasing the redox potential - Eh, and consequently affects the benthos and benthic ecosystem functions (McKindsey et al., 2011). Our data showed that the bay scallop farming did not affect the Eh on the muddy bottom, indicating that the decaying biodeposits on oxygen consumption rates may be balanced with the oxygen renewal rate from water exchange. Sandy sediments however showed a slight decrease in the Eh under the scallop farms, yet it still remained under oxic conditions, indicating no prevalence of anaerobic pathways (Chapter 2).

Next to these minor effects on oxygen level, the meiobenthic community structures in the sediment were affected at sites with high scallop farming densities (Chapter 3). Those stations with a density of 200 ind. m^{-2} also showed an enhanced BPC, indicating an enhanced flow of labile organic matters to the benthos (Chapter 2). This may affect the community structures and trophic groups of benthos by increasing the food availability to benthos (Pearson and Rosenberg, 1987; Rosenberg 2001). More specifically, the two stations of high- scallop density showed a different meiobenthic community response. In the sandy shallow area, meiobenthic community structure was only influenced at higher taxa level with an increase in nematode abundance. Furthermore, the nematodes consumed the biodeposits and their trophic levels indicated a change in feeding behavior under scallop farms, which is a typical behavior of opportunistic species (Chapter 4). In the muddy sediments of the deeper area, biodeposit inputs changed the copepod assemblages as well as increased their total density and species richness. The opportunistic copepod families e.g. Ectinosomatidae, Canuellidae, Miraciidae (specifically genus *Stenhelia*) were promoted (Chapter 3). The enhancement of copepods' density and species richness by scallop farming can be a consequence of consuming the biodeposits by those copepods (Chapter 3). We suggest that biodeposits alleviate the inter-specific competition within the copepod community. The high amount of biodeposits produced by the densely cultured bivalve is suggested to affect the functioning of the benthic ecosystem, e.g. fuel the secondary production (Callier et al., 2013; McKindsey et al., 2011; Wang et al., 2018). As expected, we demonstrated that biodeposits from scallops were consumed by harpacticoid copepods. Biodeposits were also incorporated into the diet of nematodes that occupy a lower trophic level

(TL c.a. 2). As such, the results imply that the mariculture-derived organic matter was incorporated into the basal food web of the benthic ecosystem (Chapter 4). As a matter of fact, biodeposits can be rich in nutrients (C:N ratio c.a. 2.5 in this study) and contain a high proportion of labile organic matter (McKindsey et al. 2010 and reference therein), making it an energy-enriched food source available for the meiobenthos.

Furthermore, we found that the consumption of biodeposits increased the PUFA level (in particular, DHA, Chapter 4) in the copepod family Canuellidae, which represented the dominant copepods in the scallop farming area and the main consumers of biodeposits. This may have implications beyond the observed benthic basal food web: bivalve mariculture is commonly conducted in coastal waters that usually serve as nursery ground for fish (Jin 2008; Weise et al., 2009). Since copepods are fatty acids conveyors to fish (Iverson, 2009), the PUFAs derived from bay scallop mariculture may transfer to the higher level of the marine coastal food web.

Above all, the effects of mariculture of bay scallop *A. irradians* have minimal effects on abiotic and biotic characteristics. In fact, the effect is rather positive in terms of the improved energy transfer to higher level of the aquatic food web. The lack of negative effects of culturing NIS *A. irradians* may be attributed to (1) the meso-oligo nature of the benthic environment in Laizhou Bay (Chapter 2; Wang et al. 2018), (2) the consumption of biodeposits by consumers from the basal food web (Chapter 4), and (3) the higher velocity of water flow (20 cm^{-1}) compared with those of bivalve farms with negative effects (Chapter 3). Thus, we highlight the importance of the hydrodynamics and the background of organic matter for choosing the site of bivalve farms, which can potentially avoid the benthic organic pollution.

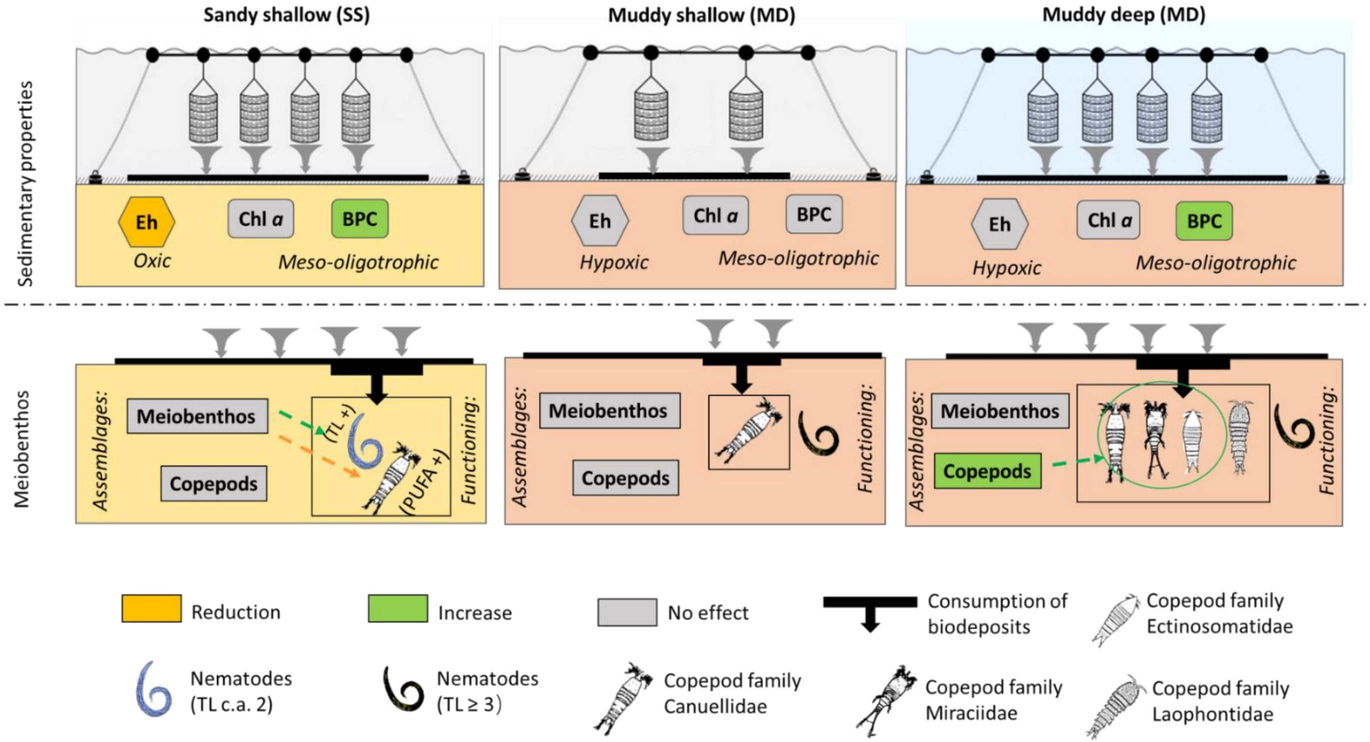

Figure 6.1 overview of effects of bay scallop farming on the benthic ecosystem. Eh = redox potential; Chl *a* = chlorophyll *a*; PUFA = Polyunsaturated fatty acids; TL = trophic level

6.1.2 Case 2: coastal ponds farming *M. japonicus* and *L. vannamei*

The state of the benthic environment in shrimp ponds is essential to the growth and survival of cultured shrimps (Avnimelech and Ritvo, 2003). Here we found different temporal patterns of environmental variables and meiobenthic community structure during the rearing time in *M. japonicus* and *L. vannamei* ponds.

First, in the early stage, there has seldom water exchange for both ponds. Feeding had been started in *L. vannamei* ponds (feed: frozen *Artemia* and smashed soybean, at 0.7 kg/ha/day and 1.8 kg/ha/day (both express as dry weight), respectively), while no external feed was added for juvenile *M. japonicus*. Increased feed addition in *L. vannamei* ponds went along with a rise in bulk organic contents, (TOC and TN), which was not observed in *M. japonicus* ponds, where feeding was not started during the early rearing stages (Chapter 2). Sedimentary Chl *a* largely increased in both ponds (Chapter 2). Application of N-P-K fertilizers right before the stocking may be a reason for this increase since the elevated nutrient levels can promote the growth of microalgae (Gamboa-Delgado 2014). The BPC levels were consequently elevated as well (Chapter 2). BPC accumulated more in *L. vannamei* ponds, due to the addition of shrimp feeds (Chapter 2), resulting in a eutrophic and a hypertrophic benthic state in *M. japonicus* and *L. vannamei* ponds, respectively. The sedimentary redox potential – Eh showed anoxic conditions in both ponds. Next to the sediment properties, the abundance of meiobenthos increased in both ponds (Fig 6.2 a, b). This increase was mainly due to an increased abundance of nematodes (Chapter 5), which are known to tolerate anoxic environments (Giere, 2009) and can be facilitated by the increasing food availability (Mirto et al., 2014).

During heavy feed addition in the late rearing stage (19-38 kg/ha/day trash fish and 18-27 kg/ha/day commercial feeds, both expressed as dry weight), *M. japonicus* ponds also started to show bulk organic matter (TOC and TN) enrichment and continued to accumulate BPC, reaching a hypertrophic state at the end of rearing period (Fig 6.2 c). Bulk organic matter and BPC leveled off in *M. japonicus* ponds. The redox potential of both ponds became more reduced (Fig 6.2 c, d). Meanwhile, the meiobenthic community structure and diversity however remained unchanged in the *M. japonicus* ponds (Fig c), while meiobenthos in *L. vannamei* ponds decreased in terms of richness and total abundance. At the end of the rearing cycle, meiobenthos in *L. vannamei* ponds formed a low-diversity community that was strongly dominated by nematodes (Fig 6.2 d), suggesting a disturbed condition (Chapter 5).

By comparing two types of ponds in the same stage, we observed that BPC levels, indicating labile organic enrichment, were significantly higher in *L. vannamei* ponds compared to *M. japonicus* ponds during all rearing stages. This may in part be attributed to the different feeding practices in the two pond types (see above for early stage). The lower stocking density (which generates less waste) and higher water renewal frequency especially in the late stage (which flushes out the nutrients and adds dissolved oxygen) in *M. japonicus* ponds may also affect labile organic enrichment. Other than those, the burrowing behavior of *M. japonicus* and transplanted *Corophium* (commercially raised and introduced to *M. japonicus* ponds in the early stage, expected to serve a food source to adult shrimp) may increase the mineralization process in the sediments of *M. japonicus* ponds.

The above discussion suggests that shrimp farming practices could be a reasonable explanation for the temporal changes of environmental variables and meiobenthic community structure in the pond bottoms. To further link those changes directly with shrimp rearing, control ponds without any rearing activity in the same area are needed for comparison.

The disease problems in shrimp farming industry have escalated throughout the recent decades. The outbreaks of viral and bacterial shrimp diseases (e.g. white spot syndrome) has caused huge economic losses, e.g. 750 million US dollars in China in 1993 (Primavera, 1998). The poor pond environment (e.g. excessive stocking density and poor water quality) has been closely linked with the occurrence of shrimp diseases (Spaargaren, 1998). The low oxygen levels, acid sulfide soils, the release of aluminum and iron precipitation, increase the physiological stresses of cultured shrimps and their susceptibility to pathogens (Kausty et al., 2000; Stevensson, 1997). Although the exchange of water may improve oxygen concentrations in the pond (Monhanty et al., 2015), the discharge of non-treatment effluent may spread among farms, which consequently causes self-pollution (Kausty et al., 2000), and furthermore, affect the wild stocks (Belak et al., 1999).

We also observed changes of the benthic energy flow. At the functional level, we demonstrated that meiobenthos represents a crucial component of the shrimps' diets, especially for adult shrimps. Interestingly, we also found that copepods and tranplanted *Corophium* in *M. japonicus* ponds and nematodes in *L. vannamei* ponds had high levels of PUFAs (especially high ω3-fatty acids) as well as high

DHA/EPA ratios in the late stage of the rearing cycle (Fig 6.2 c, d), pointing at an enhanced quality of these food items for adult shrimps (Chapter 5). This may be explained by shrimp feeds that were rich in ω3-PUFAs, i.e. fishmeal for *L. vannamei* and commericial feeds for *L. vannamei*, that were indirectly made available to the meiobenthos through the bacterial loop (Lytle et al., 1990; Ouraji et al., 2011). In fact, penaeid shrimps may benefit from consuming the PUFA-rich organisms since ω3 PUFAs can stimulate their maturation (Lytle et al., 1990). The stable isotopic modelling suggests that both *M. japonicus* and *L. vannamei* assimilated more meiobenthic food sources than external feeds. Additional analyses such as gut content analysis or fatty acids profiling are required to confirm this result and to further optimize shrimp feeding. Since our sampling design did not consider *Corophium* larger than 500 µm (ca. 20% of *Corophium* in *M. japonicus* ponds), future studies could be improved by using sieve mesh sizes larger than 500 µm, to estimate the full amount of food made available to shrimps by the transplantation of *Corophium*.

Above all, we draw conclusions that may apply for the extenstive farming of *M. japonicus* and *L. vannamei*: 1. Maintaining the pond environment at a less organic/nutrient enriched environment requires optimised rearing practices, e.g. a proper water exchange strategy and an appropriate provision of food sources (depend on the feeding preference of the shrimps). However, it should also be noticed that the large amount of organic and inorganic nutrients flushed out from the pond may cause self-pollution among ponds and furthermore deteriorate the receiving coastal marine areas (Holmer et al. 2008; Kausty et al., 2000; Yang et al. 2017). Thus, highly biotechnical approach (e.g.

bioflocs; see 6.4) can be a solution for the waste treatment (Balasubramanian et al., 2004). 2. Meiobenthos is a good food item for shrimps, being largely consumed by shrimps and representing a food soruce of high nutritional value (in terms of FA). 3. Transplanting benthos (such as *Corophium*) to shrimp ponds provide a nutritious food item for shrimps. Also, their bioturbation activity may lead to a lower extent of organic accumulation at the pond bottom.

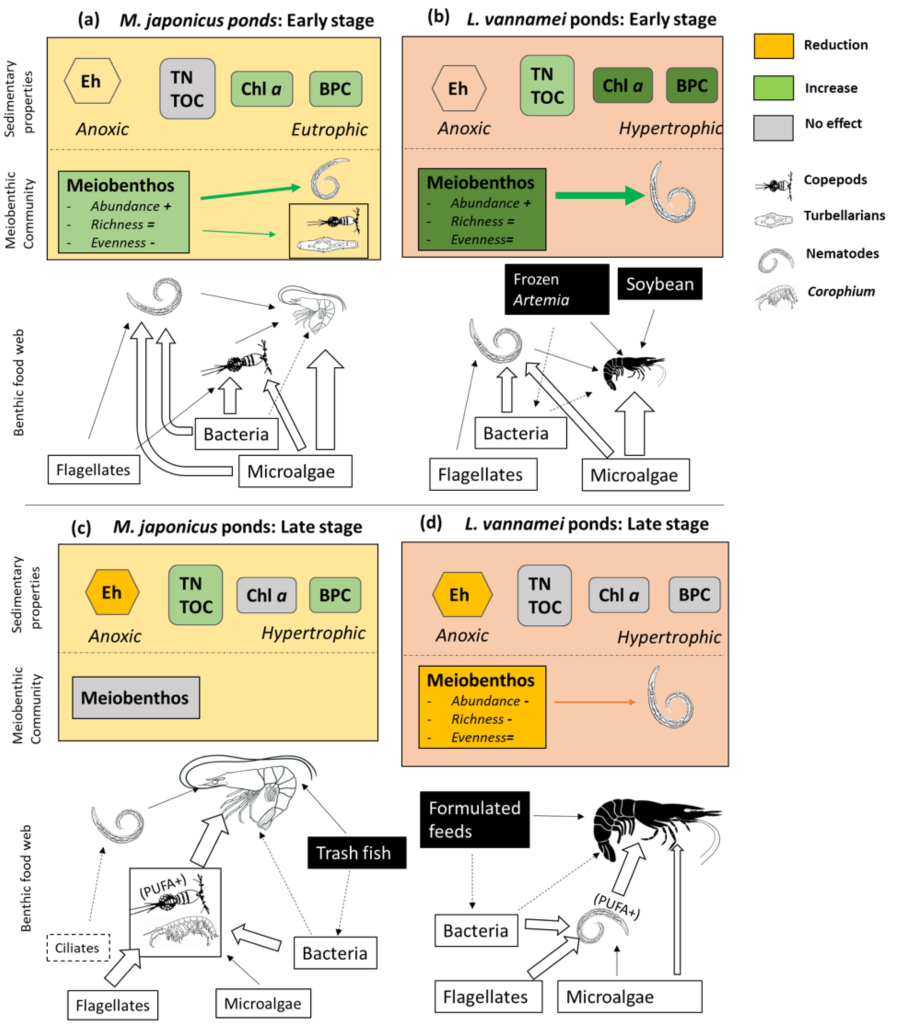

Figure 6.2 Overview of effects of farming *Penaeus japonicus* and *Penaeus vannamei* on the benthic environment and the benthic food web in each type of pond from the early and late rearing stages. Eh = redox potential; Chl *a* = chlorophyll *a*; PUFA = Polyunsaturated fatty acids; TL = trophic level; the size of the arrows indicates the proportions of contributions. The dashed line indicates uncertain roles of items or their contributions. The intensity of green color indicates the extent of

increase.

6.2 Evaluation of approaches for testing the environmental effects of mariculture

Due to the wide range of environmental effects of mariculture, there are also many methods to quantify those effects and assess the status of environment, including physio-chemical and ecological indicators (e.g. community structure and diversity of benthos) (Domínguez and Martín, 2004). We used physico-chemical and biochemical indicators (sedimentary redox potential, bulk organic concentration, biochemical composition, protein to carbohydrate ratio, chlorophyll *a*) as well as bioindicators (meiobenthic community structure and diversity and copepod to nematode ratio) to test in two mariculture systems, i.e. open-water scallop farms and coastal shrimp ponds. We also included biomarkers (stable isotopes and fatty acids) to assess the functional changes in the recipient ecosystems. We found that the assessment of both abiotic, biotic and functional effects are rather system-specific and lacked generality. Also, within a system, several indicators were not able to produce consistent conclusions in terms of organic enrichment (see further). Nevertheless, within these limitations, we were able to produce an evaluation and recommendation of the indicators based on the two referred cases.

6.2.1 Physio-chemical indicators

As expected, redox potential is indicative of the temporal changes that consistent with the heavy organic loading of shrimp ponds (Chapter 2). However, the relevance of redox potential as an indicator of mariculture effects is reduced when the organic enrichment is relatively low. For instance, redox potential did not respond to the scallop biodeposition in a muddy bottom environment (Chapter 2). Thus, when applying redox potential as an indicator in low organic accumulation systems, results must be interpreted with caution. We also found bulk organic contents (e.g. TN and TOC) is not able to detect the weak organic enrichment, which agrees with the finding when testing the benthic eutrophication of coastal ecosystems (Dell'Anno et al., 2002).

The lack of robustness of redox potential and the conservative nature of bulk organic matter make BPC the most suitable indicator to test the ecological status of mariculture ecosystems. In this research, the application of BPC proved to be reliable in both cases, in the sense that BPC not only promptly reacted to the mariculture activities but also managed to detect the modest organic accumulation in scallop farms (Chapter 2). In fact, BPC also provided indication that mariculture effects link to consumers, e.g., trophic status and food availability to the benthos (Chapter 3, 4, 5). The threshold of protein to carbohydrate ratio (PRO: CHO) to classify the level of eutrophication did not provide accurate information in the case of the shrimp ponds, since the PRO:CHO ratio was possibly biased by strong microbial activities during shrimp farming (Chapter 2, 5). Although not as sensitive as BPC, chlorophyll *a* can also provide information on photosynthetic primary production of a system. However, determining whether the source of Chl *a* is from allochthonous or autochthonous sources is impossible when using this indicator in a high organic deposition environment (Chapter 2).

We did not observe that sedimentary pH had clear pattern to indicate the mariculture effect. Since pH from sediment surface is very sensitive and can vary largely even within 0.5 cm (Brackmen et al., 2014; Mevemkamp et al., 2017). It is possible that the size of pH probe we used is too large that mixed the top 1 cm sediment layer and biased the result. Thus, a micro-sensor (tip size 500 μm) is needed for the accurate pH measurement for the top layer of sediment (Braeckman et al., 2014). Also, a pH profile can be helpful to interpret the result.

6.2.2 Bioindicators

Multivariate approaches provided a comprehensive view of community changes in relation to mariculture activities. Conversely, single indices (e.g. abundance, richness and evenness) are only able to reflect specific aspects of the community composition and can be masked by various factors, e.g. a small sample size can reduce the accuracy of richness index (Sofia, 2010), and the total abundance of the meiobenthic community remained unchanged despite changes in community structure (see sandy bottom in scallop farms in Chapter 3). However, the single community indices can complement the multivariate approaches for an interpretation of changes in community composition (See 6.1)

As for what taxonomic level of meiobenthic bioindicators should be applied, many studies suggested higher taxon levels as a powerful and rapid biomonitoring approach for fish farms (Grego, 2010; Mirto et al., 2012). We showed that analyzing meiobenthic community structure at higher-taxon level was able to indicate the temporal changes that likely link to shrimp farming practices (Chapter 5). Also, the meiobenthic community structure may fail to detect the modest effect of organic accumulation (Chapter 3). In addition, we found that both the community structure and diversity of meiobenthos did not show a consistent response at two taxonomic levels: higher-taxon- and of copepods at species-level (Chapter 3). This is in contrast to some impact studies (Daudi et al. 2012; Kennedy and Jacoby, 1999 and reference therein), which may due to the different types of pollution and the intensity of organic loading. However, assessment at copepod species level only showed higher resolution on muddy benthic environment than at meiobenthic higher-taxa level (Chapter 3), which reason is uncertain yet and need to be proved in further mesocosm experiments.

The Nematode/Copepod ratio (Ne/Co) showed a similar pattern as the meiobenthic community changes in the case of scallop farms. Thus, the sensitivity of the Ne/Co ratio is also limited in the sense that it only responds to biodeposition in sandy bottoms (Chapter 3). In the shrimp farming case, many samples did not allow to calculate Ne/Co ratios due to the absence of copepods. In such case, the Ne/Co ratio would reflect an extremely polluted condition, which make little sense for those sampled before shrimp farming. These results indicate that Ne/Co may be oversimplified and it should not be applied as a stand-alone indicator for monitoring environmental quality as many studies suggested (Lee et al., 2001; Sutherland et al., 2007).

Pearson and Rosenberg (1978) showed that the response of marine macrobenthic communities (in terms of their number of species – S, abundance – A and biomass – B; See SAB curve in Fig 1.5) along an organic input gradient is predictable, which was mainly attributed to the changes of oxygen (see Chapter 1 Section 1.2.2.2). We also plotted the abundance (a.k.a. density) and number of taxa/species of meiobenthos and copepods in response to the total organic carbon and biopolymeric carbon content across all sites in two sampling events (Appendix V Fig S1 and S2), to test if the response of meiobenthos and copepods corresponded to the SAB curve by Pearson and Rosenberg (1978). We did not observe any correlation between the abundance and number of taxa or species and the total organic carbon (TOC) or biopolymeric carbon (BPC) content (Fig S1 and S2). Only the abundance of meiobenthos seemed to increase with TOC initially when the TOC content ranged between 0.04% and 1.2% and then showed a decreasing trend with TOC contents above 1.2% (Fig. 6.3). However, due to a high variance among samples within the same TOC range and the short TOC gradient, we are not able to draw a response curve based on that. It would be interesting for future studies to obtain samples from a full spectrum of organic enrichment and oxygen gradient and compare the meiobenthos' response curve with that expected from the SAB model

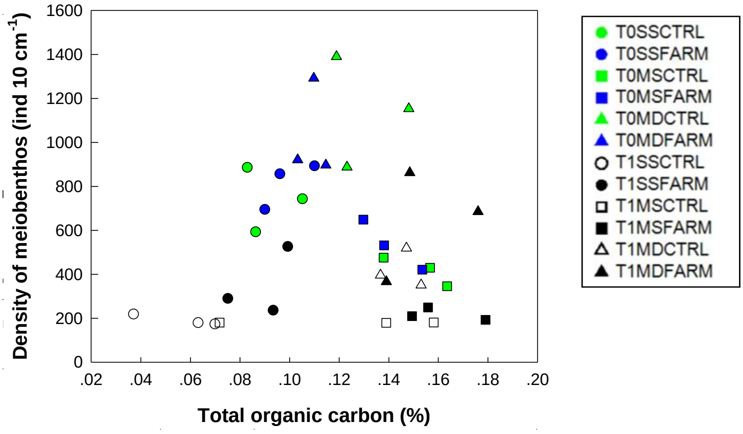

Figure 6.3 The response of meiobenthos density (a.k.a. abundance) total organic matter content in a scallop farming area in Laizhou Bay, China. T0 = before farming; T1 = during heavy biodeposition; CTRL = Control site; FARM = scallop

farming site. SS = sandy shallow station, MS = muddy sallow station, MD = muddy deep station.

6.2.3 Trophic analysis

While the above indicators mostly focus on describing an environmental state (for instance, whether the environment is eutrophic), we showed applying trophic analysis in mariculture assessment can further explain the state of environment, e.g. sediment properties and meiobenthic communities. More specifically, by using C and N stable isotopes to trace the fate of biodeposits from scallop farms, we could relate changes in the meiobenthic community to the increased food provision. Also, applying stable isotopes and fatty acids to depict the benthic energy flow in shrimps ponds helped us to unravel overfeeding as the driver of benthic eutrophication. Thus, trophic analyses may provide a better understanding of a system under stress that not only targets the effects of mariculture, but also depicts the broader impacts of mariculture activities on the ecosystem. Moreover, fatty acids profiles (in particular, concentrations of PUFAs, EPA, and DHA) directly quantified the quality of meiobenthos as food items for higher consumers. Incorporating trophic analyses is therefore recommended as an indicator to test mariculture effects on the functioning of ecosystems.

Above all, we agree with Keeley et al. (2012) that environmental assessment should focus on regionally validated measures, since the indicators provide different indications across ecosystems. We suggest that the environmental assessment of mariculture activities should have system-specific criteria. For instance, the shrimp farming activities, which are conducted in a semi-closed and artificial space with heavy feeding, do not require the same monitoring approaches as open-water mariculture systems. In addition, we suggest to interpret information from indicators at abiotic, biotic and functional levels that complement each other, especially at the initial phase of monitoring that the clear pattern are more difficult to detect. We also stress the importance of functional measurements, which provide a more integrated understanding of ecosystem changes.

6.3 NIS Mariculture

Next to the risk of biological invasion (mariculture escapees, see Miller et al. (2002)), culturing NIS can generate adverse effect on the local ecosystem due to their potential aggressive biological traits (See Chapter 1 Section 4). Although the NIS *A. irradians* has a relatively high biodeposition among those of the reported bivalves, including the native scallop *C. farreri* (Wang et al. 2018 and the reference there in), we did not find them to pose any ecological threat to the local benthic environment. Moreover, the biodeposits may potentially improve the local food web in terms of enhancing PUFA levels of copepods (see 6.1). However, this does not mean that we should encourage the wide-scale of culturing *A. irradians* to other marine coastal zones in China. After all, the effects of mariculture on recipient ecosystem can also depend on the localized factors, e.g. hydrodynamics, the types of sediments, the background organic concentrations and the benthic communities (See 6.1 case 1).

For the shrimp ponds, we found the *L. vannamei* ponds to be more polluted than the *M. japonicus* ponds in terms of heavier organic accumulation, which can be attributed to different breeding practice, e.g.

higher stocking density and lower frequency of water exchange in *L. vannamei* ponds (Chapter 2). *L. vannamei* is introduced to mariculture because of its high environmental tolerance which allows to rear very high densities (up to 400 shrimps m^{-2}), and also the high resistance to shrimp diseases (See Box 1.9). The densities of *L. vannamei* in our sampling ponds was 15-30 shrimps m^{-2}, which was 3-10 times higher than those of *M. japonicus*. Higher density of reared species generates more waste and consequently leads to a more polluted environment. Also, higher quantity of feeds input for *L. vannamei* was another reason that leads to heavier organic accumulation. It is noteworthy that in the early phase, *L. vannamei* ponds were already hypertrophic, which may be due to the deterioration of processed *Artemia* as a feed (Chapter 5). Moreover, the heavier benthic organic accumulation implies higher risk to pollute the adjacent ecosystem when disposing the pond sediments (Wu et al., 2014). However, the issues above are more relevant for the pond management rather than whether a species is non-indigenous or not, and can be solved by optimization of the feeds, applying new shrimp farming techniques (e.g. bioflocs, see further in 6.4). Thus, we cannot use the above issues as a reason to block farmers to culture NIS *L. vannamei*.

Another important issue of culturing NIS is that the escaping of broodstocks, in some cases, could manage to establish and become invasive to the local ecosystem, adversely affect aquatic biodiversity, ranging from polluting native wild genetic resources to reducing the resilience of local communities (Miller et al., 2002; Pillay, 2008). To the best of our knowledge, there is no record to show that *A. irradians* has an established population (i.e. naturalized, feral and breeding successfully; or released and breeding successfully) in the local ecosystems yet. In fact, Zhang et al. (2000) suggested that *A. irradians* is unlikely to establish a natural population in its major culture region in China (i.e. Yellow-Bohai Sea), because several local macrobenthos, e.g. *Asterias amurensis*, *Portunus trituberculatus*, could predate on *A. irradians* and control the population. For *L. vannamei*, its presence has been recorded in Yellow-Bohai Sea in the recent decade (Wu et al., 2016). However, there is no evidence that *L. vannamei* population in the wild is established from repeated releases from shrimp farms or from the successful natural reproduction. There is also a lack of research to evaluate its potential of invasiveness in the local environment. Further research should focus on tracing where those *L. vannamei* originate from, e.g. if *L. vannamei* in the wild could reach sexual maturation and manage to reproduce (Senanan et al., 2010). Also, the abundance of the *L. vannamei* in Yellow-Bohai Sea should be monitored regularly. NIS can potentially compete with local species though food consumption (Orlova et al., 2005) and may cause long-term consequences on the structure and function of aquatic ecosystems (Gallardo et al., 2016). In our study, the major food sources of *L. vannamei* from the shrimp ponds were particulate organic matter and meiobenthos. This diet has overlap compared with some of the native shrimps in Yellow-Bohai Sea, *Palaemon serrifer*, *Exopalaemon carinicauda*, and *Trachysalambria curvirostris* that were observed to largely ingest small crustaceans and phytoplankton (Yang, 2001). Also, other studies found *L. vannamei* was a voracious species that adaptable to any organic food items present in the environment (Bojórquez-Mascareño and Soto-Jiménez, 2013; Briggs et al., 2004; Dittel et al., 1997), as well as having a high food consumption rate (Chavanich et al., 2016). These evidences may suggest that *L. vannamei* could pose a threat to Yelow-Bohai Sea if it manages to establish reproductive populations in the wild. However, in order to draw further conclusions, laboratory experiments evaluating the feeding interactions between *L. vannamei* and other local shrimps should be established. So far, there is no direct data on the impact of escaped *L. vannamei* in China. Actually, many NIS cultures in China are similar as no negative effect has been found yet, but this does not mean that management actions can be postponed, because the risks may still remain due to a lag phase of bioinvasion (Lin et al., 2015). However, no policy or standardized risk assessment is available to manage and regulate these NIS (Zhan et al., 2017). Risk assessment and regular monitoring are necessary and of high priority for the early detection of bioinvasion when NIS has not established yet (Lin et al., 2015; Lodge et al., 2006). So far, the methodology developed for the Weed Risk Assessment (WRA) serves as a good example to follow up the risk assessment after introducing NIS (Lin et al., 2015; Pheloung et al., 1999). Models and tools such as Fresh-water Fish Invasiveness Scoring Kit (FISK) have been developed based on WRA (Lin et al., 2015). Such methodologies need to be modified, adjusted, and adopted to apply for different species and different condition of water (Lin et al., 2015; Zhan et al., 2017).

As for the question whether China should continue to import NIS for future mariculture, the outcome of this research is not in favor of a wide-scale introduction since the regulation and legislation associated with screening and monitoring of NIS are not developed enough (Lin et al., 2015). However, a wider debate should be encouraged to judge culturing

NIS in order to evaluate the risks and benefits from the socio-economic perspective, e.g. what are the market characteristics, what is the preference of consumers, how much profits can it bring to the stakeholders. Taking those cautious estimations into account, careful risk assessment should be applied before the introduction, including assessing the probability of establishment, environmental and economic consequences of a possible establishment (Hill, 2009). Since high variations of effects are observed across species and ecosystems due to the complex interactions between the functional traits of NIS and the local ecosystem (Liao et al., 2008; Vilà et al., 2011), we should also incorporate the functional diversity metrics and ecosystem processes into the assessment framework (Kuebbing et al., 2018). After the introduction, a rigid monitoring program should be applied to detect the mariculture effects on the local ecosystem as well as to record the escapees from farms. Nevertheless, more funding should be distributed on developing techniques for culturing native species (Pérez et al., 2003).

In conclusion, assessment of mariculture of NIS should include the risk assessment in the environmental and socio-economic context before the introduction. After the introduction, NIS culturing should be monitoring frequently and evaluated in terms of impacts on physio-chemical environment, structural composition of native benthic/pelagic organisms, marine food web (functional impact), and effect of the establishment of an escapee community.

6.4 Limitations and future perspectives

The outcome of this PhD research allowed to move a step forward in understanding the effects of mariculture NIS on benthic environment, using open-water and coastal ponds systems as two case-studies. However, this research also included some limitations and highlighted new perspectives for the future studies:

- **Improving the field sampling design – temporal scale**

Our sampling collection followed the planning of mariculture, i.e., scallop farming: before culture (May) vs. during heavy biodeposition phase (October-November), and shrimp farming: before culture (January), the early (May) and the late rearing stage (August). However, seasonal changes can also play a role for the abiotic and biotic components of the recipient ecosystem (Dell'Anno et al., 2002; Giere, 2009). This aspect was not covered in the case of scallop farming which culture activity includes summer, autumn, and early winter. We therefore recommend for future studies to take into account seasonal patterns in order to understand whether the impact of mariculture differs according to the season.

In Bohai Sea, algal blooms and sometimes even harmful algal bloom (HAB) occasionally happen in spring and autumn (Tang et al., 2006; Wei et al., 2004). Algae blooms will not only influence the behavior, health, and survival of cultured bivalves (Shumway, 1990), but also change the biochemical cycles in the benthic environment (Rozan et al., 2002). As far as we know, there is not such event in our studied regions during the time of sampling. However, it may be interesting to extend the mariculture-impact study to a longer term in order to incorporate the algal bloom.

- **Improving the field sampling design - spatial scale**

Another interesting research question for mariculture impact assessment is identifying weather the effect is localized or has been extended to a broader spatial scale. For instance, for the open-water mariculture, it is important to know whether and how far the farm-derived effluents can disperse. Also, it may be interesting to involve the organisms from the water column (plankton, nekton) to assess a more overall mariculture effect on benthic-pelagic coupling. For the coastal ponds system, we found that the shrimp ponds abundantly accumulated organic matter during the rearing season. This sediment usually disposes to the adjacent wetland or bay without any treatment, which might become a pollution source. To date, many studies about pond effluent affecting nearby coastal zones focus more on the water column (Wu et al., 2014; Yang et al., 2017), yet further studies should also include the benthic assessment (e.g. community and functional changes of (meio)benthos).

In addition, the sampling conducted in this research was applied at local and regional scales. Therefore, the effects can be idiosyncratic, for instance, our scallop farming region is characterized by meso-oligotrophic benthic environment. To generate more general conclusions, comparison studies should conduct in multiple regions and ecosystems for the similar type of mariculture installation. Such studies can apply in China that similar mariculture method is conducted on a long coastal line covered nine provinces with various hydrological and benthic characteristics (Wartenberg et al., 2017).

- **Applying lab experiments to unravel the underlying mechanisms**

Since this research is based on field sampling, the results and conclusions are highly ecological relevant but have low interpretability at the individual or species level (Höss and Williams, 2009). For instance, we found that copepods may accumulated PUFAs from the mariculture source, e.g. biodeposits and feeds. Future feeding experiments on single or multiple species may give insight on how these mariculture-derived PUFAs could be transferred to higher levels of consumers (e.g. fishes and shrimps). As such, feeding experiments can allow to further unravel the functional impact of mariculture. Yet, these experiments will be limited to include the overall setting from the field. Further, better functional insight can provide more information on how we can use copepods as mariculture feed at a commercial scale of production. The use of natural feed can lead towards a more sustainable mariculture with limited to no eutrophication effects.

- **Incorporating modeling studies to predict the mariculture effects**

The ultimate goal to support sustainable mariculture is to have good tools to predict any effects of mariculture on the environment. Therefore, monitoring the environmental effects from mariculture will allow to develop models in order to predict the relationship between culture activities and the environment. This information can be used by both farmers and public authorities (Domínguez and Martín, 2004). To date, several models can be applied or potentially applied for the assessment of mariculture effect, coupling the compartments of abiotic and biotic variables and ecosystem functioning (Piroddi et al., 2015 and the references therein). Future studies should be devoting to improve the robustness of these models so that they can be applied to different mariculture systems. Besides, using artificial intelligence for ecosystem services (ARIES) may also be applied for the future mariculture-derived impact assessment (Bagstad et al., 2013).

- **Promoting integrated multitrophic aquaculture (IMTA)**

Although this thesis focused on the mono-culture method, we highlighted that the consumers from the basal food web utilized the mariculture waste. This actually agrees with a recently popular concept of aquaculture - integrated multitrophic aquaculture (IMTA), as a means of practicing sustainable aquaculture by reducing nutrients though co-cultured species from different trophic levels (Chopin et al., 2008). IMTA has been practiced in China for a long time and there are many successful cases especially along the Yellow Sea coast in Shandong province (Fang et al., 2016; Mao et al, 2009; Yang et al., 2000; Zhou et al. 2006;). It would be interesting to compare the mariculture effects between mono-culture and IMTA systems, especially from the perspective of energy flow. Biomarkers (stable isotopes and fatty acids) proved to be a useful tool to study the trophic interactions (as in Irisarri et al., 2014; Sanz-Lazaro and Sanchez-Jerez, 2017), which can further be applied to assess the efficiency of IMTA.

- **Applying bioflocs technique in the shrimp farming industry**

Although water exchange may alleviate the organic accumulation in the shrimp ponds, the discharge of high nutrients pond water poses a threat to the receiving environment (Yang et al., 2017). One technique that has been developed to mitigate the negative effects of discharging shrimp farming effluents to the adjacent environment is the use of bioflocs technology, especially for the semi-intensive and intensive farming (Crab et al., 2012). This system produces microbial cells that aggregate into flocs (Avnimelech, 2009). Microbial biomass rapidly takes up the ammonium and this promotes the water quality in the shrimp pond (Burford, 2003; Hargreaves, 2006). The bioflocs also serve as a feed for reared shrimps since bioflocs have high nutritional values in terms of protein, lipid, and PUFA content, which on the other hand lower the feed input, especially fishmeal input (Crab et al., 2010; De Schryver et al., 2008). The short-chain fatty acids produced by bioflocs that protects against pathogenic disease is another advantage to use this technology (De Schryver et al., 2008). As the nutrients are well balanced in the system, water exchange reduces to a minimal level. Studies have found that in comparison to conventional ponds, bioflocs are a low-cost sustainable method (Avnimelch, 2009; De Schryver et al., 2008). Thus, this technique can apply to our study area as well as it will be widely used in future aquaculture.

- **Incorporating socio-economic context for the mariculture assessment**

Mariculture activities not only depend and impact on the environment, but also interact with socio-economic factors, since most of the farmers choose the culture method based on the financial costs and benefits. To date, aquaculture management (including mariculture) often focuses to maximize the output of targets (e.g. the production) instead of profits maximization, which is rather economically inefficient and include economic and ecological risks (Tsani and Koundouri, 2018). Therefore, it is necessary to combine environmental and socio-economic effects into mariculture assessment. Although the information of mariculture based in socio-economic context is relatively undeveloped compared with biological and technical aspect, there are a few frameworks taking both environmental and socio-economic parameters into account, for instance, “Emergy” from Odum (1996), that may apply in the future assessment. However, the methodologies are still needed for improvement especially when tackling with various small-scale mariculture activities in China.

- **Comparing the environmental effects of culturing native species and non-indigenous species**

In this thesis, in the scallop farming case, we aimed to study the benthic effects of culturing non-indigenous scallop *A. irradians*. It would be interesting to conduct the similar study in native scallop farms (e.g. *C. farreri*) for comparison. This may help the decision maker to choose one species over another for sustainable mariculture. In the shrimp farming case, we acknowledge that the factor of farming practices (e.g. stocking density of shrimps, water renewal, feeding addition) is crucial to determine the pond environment. Due to different farming practices of *M. japonicus* and *L. vannamei* in this study, the factor of biological traits of shrimp cannot be separated and discussed alone regarding whether the native *M. japonicus* is better than non-indigenous *L. vannamei* or not. Future mesocosm experiment for the two species with the same rearing practice is needed in order to further compare biological traits (e.g. bioturbation, food preference) and link these traits to the changes of pond environment.

Appendix I – supporting material Chapter 2

Table S1 The sediment composition and the mass median diameter (D50) (mean±SD) in the sampled area in T1 (October-November, 2016) in Laizhou Bay, China. Values represent average of 3 replicates. The sand fractions in this study are all within a range of 63-250 μm (i.e., fine sand). Farm = farm site, Ctrl = control site.; SS = sandy shallow station, MS = muddy sallow station, MD = muddy deep station

Site	**Clay (%)**	**Silt (%)**	**Fine sand (%)**	**D50 (μm)**
Farm SS	2.0±0.1	26.9±0.9	71±1.0	76.2±0.7
Ctrl SS	1.8±0.1	24.4±0.9	73.6±0.8	77.6±0.5
Farm MS	4.4±0.5	54.6±1.5	40.8±1.8	56.1±1.8
Ctrl MS	4.7±0.4	54.2±0.4	41.0±0.8	57.8±1.2
Farm MD	4.4±0.6	52.4±0.3	43.0±0.8	58.9±1.0
Ctrl MD	4.3±0.4	52.7±1.5	42.8±1.6	58.7±1.1

Table S2 The sediment composition and the mass median diameter (D50) (mean±SD, with n replicates) in the sampled *Marsupenaeus japonicus* and *Litopenaeus vannamei* ponds in Bohai Bay coast, China. Values represent average of sediments sampled in the early (May, 2016) and the late rearing stages (August, 2016). The sand fractions in this study are all within a range of 63-500 µm. NAT P = *M. japonicus* pond, NIS P = *L. vannamei* pond.

Pond	**Clay (%)**	**Silt (%)**	**Fine sand (%)**	**Medium sand (%)**	**D50 (µm)**
NAT P1	81.9±3.1 (5)	13.4±1.5 (5)	3.73±2.48 (5)	0.82±0.21 (5)	25.6±2.0 (5)
NAT P2	82.2±4.3 (6)	11.1±1.8 (6)	6.21±2.42 (6)	0.34±0.27 (6)	28.4±1.2 (6)
NAT P3	87.8±2.2 (6)	10.4±2.0 (6)	1.50±1.24 (6)	0.15±1.00 (6)	25.8±1.9 (6)
NIS P4	95.7±0.8 (3)	4.27±0.8 (3)	0.02±0.01 (3)	0	16.9±2.5 (3)
NIS P5	93.1±1.8 (5)	5.72±1.3 (5)	1.17±1.72 (5)	0	19.5±2.6 (5)
NIS P6	90.6±1.7 (6)	7.96±1.7 (6)	1.40±0.92 (6)	0	24.7±6.9 (6)

Table S3 Results of PERMANOVA tests for the differences in measured variables in the water column and in the sediments, from a scallop farming area of Laizhou Bay and in two types of shrimp ponds from Bohai Bay coast, China. Factors and/or interactions significantly (at *= $p < 0.05$; **= $p < 0.001$; ***= $p < 0.001$) distinguishing groups are displayed. df = degree of freedom; MS = mean square; P-F = Pseudo-F. Scallop farms: T0 = before scallop farming, T1 = high biodeposition period; SS = sandy shallow station, MS = muddy sallow station, MD = muddy deep station; Shrimp ponds: T0 = before shrimp farming; Early = the early rearing stage, Late = the late rearing stage; NAT P = *Marsupenaeus japonicus* ponds; NIS P = *Litopenaeus vannamei* ponds.

Compartment	**Parameter**	**Factor**	**df**	**MS**	**P-F**	**P (MC)**
Scallop farms: water column	Temperature	Time	1	2.7	396.33	**
		Station	2	0	4.43	*
		Time×Station	2	0.01	85.85	***
	Salinity	Time	1	7.77	58.62	*
		Station	2	0.23	463.93	***
		Time×Station	2	0.13	272.85	***
		Site×Station	2	0.01	12.55	***
		Time×Site×Station	2	0	5.16	*
	DO	Time×Station	2	1.28	25.35	***
	pH	Station	2	0.05	41.55	***
		Time×Station	2	0.02	14.36	***
Scallop farms: sediment	pH	Time	1	0.14	28.478	*
		Station	2	0.01	12.378	***
		Time×Station	2	0.01	6.25	**
	Eh	Station	2	124870	146.97	***
		Time×Station	2	16517	19.439	**
		Time×Site×Station	2	3465.7	4.0789	*
	Total nitrogen	Station	2	0.43	7.3	**
		Time×Station	2	1.14	19.2	***
	Total organic carbon	Time×Station	2	0.22	101.98	*
	Carbon to nitrogen ratio	Station	2	1.12	33.28	***

		Time×Station	2	0.22	6.46	**

	Parameter	Factor	df	MS	P-F	P (MC)
	Chlorophyll *a*	Time	1	18.06	40.95	*
		Station	2	0.76	11.37	***
		Site	1	1.21	850.67	**
		Time×Station	2	0.44	6.57	**
	Protein	Station	2	1.8276	148.95	***
		Time×Station	2	0.17792	14.5	***
		Site×Station	2	0.052289	4.2616	*
		Time×Site×Station	2	0.04	3.31	*
	Carbohydrate	Site	2	4.41	66.53	***
		Time×Site	2	1.05	15.85	***
		Site×Station	2	0.39	5.85	**
		Time×Site×Station	2	0.44	6.59	**
	Lipid	Time×Site	2	0.08	35.5	***
	Biopolymeric carbon	Station	2	1.48	136.10	***
		Time×Station	2	0.09	7.93	**
		Site×Station	2	0.07	6.41	**
		Time×Site×Station	2	0.08	6.95	**
	Algae C to BPC	Time	1	25.48	27.79	*
		Station	2	0.27	3.66	*
		Time×Station	2	0.92	12.43	***
Shrimp ponds: water column						
	Temperature	Stage	1	11	220.07	***
		Pond(Shrimp)	4	0.07	23.46	***
		Stage×Pond(Shrimp)	4	0.05	16.04	***
	Salinity	Shrimp	1	0.38	54.84	**
		Pond(Shrimp)	4	0.01	295.15	***
		Stage×Shrimp	1	0.02	8.5	*
		Stage×Pond(Shrimp)	4	0	96.71	***
	DO	Stage	1	107.8	26.55	**
		Stage×Shrimp	1	32.27	7.95	*
		Stage×Pond(Shrimp)	4	4.06	5.13	**

	pH	Shrimp	1	0.9	11.75	*

Compartment	**Parameter**	**Factor**	**df**	**MS**	**P-F**	**P (MC)**
Shrimp ponds: sediment	pH	Pond(Shrimp)	4	0.29	8.1902	***
		Stage×Pond(Shrimp)	4	0.18	5.034	**
	Eh	Stage	1	113220	37.2	**
		Pond(Shrimp)	4	12183	10.001	***
	Total nitrogen	Stage	2	0.09	35.17	***
		Stage×Shrimp	2	0.01	4.84	*
	Total organic carbon	Stage	2	3.47	33.04	***
	Chlorophyll *a*	Stage	2	1143.3	18.88	***
		Shrimp	1	1511.8	13.65	*
		Stage×Shrimp	2	406.86	6.72	*
	Protein	Stage	2	3.89	58.56	***
		Shrimp	1	1.04	8.41	*
		Pond(Shrimp)	4	0.12	7.66	***
		StagexPond(Shrimp)	8	0.07	4.12	**
	Carbohydrate	Stage	2	582.06	21.22	***
		Shrimp	1	1468.5	24.06	**
		Pond(Shrimp)	4	61.04	3.74	*
		StagexShrimp	2	387.83	14.14	**
	Lipid	Stage	2	0.28	13.96	**
		Shrimp	1	0.69	31.65	**
	Biopolymeric carbon	Stage	2	272.03	22.04	***
		Shrimp	1	528.4	22.72	**
		Pond(Shrimp)	4	23.26	3.41	*
		StagexShrimp	2	89.97	7.29	*
	Algae C to BPC	Stage	2	1.69	29.62	***
		Pond(Shrimp)	4	0.25	5.15	**
		StagexShrimp	2	1.94	34.06	***

Appendix II – supporting material Chapter 3

Table S1. Sediment characteristics at each farm and control site in three stations (SS, MS, MD) before aquaculture (T0) and during heavy biodeposition (T1). CTRL = control; TN = total nitrogen; TOC = total organic carbon; C/N = carbon and nitrogen ration; Chl *a* = chlorophyll *a*; BPC = biopolymeric carbon. CTRL = control site; FARM = scallop farming site.

		TN (%)	TOC (%)	C/N	Chl *a* (μg g^{-1})	Protein (mg C g^{-1})	Carbohydrate (mg C g^{-1})	Lipid (mg C g^{-1})	BPC (mg C g^{-1})
T0	SS-CTRL	0.03±0.004	0.09±0.011	3.66±0.22	0.75±0.364	0.41±0.022	0.81±0.069	0.21±0.026	0.72±0.042
	SS-FARM	0.03±0.001	0.1±0.010	3.66±0.368	1.01±0.257	0.4±0.043	0.78±0.087	0.19±0.018	0.68±0.073
	MS-CTRL	0.03±0.007	0.15±0.013	4.92±0.744	1.49±0.102	0.78±0.065	1.54±0.134	0.31±0.041	1.3±0.100
	MS-FARM	0.03±0.008	0.14±0.012	4.53±0.758	1.71±0.216	0.72±0.052	1.44±0.077	0.32±0.039	1.23±0.061
	MD-CTRL	0.03±0.003	0.13±0.016	3.96±0.509	1.45±0.412	0.66±0.062	1.21±0.124	0.27±0.022	1.06±0.098
	MD-FARM	0.03±0.005	0.11±0.006	3.45±0.379	1.7±0.299	0.62±0.049	1.07±0.050	0.27±0.034	0.98±0.044
T1	SS-CTRL	0.07±0.02	0.06±0.017	0.86±0.071	0.24±0.062	0.27±0.030	0.40±0.005	0.35±0.126	0.57±0.087
	SS-FARM	0.11±0.055	0.09±0.013	0.94±0.458	0.36±0.117	0.53±0.016	1.06±0.030	0.46±0.045	1.08±0.055
	MS-CTRL	0.03±0.009	0.12±0.045	3.84±0.509	0.31±0.016	0.65±0.126	2.37±0.525	0.19±0.005	1.56±0.211
	MS-FARM	0.04±0.006	0.16±0.016	4.14±0.586	0.54±0.118	0.9±0.147	2.32±0.343	0.21±0.004	1.65±0.181
	MD-CTRL	0.03±0.007	0.15±0.008	4.61±0.897	0.19±0.023	0.85±0.063	1.48±0.358	0.23±0.038	1.24±0.175
	MD-FARM	0.04±0.003	0.15±0.019	4.34±0.208	0.34±0.09	1.05±0.118	2.91±0.464	0.3±0.034	2.07±0.269

Table S2 Univariate of PERMANOVA to analyze variations in total density of meiobenthos, biodiversity indexes of meiobenthos: number of species (S), Shannon-Wiener diversity index (H'), Peilou's evenness (J’) and dominance (λ), and the ratio of nematodes to copepods (Ne/Co) among the levels of the three factors: Time (T0 vs T1), Site (Farm vs Control), and Station (SS, MS, and MD). T0 = before aquaculture; T1 = during heavy biodeposition. df = degree of freedom; MS = mean square; P-F = Pseudo-F.

S (Number of ta×a)

Source	df	MS	P-F	P(MC)
Time	1	77.21	0.07	ns
Station	2	454.35	3.12	ns
Site	1	85.60	0.69	ns
Time×Station	2	1173.20	8.05	**
Time×Site	1	416.36	6.00	ns
Station×Site	2	124.77	0.86	ns
Time×Station×Site	2	69.37	0.48	ns
Res	24	145.76		
Total	35			

N (total density)

Source	df	MS	P-F	P(MC)
Time	1	12843.00	10.43	*
Station	2	3780.10	28.87	**
Site	1	846.16	13.02	*
Time×Station	2	1231.80	9.41	**
Time×Site	1	288.22	1.02	ns
Station×Site	2	64.97	0.50	ns
Time×Station×Site	2	283.68	2.17	ns
Res	24	130.96		
Total	35			

λ (dominance)

Source	df	MS	P-F	P(MC)
Time	1	959.83	6.38	ns
Station	2	31.01	1.35	ns
Site	1	3.95	0.06	ns
Time×Station	2	150.44	6.53	**
Time×Site	1	7.53	0.22	ns
Station×Site	2	63.38	2.75	ns
Time×Station×Site	2	33.82	1.47	ns
Res	24	23.04		
Total	35			

H' (Shanon-Wiener diversity)

Source	df	MS	P-F	P(MC)
Time	1	9803.30	3.50	ns
Station	2	1228.30	4.33	*
Site	1	176.37	0.34	ns
Time×Station	2	2797.50	9.87	**
Time×Site	1	55.72	0.41	ns
Station×Site	2	522.60	1.84	ns
Time×Station×Site	2	134.83	0.48	ns
Res	24	283.45		
Total	35			

J' (Peilou's eveness)

Source	df	MS	P-F	P(MC)
Time	1	9796.60	5.98	ns
Station	2	1424.30	6.85	**
Site	1	324.63	0.66	ns
Time×Station	2	1637.90	7.88	**
Time×Site	1	434.12	2.10	ns
Station×Site	2	492.82	2.37	ns
Time×Station×Site	2	207.03	1.00	ns
Res	24	207.89		
Total	35			

Ne/Co (Nematode to copepod ratio)

Source	df	MS	P-F	P(MC)
Time	1	7701.30	1.82	ns
Station	2	5459.10	13.23	**
Site	1	554.15	0.51	ns
Time×Station	2	4228.10	10.25	**
Time×Site	1	420.80	0.26	ns
Station×Site	2	1094.80	2.65	*
Time×Station×Site	2	1604.20	3.89	**
Res	24	412.57		
Total	35			

Table S3 Univariate of PERMANOVA to analyze variations in total density of harpacticoid copepods and the biodiversity indexes of harpacticoid copepods: number of species (S), Shannon-Wiener diversity index (H'), Peilou's evenness (J') and dominance (λ) among the levels of the three factors: Time (T0 vs T1), Site (Farm vs Control), and Station (SS, MS, and MD). T0 = before aquaculture; T1 = during heavy biodeposition. df = degree of freedom; MS = mean square; P-F = Pseudo-F.

S (Number of species)					*N* (total density)					λ (dominance)				
Source	df	MS	P-F	P(MC	Source	df	MS	P-F	P(MC	Source	df	MS	P-F	P(MC
Time	1	40.11	0.30	ns	Time	1	657.70	0.17	ns	Time	1	8033.2	3.25	ns
Station	2	252.3	62.6	**	Station	2	11731.0	34.1	**	Station	2	9664.1	43.7	**
Site	1	21.78	1.80	ns	Site	1	172.43	0.21	ns	Site	1	38.44	0.14	ns
Time×Station	2	133.4	33.1	**	Time×Station	2	3893.60	11.3	**	Time×Station	2	2468.2	11.1	**
Time×Site	1	25.00	0.99	ns	Time×Site	1	238.93	0.17	ns	Time×Site	1	811.72	2.49	ns
Station×Site	2	12.11	3.01	ns	Station×Site	2	808.77	2.35	ns	Station×Site	2	270.11	1.22	ns
Time×Station×Si	2	25.33	6.29	**	Time×Station×Si	2	1366.10	3.97	**	Time×Station×Si	2	326.03	1.47	ns
Res	2	4.03			Res	2	343.87			Res	2	221.15		
Total	3				Total	3				Total	3			

H' (Shanon-Wiener diversity)					J' (Peilou's eveness)				
Source	df	MS	P-F	P(MC	Source	df	MS	P-F	P(MC
Time	1	1.33	2.57	ns	Time	1	340.29	4.65	ns
Station	2	1.27	30.6	**	Station	2	146.53	3.90	*
Site	1	0.01	0.57	ns	Site	1	0.43	1.12	ns
Time×Station	2	0.52	12.4	**	Time×Station	2	73.24	1.95	ns
Time×Site	1	0.07	22.8	ns	Time×Site	1	4.89	1.74	ns
Station×Site	2	0.01	0.35	ns	Station×Site	2	0.38	0.01	ns
Time×Station×Si	2	0.00	0.07	ns	Time×Station×Si	2	2.81	0.07	ns
Res	2	0.04			Res	2	37.62		
Total	3				Total	3			

Table S4 Main tests and pairwise tests of PERMANOVA to analyze variations in the composition of biopolymeric carbon (BPC): proteins, carbohydrates, lipids, among the levels of the three factors: Time (T0 vs T1), Site (Farm vs Control), and Station (SS, MS, and MD). T0 = before aquaculture; T1 = during heavy biodeposition, df = degree of freedom; MS = mean square; P-F = Pseudo-F.

Source	df	MS	P-F	P (MC)
Time	1	3.671	0.258	ns
Station	2	22.535	50.925	**
Site	1	4.601	4.431	ns
TimexStation	2	14.228	32.151	**
TimexSite	1	8.029	6.484	*
StationxSite	2	1.038	2.346	ns
TimexStationxSite	2	1.238	2.798	*
Res	24	0.443		
Total	35			
pairwise test: FARM VS CTRL			t	P (MC)
T0-SS			1.111	ns
T0-MS			0.585	ns
T0-MD			0.706	ns
T1-SS			3.171	**
T1-MS			1.826	ns
T1-MD			3.013	*

Table S5 Correlation coefficients and percent significance level (%) associated with meiobenthic community structure and harpacticoid copepod assemblage (RELATE test for seriation). TN = total nitrogen; TOC = total organic carbon; Chl *a* = Chlorophyll *a*; PRO = proteins; CHO = carbohydrates; LIP = lipids; BPC = biopolymeric carbon; Eh = redox potential.

Variable	Meiobenthos community		Harpacticoid copepod assemblage	
	Rho	Sig. level %	Rho	Sig. level %
TN	0.067	17.1	0.022	33.2
TOC	0.231	0.2	0.059	12.5
Chl *a*	0.159	0.5	0.155	1.4
BPC	0.327	0.1	0.120	3.2
PRO	0.255	0.1	0.129	1.9
CHO	0.356	0.1	0.119	1.9
LIP	0.050	19.5	0.151	0.7
pH	0.273	0.1	0.096	5.6
Eh	0.171	0.3	-0.018	59.1

Table S6. Summary of results from the BIO-ENV analysis showing best 10 combinations of environmental variables associated with the highest correlation between meiobenthos (a)/copepods (b) and environmental matrices. Correlation values correspond to Spearman's rank correlation coefficient (ρ). TOC = total organic carbon; Chl *a* = Chlorophyll *a*; PRO = proteins; CHO = carbohydrates; LIP = lipids; Eh = redox potential.

No. of variables	Correlation	Environmental variables
(a) Meiobenthos		
2	0.453	PRO, CHO,
2	0.452	CHO, pH
2	0.445	TOC, CHO
4	0.437	TOC, PRO, CHO, pH
4	0.42	Chl *a*-CHO, pH
5	0.415	TOC-CHO, pH
4	0.413	TOC, Chl *a*, CHO, pH
1	0.409	CHO
3	0.408	Chl *a*, CHO, pH
4	0.407	PRO, CHO, Eh, pH
(b) Copepods		
2	0.223	Chl *a*, PRO
3	0.222	Chl *a*, Eh, pH
3	0.218	Chl *a*, PRO, pH
2	0.217	Chl *a*, Eh
3	0.217	Chl *a*-CHO
4	0.214	Chl *a*, PRO, Eh, pH
4	0.211	Chl *a*-CHO, Eh
3	0.21	Chl *a*, PRO, Eh
4	0.209	Chl *a*-CHO, pH
5	0.202	Chl *a*-CHO, Eh, pH

Appendix III – supporting material Chapter 4

Table S1. T-test for the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values of the organic matter of sediment surface (SSOM) between each pair of farm and control site in Station SS, MS and MD. Significance level: NS=not significant, *p < 0.05; **p < 0.01; ***p < 0.001, was applied for t-test.

Year		SS			MS			MD		
		df	F	p	df	F	p	df	F	p
2015	$\delta^{13}C$	4	0.565	0.602^{NS}	2	0.317	0.781^{NS}	4	0.044	0.967^{NS}
	$\delta^{15}N$	4	6.262	0.067^{NS}	4	0.521	0.630^{NS}	4	2.226	0.690^{NS}
2016	$\delta^{13}C$	n/a	n/a	n/a	4	1.126	0.323^{NS}	4	0.798	0.422^{NS}
	$\delta^{15}N$	n/a	n/a	n/a	4	0.039	0.971^{NS}	4	0.718	0.445^{NS}

Table S2 T-test comparison between farm and control site for the fatty acid (FA) markers in relative FA composition or FA ratios in harpacticoid copepod family Canuellidae in Station SS in 2016 during aquaculture activities. EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; PUFA: polyunsaturated fatty acids. EPA, DHA, and PUFA are indicative for quality of food. Other biomarkers relevant for meiobenthos diet are 16:1ω7 and 16:1ω7/16:0: diatoms; EPA/DHA: diatom/dinoflagellate; SUM 15:0, 17:0: bacteria; 18: 2ω6: terrestrial detritus or green algae; 20:1ω9 and PUFA/SFA ratio: carnivory. Significance level: NS=not significant, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, was applied for t-test.

Marker FA	t	df	*p*
EPA	0.061	5	0.954^{NS}
DHA	4.701	5	0.005^{**}
PUFA	4.236	5	0.008^{**}
16:1ω7	1.176	5	0.292^{NS}
SUM 15,17	0.188	5	0.858^{NS}
20:1ω9	4.858	5	0.004^{**}
18:2ωw6	0.633	5	0.554^{NS}
16:1ω7/16:0	1.654	5	0.158^{NS}
EPA/DHA	3.100	5	0.026^{*}
PUFA/SFA	4.523	5	0.006^{**}

Appendix IV – supporting material Chapter 5

Table S1 Permutational analysis of variance (PERMANOVA) results testing the variations of meiobenthic number of taxa, total abundance, and diversity indices (Shannon *H'*) and equitability (*J'*, Pielou's evenness) among rearing stages (Stage), types of shrimp ponds (Shrimp), and pond (nested in Shrimp). Significance level: $p < 0.05$; $*p < 0.01$; $**p < 0.001$ and are given in bold.

Source	df	MS	P-F	P(perm)	perms	P(MC)
Number of taxa						
Stage	2	2355.7	2.1	0.183	9959.000	0.156
Shrimp	1	1931.3	2.2	0.335	720.000	0.173
Pond (Shrimp)	4	914.6	2.6	**0.024**	9943.000	**0.025**
Stage×Shrimp	2	5272.6	4.8	0.050	9960.000	**0.031**
Stage×Pond(Shrimp)**	6	1121.3	3.2	**0.004**	9940.000	**0.004**
Res	35	346.2				
Total	50					
Total abundance						
Stage	2	21489.0	15.2	**0.000**	9960.000	**0.000**
Shrimp	1	21757.0	3.1	**0.015**	719.000	0.081
Pond (Shrimp)	4	7595.7	15.8	**0.000**	9933.000	**0.000**
Stage×Shrimp	2	9881.1	7.0	**0.004**	9954.000	**0.001**
Stage×Pond(Shrimp)**	6	1437.6	3.0	**0.000**	9897.000	**0.000**
Res	35	479.4				
Total	50					
Shannon *H'*						
Stage	2	3586.3	6.1	**0.028**	9946.000	**0.018**
Shrimp	1	321.6	0.3	0.873	720.000	0.699
Pond (Shrimp)	4	1114.1	3.5	**0.008**	9949.000	**0.008**
Stage×Shrimp	2	3792.4	6.5	**0.030**	9964.000	**0.015**
Stage×Pond(Shrimp)**	6	593.5	1.8	0.090	9940.000	0.084
Res	35	322.6				
Total	50					
Equitability (*J'*))						
Stage	2	1435.1	0.5	0.865	9936.000	0.858
Shrimp	1	26748.0	29.4	**0.004**	718.000	**0.000**
Pond (Shrimp)	4	865.7	0.7	0.703	9917.000	0.713
Stage×Shrimp	2	1849.2	0.6	0.732	9954.000	0.738
Stage×Pond(Shrimp)**	5	3249.4	2.8	**0.001**	9907.000	**0.001**
Res	25	1162.2				
Total	39					

Table S2 Non-parametric Kruskal-Wallis tests to compare the relative contribution of selected fatty acids between stages in meiobenthos belong to the same taxon. EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, PUFA = polyunsaturated fatty acids, FA = fatty acids, NAT = *Penaeus japonicus*, *NIS* = *Penaeus vannamei*. The significant level is $p < 0.05$ and the significant p is given in bold. The H and p values are adjusted for ties.

	Copepods_NAT ponds			Nematodes_NAT ponds			Nematodes_NIS ponds		
	df	H	p	df	H	p	df	H	p
Σ 15, Σ 17	1	0.381	0.537	1	5.00	**0.025**	1	3.86	**0.05**
Σi-FA+ai-FA	1	7.714	**0.005**	1	5.00	**0.025**	1	0.43	0.531
18:1ω7	1	11.508	**0.001**	1	5.00	**0.025**	1	1.19	0.275
16:1ω7	1	7.714	**0.005**	1	5.00	**0.025**	1	3.86	**0.05**
EPA	1	7.714	**0.005**	1	5.00	**0.025**	1	1.19	0.275
DHA/EPA	1	7.714	**0.005**	1	0.20	0.655	1	3.86	**0.05**
18 PUFA	1	5.357	**0.021**	1	5.00	**0.025**	1	0.43	0.513
18:4ω3	1	11.508	**0.001**	1	1.14	0.285	1	1.00	0.317
18:1ω9	1	11.508	**0.001**	1	0.02	0.881	1	2.33	0.127
DHA	1	7.714	**0.005**	1	5.00	**0.025**	1	2.33	0.127
18.2ω6	1	11.508	**0.001**	1	0.56	0.456	1	0.43	0.513
18.3ω3	1	0.095	0.758	1	5.00	**0.025**	1	0.43	0.513
18:1ω9/18:1ω7	1	11.508	**0.001**	1	0.56	0.456	1	0.43	0.513
PUFA	1	7.714	**0.005**	1	5.00	**0.025**	1	3.86	**0.05**
ω3 FAs	1	7.977	**0.005**	1	5.00	**0.025**	1	2.33	0.127
ω6 FAs	1	1.789	0.181	1	5.60	**0.018**	1	0.43	0.513

Table S3 Permutational analysis of variance (PERMANOVA) results testing the variations of stable isotopic values in primary organic matter (i.e. particulate organic matters: POM and microphytobenthos: MPB) among rearing stages (Stage), types of shrimp ponds (Shrimp), and pond (nested in Shrimp). Significance level: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. OM = organic matters.

Main test of $\delta^{13}C$

Source	df	SS	MS	Pseudo-F	P(MC)
Stage	1	435.59	435.59	1.2115	0.383
Shrimp	1	2520.7	2520.7	18.387	**0.021***
Primary OM	1	2.8578	2.8578	7.92E-03	0.993
Pond	2	274.33	137.16	2.517	0.080
Stage*Shrimp	1	3007.4	3007.4	8.3644	0.078
Stage*Primary OM	1	1068.9	1068.9	9.3066	**0.051**
Shrimp*Primary OM	1	862.13	862.13	2.39	0.240
Stage*Pond (Shrimp)	2	719.67	359.84	6.6032	**0.003****
Primary OM*Pond (Shrimp)	2	722	361	6.6246	**0.002****
Stage*Shrimp*Primary OM	1	989.15	989.15	8.6119	**0.051**
Stage*Primary OM*Pond (Shrimp)	2	229.83	114.91	2.1087	0.117
Res	31	1689.3	54.494		
Total	46	12740			

Main test of $\delta^{15}N$

Source	df	SS	MS	Pseudo-F	P(MC)
Stage	1	3.7007	3.7007	1.9446	0.303
Shrimp	1	31.409	31.409	214.27	**0.005****
Primary OM	1	3.8903	3.8903	4.3654	0.172
Pond	2	0.29292	0.14646	0.51429	0.605
Stage*Shrimp	1	3.2761	3.2761	1.7215	0.319
Stage*Primary OM	1	2.4515	2.4515	19.647	**0.046***
Shrimp*Primary OM	1	29.438	29.438	33.033	**0.030***
Stage*Pond (Shrimp)	2	3.809	1.9045	6.6876	**0.004****
Primary OM*Pond (Shrimp)	2	1.7834	0.89172	3.1312	**0.058**
Stage*Shrimp*Primary OM	1	2.4946	2.4946	19.992	**0.048***
Stage*Primary OM*Pond (Shrimp)	2	0.24926	0.12463	0.43764	0.659
Res	31	8.8283	0.28478		
Total	46	93.64			

Appendix V – supporting material Chapter 6

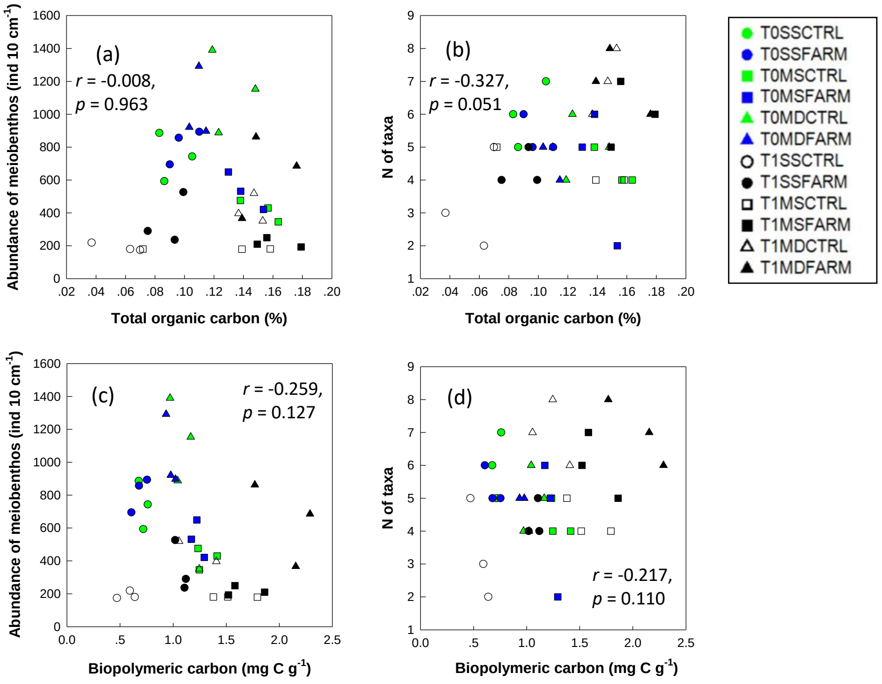

Figure S1 The response and the Pearson correlation analysis of abundance of meiobenthos and number of meiobenthos' taxa (N of taxa) to the total organic matter and biopolymeric carbon in scallop farming area in Laizhou Bay, China. T0 = before farming; T1 = during heavy biodeposition; CTRL = Control site; FARM = scallop farming site. SS = sandy shallow station, MS = muddy sallow station, MD = muddy deep station.

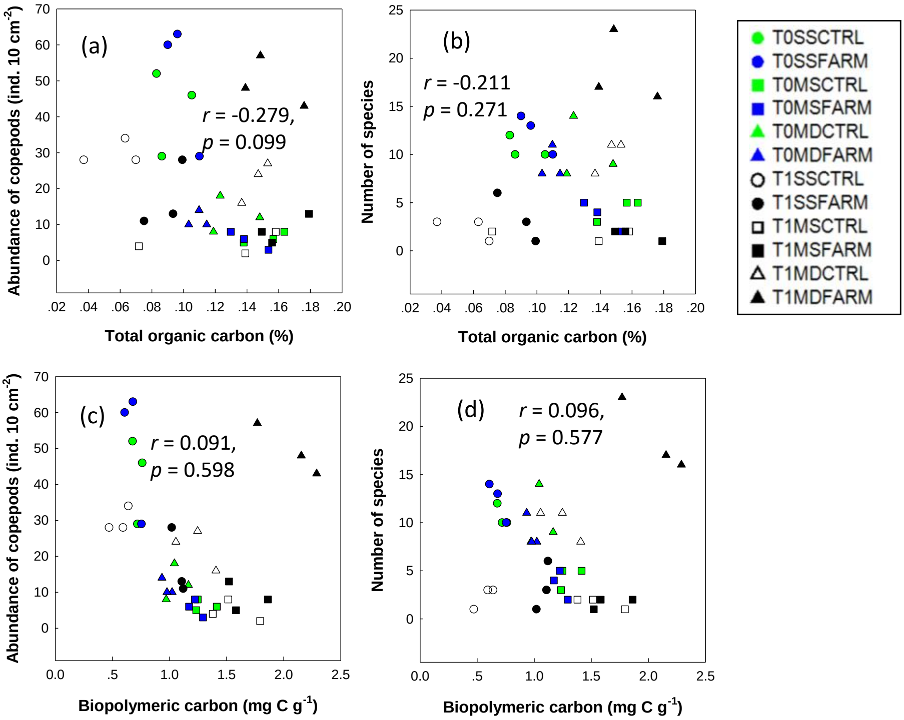

Figure S2 The response and the Pearson correlation analysis of abundance of copepod and number of copepod species to the total organic matter and biopolymeric carbon in scallop farming area in Laizhou Bay, China. T0 = before farming; T1 = during heavy biodeposition; CTRL = Control site; FARM = scallop farming site. SS = sandy shallow station, MS = muddy sallow station, MD = muddy deep station

Reference

Abe H, Hirai S, Okada S (2007) Metabolic responses and arginine kinase expression under hypoxic stress of the kuruma prawn *Marsupenaeus japonicus*. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 146:40-46

Alkemade R, Wielemaker A, Hemminga MA (1992) Stimulation of decomposition of *Spartina anglica* leaves by the bacterivorous marine nematode *Diplolaimelloides bruciei* (Monhysteridae). Journal of Experimental Marine Biology and Ecology 159:267-278

Alonso-Rodríguez R, Páez-Osuna F (2003) Nutrients, phytoplankton and harmful algal blooms in shrimp ponds: a review with special reference to the situation in the Gulf of California. Aquaculture 219:317-336

Anderson M, Braak CT (2003) Permutation tests for multi-factorial analysis of variance. Journal of statistical computation and simulation 73:85-113

Anderson M, Gorley R, Clarke K (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62:245-253

Anderson MR, Tlusty MF, Pepper VA (2005) Organic enrichment at cold water aquaculture sites—the case of coastal Newfoundland. In: Hargrave BT (ed) Environmental effects of marine finfish aquaculture. Springer, Berlin, Germany, p 99–113

Anderson RK, Parker PL, Lawrence A (1987) A $^{13}C/^{12}C$ tracer study of the utilization of presented feed by a commercially important shrimp *Penaeus vannamei* in a pond growout system. Journal of the World Aquaculture Society 18:148-155

Andersson G, Granéli W, Stenson J (1988) The influence of animals on phosphorus cycling in lake ecosystems. In: Phosphorus in Freshwater Ecosystems. Hydrobiologia 170: 267-284

APFIC (Asia-Pacific Fishery Commission) (2014) Regional overview of aquaculture trends in the Asia-Pacific Region 2014, RAP Publication 2014/26, Bangkok, 45 p

Arismendi I, Soto D, Penaluna B, Jara C, Leal C, Leon-Munoz J (2009) Aquaculture, non-native salmonid invasions and associated declines of native fishes in Northern Patagonian lakes. Freshwater Biology 54:1135-1147

Armenteros M, Pérez-García JA, Ruiz-Abierno A, Díaz-Asencio L, Helguera Y, Vincx M, Decraemer W (2010) Effects of organic enrichment on nematode assemblages in a microcosm experiment. Marine Environmental Research 70:374-382

Aryuthaka C, Kito K (2002) The meiofauna communities in shrimp culture ponds in Thailand. Fisheries Science 68:1018-1019

Avnimelech Y (2009) Biofloc technology. A practical guide book. 2nd ed. The World Aquaculture Society, p 272

< REFERENCE >

Avnimelech Y, Ritvo G (2003) Shrimp and fish pond soils: processes and management. Aquaculture 220:549-567

Avnimelech Y, Ritvo G, Kochva M (2004) Evaluating the active redox and organic fractions in pond bottom soils: EOM, easily oxidized material. Aquaculture 233:283-292

Bagstad KJ, Semmens DJ, Waage S, Winthrop R (2013) A comparative assessment of decision-support tools for ecosystem services quantification and valuation. Ecosystem Services 5:27-39

Balasubramanian CP, Pillai SM, Ravichandran P (2004) Zero-water exchange shrimp farming systems (extensive) in the periphery of Chilka lagoon, Orissa, India. Aquaculture International 12:555-572

Baldi F, Marchetto D, Pini F, Fani R, Michaud L, Lo Giudice A, Berto D, Giani M (2010) Biochemical and microbial features of shallow marine sediments along the Terra Nova Bay (Ross Sea, Antarctica). Continental Shelf Research 30:1614-1625

Ballester ELC, Wasielesky Jr W, Cavalli RO, Abreu PC (2007) Nursery of the pink shrimp *Farfantepenaeus paulensis* in cages with artificial substrates: biofilm composition and shrimp performance. Aquaculture 269:355-362

Belak J., Dhar A.K., Primavera J.H., dela Pena L.D., Pettit P., Alcivar-Warren A. (1999) Prevalence of viral diseases IHHNV and WSSV in *Penaeus monodon* from the Philippines and its association with mangrove status and shrimp culture systems. In: Alcivar-Warren A (ed) Proceedings of the Aquaculture nd Conservation of Marine Shrimp Biodiversity Symposium, Tufts University School of Veterinary Medicine, December 10.

Bell J, McEvoy L, Estevez A, Shields R, Sargent J (2003) Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture 227:211-220

Bell MV, Henderson R, Sargent J (1985) Changes in the fatty acid composition of phospholipids from turbot (*Scophthalmus maximus*) in relation to dietary polyunsaturated fatty acid deficiencies. Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology 81:193-198

Bell MV, Tocher DR (2009) Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In: Arts MT, Brett MT, Kainz MJ (eds) Lipids in aquatic ecosystems. Springer, New York, NY, p 211–236

Bianchelli S, Gambi C, Pusceddu A, Danovaro R (2008) Trophic conditions and meiofaunal assemblages in the Bari Canyon and the adjacent open slope (Adriatic Sea). Chemistry and Ecology 24:101-109

Black KD (2001) Environmental impacts of aquaculture. Sheffield Academic Press, Sheffield

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 42:411-440

Bojórquez-Mascareño E, Soto-Jiménez M (2013) Effects of natural diet on growth on white-leg shrimp *Litopenaeus vannamei* under experimental mesocosms emulating an intensive culture system. Journal of Aquaculture Research and Development 4:163

Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, Tan Z, Shariff

M (2005) Disease and health management in Asian aquaculture. Veterinary parasitology 132:249-272

Bongiorni L, Mirto S, Pusceddu A, Danovaro R (2005) Response of benthic protozoa and thraustochytrid protists to fish Farm impact in seagrass (*Posidonia oceanica*) and soft-bottom sediments. Microbial Ecology 50:268-276

Boon A, Duineveld G (1996) Phytopigments and fatty acids as molecular markers for the quality of near-bottom particulate organic matter in the North Sea. Journal of Sea Research 35:279-291

Borja A, Bricker SB, Dauer DM, Demetriades NT, Ferreira JG, Forbes AT, Hutchings P, Jia X, Kenchington R, Marques JC (2008) Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Marine pollution bulletin 56:1519-1537

Borja A, Ranasinghe A, Weisberg SB (2009) Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: challenges for the future. Marine Pollution Bulletin 59:1-4

Boucher G, Lambshead PJD (1995) Ecological Biodiversity of Marine Nematodes in Samples from Temperate, Tropical, and Deep-Sea Regions. Conservation Biology 9:1594-1604

Boxshall GA, Halsey SH (2004) An introduction to copepod diversity, Organic Geochemistry. The Ray Society, Andover, UK

Boyd CE (1995) Bottom soils, sediment, and pond aquaculture. Chapman and Hall, New York

Boyd CE (2003) Guidelines for aquaculture effluent management at the farm-level. Aquaculture 226:101-112

Boyd CE (2015) Water quality: an introduction. Springer

Boyd CE, Clay JW (1998) Shrimp aquaculture and the environment. Scientific American 278:58-65

Braeckman U, Van Colen C, Guilini K, Van Gansbeke D, Soetaert K, Vincx M, Vanaverbeke J (2014) Empirical evidence reveals seasonally dependent reduction in nitrification in coastal sediments subjected to near future ocean acidification. PLOS ONE 9:e108153

Brett M, Muller-Navarra D (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483-499

Briggs M, Funge-Smith S, Subasinghe R, Phillips M (2004) Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. RAP publication 10:92

Briggs M, Funge-Smith S, Subasinghe R, Phillips M (2004) Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. RAP publication 2004/10, Bangkok

Britton-Simmons KH (2004) Direct and indirect effects of the introduced alga *Sargassum muticum* on benthic, subtidal communities of Washington State, USA. Marine Ecology Progress Series 277:61-78

Brooks KM, Stierns AR, Mahnken CVW, Blackburn DB (2003) Chemical and biological remediation of the benthos near Atlantic salmon farms. Aquaculture 219:355-377

Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Organic Geochemistry 29:1547-1559

Buffan-Dubau E, Carman KR (2000) Diel feeding behavior of meiofauna and their relationships with microalgal resources. Limnology and Oceanography 45:381-395

Bulbul M, Kader MA, Asaduzzaman M, Ambak MA, Chowdhury AJK, Hossain MS, Ishikawa M, Koshio S (2016) Can canola meal and soybean meal be used as major dietary protein sources for kuruma shrimp, *Marsupenaeus japonicus*? Aquaculture 452:194-199

Bulbul M, Koshio S, Ishikawa M, Yokoyama S, Kader MA (2013) Performance of kuruma shrimp, *Marsupenaeus japonicus* fed diets replacing fishmeal with a combination of plant protein meals. Aquaculture 372:45-51

Burford MA, Longmore AR (2001) High ammonium production from sediments in hypereutrophic shrimp ponds. Marine ecology progress series 224:187-195

Burford MA, Pearson DC (1998) Effect of different nitrogen sources on phytoplankton composition in aquaculture ponds. Aquatic Microbial Ecology 15:277-284

Burford MA, Preston NP, Minh TH, Hoa TTT, Bunn SE, Fry VM (2004) Dominant sources of dietary carbon and nitrogen for shrimp reared in extensive rice-shrimp ponds. Aquaculture Research 35:194-203

Burford MA, Thompson PJ, McIntosh RP, Bauman RH, Pearson DC (2003) Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219:393-411

Burreson EM, Stokes NA, Carnegie RB, Bishop MJ (2004) *Bonamia* sp.(Haplosporidia) found in nonnative oysters *Crassostrea ariakensis* in Bogue Sound, North Carolina. Journal of Aquatic Animal Health 16:1-9

Cai S, Wang Q (1999) Some Aspects of the shrimp farming industry in China: constraints and priorities. In: Smith P (ed) Towards sustainable shrimp culture in Thailand and the region. Report on a reginal study and workshop on aquaculture sustainablility and the environment. Asian Development Bank and Network of Aquaculture Centres in Asia-Pacific (ADB/NACA), Bangkok, p 236-241

Callier MD, Lefebvre S, Dunagan MK, Bataille M-P, Coughlan J, Crowe TP (2013) Shift in benthic assemblages and organisms' diet at salmon farms: community structure and stable isotope analyses. Marine Ecology Progress Series 483:153-167

Callier MD, McKindsey CW, Desrosiers G (2007) Multi-scale spatial variations in benthic sediment geochemistry and macrofaunal communities under a suspended mussel culture. Marine Ecology Progress Series 348:103-115

Callier MD, McKindsey CW, Desrosiers G (2008) Evaluation of indicators used to detect mussel farm influence on the benthos: two case studies in the Magdalen Islands, Eastern Canada. Aquaculture 278:77-88

Callier MD, Weise AM, McKindsey CW, Desrosiers G (2006) Sedimentation rates in a suspended mussel farm (Great-Entry Lagoon, Canada): biodeposit production and dispersion.

Marine Ecology Progress Series 322:129-141

Cam D, Rollet P-E, Mariotti A, Guillaume J (1991) The relative contribution of natural productivity and formulated food in the nutrition of *Penaeus japonicus* reared in semi-intensive conditions. Aquatic Living Resources 4:175-180

Cao L, Wang W, Yang Y, Yang C, Yuan Z, Xiong S, Diana J (2007) Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. Environmental Science and Pollution Research-International 14:452-462

Cardozo AP, Britto VO, Odebrecht C (2011) Temporal variability of plankton and nutrients in shrimp culture ponds vs. adjacent estuarine water. Pan-American Journal of Aquatic Sciences 6(1):28-43

Carlsson MS, Glud RN, Petersen JK (2010) Degradation of mussel (*Mytilus edulis*) fecal pellets released from hanging long-lines upon sinking and after settling at the sediment. Canadian Journal of Fisheries and Aquatic Sciences 67:1376-1387

Carlton JT (1989) Man's Role in Changing the face of the ocean: biological invasions and implications for conservation of near-shore environments. Conservation Biology 3:265-273

Carman KR, Fry B (2002) Small-sample methods for $\delta^{13}C$ and $\delta^{15}N$ analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. Marine Ecology Progress Series 240:85-92

Cartaxana P, Mendes C, Van Leeuwe M, Brotas V (2006) Comparative study on microphytobenthic pigments of muddy and sandy intertidal sediments of the Tagus estuary. Estuarine, Coastal and Shelf Science 66:225-230

Casazza G, Silvestri C, Spada E (2002) The use of bio-indicators for quality assessments of the marine environment: examples from the Mediterranean Sea. Journal of Coastal Conservation 8:147

Casillas-Hernández R, Nolasco-Soria H, García-Galano T, Carrillo-Farnes O, Páez-Osuna F (2007) Water quality, chemical fluxes and production in semi-intensive Pacific white shrimp (*Litopenaeus vannamei*) culture ponds utilizing two different feeding strategies. Aquacultural Engineering 36:105-114

Castel J, Labourg P-J, Escaravage V, Auby I, Garcia ME (1989) Influence of seagrass beds and oyster parks on the abundance and biomass patterns of meio- and macrobenthos in tidal flats. Estuarine, Coastal and Shelf Science 28:71-85

Castel J, Labourg P-J, Escaravage V, Auby I, Garcia ME (1989) Influence of seagrass beds and oyster parks on the abundance and biomass patterns of meio-and macrobenthos in tidal flats. Estuarine, coastal and shelf science 28:71-85

Castine SA, Erler DV, Trott LA, Paul NA, de Nys R, Eyre BD (2012) Denitrification and anammox in tropical aquaculture settlement ponds: an isotope tracer approach for evaluating N_2 production. PLOS ONE 7:e42810

Caut S, Angulo E, Courchamp F (2008) Caution on isotopic model use for analyses of consumer diet. Canadian Journal of Zoology 86:438-445

Chamberlain J, Fernandes T, Read P, Nickell T, Davies I (2001) Impacts of biodeposits from suspended mussel (*Mytilus edulis* L.) culture on the surrounding surficial sediments.

ICES journal of marine science 58:411-416

Chandler GT (1986) High-density culture of meiobenthic harpacticoid copepods within a muddy sediment substrate. Canadian Journal of Fisheries and Aquatic Sciences 43:53-59

Chavanich S, Viyakarn V, Senanan W, Panutrakul S (2016) Laboratory assessment of feeding-behavior interactions between the introduced Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) (Penaeidae) and five native shrimps plus a crab species in Thailand. Aquatic Invasions 11:67–74

Chen Y, Dong S, Wang F, Gao Q, Tian X (2016) Carbon dioxide and methane fluxes from feeding and no-feeding mariculture ponds. Environmental Pollution 212:489-497

Chmura GL, Anisfeld SC, Cahoon DR, Lynch JC (2003) Global carbon sequestration in tidal, saline wetland soils. Global biogeochemical cycles 17:1-12

Chopin T, Robinson S, Troell M, Neori A, Buschmann A, Fang J (2008) Ecological engineering: multi-trophic integration for sustainable marine aquaculture. In: Jørgensen SE, Fath BD (eds) The Encyclopedia of ecology, ecological engineering, vol. 3, Elsevier, Oxford, p 2463-2475

Christensen PB, Glud RN, Dalsgaard T, Gillespie P (2003) Impacts of longline mussel farming on oxygen and nitrogen dynamics and biological communities of coastal sediments. Aquaculture 218:567-588

Cifuentes L, Sharp J, Fogel ML (1988) Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnology and Oceanography 33:1102-1115

Clarke H (1965) The scallop superspecies *Argopecten irradians* (Lamarck). Malacologia 2:161-188

Clarke K, Ainsworth M (1993) A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series 92:205-219

Clarke KR, Gorley RN (2006) PRIMER V6: user manual-tutorial. Plymouth Marine Laboratory, Plymouth

Cleland EE (2011) Biodiversity and ecosystem stability. Nature Education Knowledge 3:14

Cnudde C, Moens T, Werbrouck E, Lepoint G, Van Gansbeke D, De Troch M (2015) Trophodynamics of estuarine intertidal harpacticoid copepods based on stable isotope composition and fatty acid profiles. Marine Ecology Progress Series 524:225-239

Cnudde C, Moens T, Willems A, De Troch M (2013) Substrate-dependent bacterivory by intertidal benthic copepods. Marine Biology 160:327-341

Cnudde C, Willems A, Van Hoorde K, Vyverman W, Moens T, De Troch M (2011) Effect of food preservation on the grazing behavior and on the gut flora of the harpacticoid copepod *Paramphiascella fulvofasciata*. Journal of Experimental Marine Biology and Ecology 407:63-69

Cobo MdL, Wouters R, Wille M, Sonnenholzner S, Sorgeloos P (2015) Evaluation of frozen umbrella-stage *Artemia* as first animal live food for *Litopenaeus vannamei* (Boone) larvae. Aquaculture Research 46:2166-2173

Commito JA, Tita G (2002) Differential dispersal rates in an intertidal meiofauna assemblage. Journal of Experimental Marine Biology and Ecology 268:237-256

Coull BC (1988) Ecology of the marine meiofauna. In: Higgins RP, Thiel H (eds) Introduction to the study of meiofauna. Smithsonian Institution Press, p 18-38

Coull BC, Chandler GT (1992) Pollution and meiofauna: field, laboratory, and mesocosm studies. Oceanography and Marine Biology, An Annual Review 30:191-271

Coull BC, Dudley BW (1976) Delayed naupliar development of meiobenthic copepods. The Biological Bulletin 150:38-46

Coull BC, Greenwood JG, Fielder DR, Coull BA (1995) Subtropical Australian juvenile fish eat meiofauna: experiments with winter whiting Sillago maculata and observations on other species. Marine Ecology Progress Series 125:13-19

Crab R, Chielens B, Wille M, Bossier P, Verstraete W (2010) The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquaculture Research 41: 559-567

Crab R, Defoirdt T, Bossier P, Verstraete W (2012) Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture 356-357:351-356

Cranford PJ, Anderson R, Archambault P, Balch T, Bates SS, Bugden G, Callier MD, Carver C, Comeau L, Hargrave B (2006) Indicators and thresholds for use in assessing shellfish aquaculture impacts on fish habitat. Canadian Scientific Advisory Secretariat Research Document 2006/34, Department of Fish and Oceans, Ottawa, ON, Canada

Crawford CM, Macleod CKA, Mitchell IM (2003) Effects of shellfish farming on the benthic environment. Aquaculture 224:117-140

Cripps G, Atkinson A (2000) Fatty acid composition as an indicator of carnivory in Antarctic krill, Euphausia superba. Canadian Journal of Fisheries and Aquatic Sciences 57:31-37

Cui Y, Chen B, Chen J (2005) Evaluation on self-pollution of marine culture in the Yellow Sea and Bohai Sea. Chinese journal of Applied Ecology 16:180-185 (in Chinese with English abstract)

Da Silva CF, Ballester E, Monserrat J, Geracitano L, Wasielesky Jr W, Abreu P (2008) Contribution of microorganisms to the biofilm nutritional quality: protein and lipid contents. Aquaculture Nutrition 14:507-514

Dahlbäck B, Gunnarsson L (1981) Sedimentation and sulfate reduction under a mussel culture. Marine Biology 63:269-275

Dall W, Hill B, Rothlisberg P, Sharples D (1991) The biology of the Penaeidae. The biology of the Penaeidae. Advances in Marine Biology 27:1-489

Dalsgaard J, John MS, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46:225-340.

Dame RF (1996) Ecology of marine bivalves: An ecosystem approach, 2nd edn. CRC Marine Science Series. Boca Raton FL

Danovaro R (2009) Methods for the study of deep-sea sediments, their functioning and biodiversity, p 458, CRC Press

Danovaro R, Corinaldesi C, La Rosa T, Luna G, Mazzola A, Mirto S, Vezzulli L, Fabiano M (2003) Aquaculture impact on benthic microbes and organic matter cycling in coastal Mediterranean sediments: a synthesis. Chemistry and Ecology 19:59-65

Danovaro R, Fabiano M, Della Croce N (1993) Labile organic matter and microbial biomasses in deep-sea sediments (Eastern Mediterranean Sea). Deep Sea Research Part I: Oceanographic Research Papers 40:953-965

Danovaro R, Gambi C, Dell'Anno A, Corinaldesi C, Fraschetti S, Vanreusel A, Vincx M, Gooday AJ (2008) Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Current Biology 18:1-8

Danovaro R, Gambi C, Luna G, Mirto S (2004) Sustainable impact of mussel farming in the Adriatic Sea (Mediterranean Sea): evidence from biochemical, microbial and meiofaunal indicators. Marine Pollution Bulletin 49:325-333

Daudi LN, Lugomela C, Uku JN, De Troch M (2012) Effect of nutrient enrichment on seagrass associated meiofauna in Tanzania. Marine Environmental Research 82:49-58

De Backer A, Van Coillie F, Montserrat F, Provoost P, Van Colen C, Vincx M, Degraer S (2011) Bioturbation effects of *Corophium volutator*: Importance of density and behavioural activity. Estuarine, Coastal and Shelf Science 91:306-313

de Lima LCM, Navarro DMAF, Souza-Santos LP (2013) Effect of diet on the fatty acid composition of the copepod *Tisbe Biminiensis*. Journal of Crustacean Biology 33:372-381

De Mesel I, Derycke S, Moens T, Van der Gucht K, Vincx M, Swings J (2004) Top-down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. Environmental Microbiology 6:733-744

De Pauw N, Morales J, Persoone G (1984) Mass culture of microalgae in aquaculture systems: Progress and constraints. Hydrobiologia 116:121-134

De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W (2008) The basics of bio-flocs technology: The added value for aquaculture. Aquaculture 277:125-137

De Silva SS, Hasan MR (2007) Feeds and fertilizers: the key to long-term sustainability of Asian aquaculture. In Hasan MR, Hecht T, De Silva SS, Tacon AGF (eds) Study and analysis of feeds and fertilizers for sustainable aquaculture development, p 19-47, FAO Fisheries Technical Paper No. 497. Rome, FAO

De Silva SS, Nguyen TT, Turchini GM, Amarasinghe US, Abery NW (2009) Alien species in aquaculture and biodiversity: a paradox in food production. Ambio: a journal of the human environment 38:24-28

De Troch M, Boeckx P, Cnudde C, Van Gansbeke D, Vanreusel A, Vincx M, Caramujo MJ (2012) Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis. Marine Ecology Progress Series 465:53-67

De Troch M, Chepurnov V, Gheerardyn H, Vanreusel A, Olafsson E (2006) Is diatom size selection by harpacticoid copepods related to grazer body size? Journal of

Experimental Marine Biology and Ecology 332:1-11

De Troch M, Fiers F, Vincx M (2003) Niche segregation and habitat specialisation of harpacticoid copepods in a tropical seagrass bed. Marine Biology 142:345-355

De Troch M, Roelofs M, Riedel B, Grego M (2013) Structural and functional responses of harpacticoid copepods to anoxia in the Northern Adriatic: an experimental approach. Biogeosciences 10:4259-4272

Decamp O, Conquest L, Cody J, Forster I, Tacon AG (2007) Effect of shrimp stocking density on size-fractionated phytoplankton and ecological groups of ciliated protozoa within zero-water exchange shrimp culture systems. Journal of the World Aquaculture Society 38:395-406

Dell'Anno A, Mei M, Pusceddu A, Danovaro R (2002) Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Marine Pollution Bulletin 44:611-622

Deng J, Ma S, Niu H, Dong S, Su Y (2007) An experiment of shrimp (*Fenneropenaeus Chinensis*) culture by inputting polychaetes (*Perinereis aibuhitensis*). Transactions of Oceanology and Limnology 2:135-140

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495-506

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321:926-929

Dittel AI, Epifanio CE, Cifuentes LA, Kirchman DL (1997) Carbon and nitrogen sources for shrimp postlarvae fed natural diets from a tropical mangrove system. Estuarine, Coastal and Shelf Science 45:629-637

Doi H, Kikuchi E, Hino S, Itoh T, Takagi S, Shikano S (2003) Seasonal dynamics of carbon stable isotope ratios of particulate organic matter and benthic diatoms in strongly acidic Lake Katanuma. Aquatic Microbial Ecology 33:87-94

Domínguez LM, Martín JV (2004) Aquaculture environmental impact assessment. In: Popov, H, Brebbia CA, Kungolos S (eds) Waste Management and the Environment, WIT press, p 321-333

Dubois S, Marin-Léal JC, Ropert M, Lefebvre S (2007) Effects of oyster farming on macrofaunal assemblages associated with Lanice conchilega tubeworm populations: A trophic analysis using natural stable isotopes. Aquaculture 271:336-349

Dumbauld BR, Ruesink JL, Rumrill SS (2009) The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. Aquaculture 290:196-223

Duplisea D, Hargrave B (1996) Response of meiobenthic size-structure, biomass and respiration to sediment organic enrichment. Hydrobiologia 339:161-170

Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod Acartia tonsa. Limnology and Oceanography 40:860-867

Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod Acartia tonsa. Limnology and Oceanography 40:860-867

Elton CS (1958) The ecology of invasions by animals and plants. University of Chicago Press, Chicago

Epstein SS (1997) Microbial food webs in marine sediments. II. seasonal changes in trophic Interactions in a sandy tidal flat community. Microbial Ecology 34:199-209

Fabi G, Manoukian S, Spagnolo A (2009) Impact of an open-sea suspended mussel culture on macrobenthic community (Western Adriatic Sea). Aquaculture 289:54-63

Fabiano M, Danovaro R (1994) Composition of organic matter in sediments facing a river estuary (Tyrrhenian Sea): relationships with bacteria and microphytobenthic biomass. Hydrobiologia 277:71-84

Fabiano M, Danovaro R, Fraschetti S (1995) A three-year time series of elemental and biochemical composition of organic matter in subtidal sandy sediments of the Ligurian Sea (northwestern Mediterranean). Continental Shelf Research 15:1453-1469

Fabiano M, Danovaro R, Olivari E, Misic C (1994) Decomposition of faecal matter and somatic tissue of *Mytilus galloprovincialis*: changes in organic matter composition and microbial succession. Marine Biology 119:375-384

Fang J, Zhang J, Xiao T, Huang D, Liu S (2016) Integrated multi-trophic aquaculture (IMTA) in Sanggou Bay, China. Aquaculture Environment Interactions 8:201-205

FAO (Food and Agriculture Organization of the United Nations) (2010) The state of world fisheries and aquaculture 2010. FAO, Rome, 197 p

FAO (Food and Agriculture Organization of the United Nations) (2011) World aquaculture 2010. FAO Fisheries and Aquaculture Technical paper 500/1, FAO, Rome, 105 p

FAO (Food and Agriculture Organization of the United Nations) (2016) The state of world fisheries and aquaculture 2016. FAO, Rome, 200 p

FAO (Food and Agriculture Organization of the United Nations) (2017) FAO Yearbook. Fishery and Aquaculture Statistics. 2015. FAO, Rome, 78 p

Farkas T (1979) Adaptation of fatty acid compositions to temperature—a study on planktonic crustaceans. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 64:71-76

Fenchel T, Riedl R (1970) The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. Marine Biology 7:255-268

Feng H (1996) Focus on fish and shrimp nutrition ecology due to aquaculture calamity. China Feeds 13:18-20 (in Chinese)

Feng J, Huang Q, Chen H, Guo J, Lin G (2018) Restoration of native mangrove wetlands can reverse diet shifts of benthic macrofauna caused by invasive cordgrass. Journal of Applied Ecology 55:905-916

Feng Y, He, G., Ma, S. (1996) Systematical study of scale and coordinated development of urban infrastructure. Journal of Management Sciences in China 4:18-22

Feng YY, Hou LC, Ping NX, Ling TD, Kyo CI (2004) Development of mariculture and its impacts in Chinese coastal waters. Reviews in Fish Biology and Fisheries 14:1-10

Fernandes Da Silva C, Ballester E, Monserrat J, Geracitano L, Wasielesky Jr W, Abreu P (2008) Contribution of microorganisms to the biofilm nutritional quality: protein and lipid contents. Aquaculture Nutrition 14:507-514

Fernandes R, Millard AR, Brabec M, Nadeau M-J, Grootes P (2014) Food reconstruction using isotopic transferred signals (FRUITS): a Bayesian model for diet reconstruction. PloS One 9:e87436

Fernández de Alaiza García Madrigal R, Silva UdAT, Tavares CPdS, Ballester ELC (2017) Use of native and non-native shrimp (Penaeidae, Dendrobranchiata) in world shrimp farming. Reviews in Aquaculture

Ferriss BE, Reum JC, McDonald PS, Farrell DM, Harvey CJ (2015) Evaluating trophic and non-trophic effects of shellfish aquaculture in a coastal estuarine foodweb. ICES Journal of Marine Science 73:429-440

Fichez R (1991) Composition and fate of organic-matter in submarine cave sediments-implications for the biogeochemical cycle of organic-carbon. Oceanologica Acta 14:369-377

Forster JRM, 1976. Studies on the development of compounded diets for prawns. In: Kent SP, Shaw WN and Danberg KS (eds), Proc. of the First International Conference on Aquaculture Nutrition. Lewes/Rehaboth, Delaware, COB. Mar. Stud., University Delaware, Newark, p 229-248.

Franzo A, Cibic T, Del Negro P, Solidoro C (2014) Microphytobenthic response to mussel farm biodeposition in coastal sediments of the northern Adriatic Sea. Marine Pollution Bulletin 79:379-388

Fry B (2006) Stable isotope ecology. Springer, New York

Funge-Smith SJ, Briggs MRP (1998) Nutrient budgets in intensive shrimp ponds: implications for sustainability. Aquaculture 164:117-133

G. Moore C, Bett B (2008) The use of meiofauna in marine pollution impact assessment. Zoological Journal of the Linnean Society 96:263 - 280

Gallardo B, Clavero M, Sánchez MI, Vilà M (2016) Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology 22:151-163

Gamboa-Delgado J (2014) Nutritional role of natural productivity and formulated feed in semi-intensive shrimp farming as indicated by natural stable isotopes. Reviews in Aquaculture 6:36-47

Gamboa-Delgado J, Le Vay L (2009) *Artemia* replacement in co-feeding regimes for mysis and postlarval stages of *Litopenaeus vannamei*: Nutritional contribution of inert diets to tissue growth as indicated by natural carbon stable isotopes. Aquaculture 297:128-135

Gamboa-Delgado J, Molina-poveda C, Cahu C (2003) Digestive enzyme activity and food ingesta in juvenile shrimp *Litopenaeus vannamei* (Boone, 1931) as a function of body weight. Aquaculture research 34:1403-1411

Gamboa-Delgado J, Rojas-Casas MG, Nieto-López MG, Cruz-Suárez LE (2013) Simultaneous estimation of the nutritional contribution of fish meal, soy protein isolate and corn gluten to the growth of Pacific white shrimp (*Litopenaeus vannamei*) using dual stable isotope analysis. Aquaculture 380-383:33-40

Gao X, Li P (2012) Concentration and fractionation of trace metals in surface sediments of intertidal Bohai Bay, China. Marine Pollution Bulletin 64:1529-1536

Gao X, Yang Y, Wang C (2012) Geochemistry of organic carbon and nitrogen in surface sediments of coastal Bohai Bay inferred from their ratios and stable isotopic signatures. Marine Pollution Bulletin 64:1148-1155

Garcia Rodrigues J, Conides AJ, Rivero Rodriguez S, Raicevich S, Pita P, Kleisner KM, Pita C, Lopes PF, Alonso Roldán V, Ramos SS (2017) Marine and Coastal Cultural Ecosystem Services: knowledge gaps and research priorities. One Ecosystem 2: e12290

Gatune C, Vanreusel A, Cnudde C, Ruwa R, Bossier P, De Troch M (2012) Decomposing mangrove litter supports a microbial biofilm with potential nutritive value to penaeid shrimp post larvae. Journal of Experimental Marine Biology and Ecology 426-427:28-38

Gee J (1987) Impact of epibenthic predation on estuarine intertidal harpacticoid copepod populations. Marine Biology 96:497-510

Gee J (1989) An ecological and economic review of meiofauna as food for fish. Zoological Journal of the Linnean Society 96:243-261

Giere O (2009) Meiobenthology: the microscopic fauna in aquatic sediments, 2nd edn. Springer-Verlag Berlin Heidelberg

Giles H, Pilditch CA, Bell DG (2006) Sedimentation from mussel (*Perna canaliculus*) culture in the Firth of Thames, New Zealand: impacts on sediment oxygen and nutrient fluxes. Aquaculture 261:125-140

Goedkoop W, Sonesten L, Ahlgren G, Boberg M (2000) Fatty acids in profundal benthic invertebrates and their major food resources in Lake Erken, Sweden: seasonal variation and trophic indications. Canadian Journal of Fisheries and Aquatic Sciences 57:2267-2279

Gondwe MJ, Guildford SJ, Hecky RE (2012) Tracing the flux of aquaculture-derived organic wastes in the southeast arm of Lake Malawi using carbon and nitrogen stable isotopes. Aquaculture 350-353:8-18

Goodsell P, Underwood A, Chapman M (2009) Evidence necessary for taxa to be reliable indicators of environmental conditions or impacts. Marine Pollution Bulletin 58:323-331

Gophen M, Ochumba PB, Kaufman LS (1995) Some aspects of perturbation in the structure and biodiversity of the ecosystem of Lake Victoria (East Africa). Aquatic Living Resources 8:27-41

Graeve M, Kattner G, Wiencke C, Karsten U (2002) Fatty acid composition of Arctic and Antarctic macroalgae: indicator of phylogenetic and trophic relationships. Marine Ecology Progress Series 231:67-74

Grant J, Hatcher A, Scott DB, Pocklington P, Schafer C, Winters G (1995) A multidisciplinary approach to evaluating impacts of shellfish aquaculture on benthic communities. Estuaries 18:124-144

Grave K, Hansen MK, Kruse H, Bangen M, Kristoffersen AB (2008) Prescription of antimicrobial drugs in Norwegian aquaculture with an emphasis on “new” fish species. Preventive Veterinary Medicine 83:156-169

Gray JS, Wu RS-s, Or YY (2002) Effects of hypoxia and organic enrichment on the coastal marine environment. Marine Ecology Progress Series 238:249-279

Grego M (2010) Impact of fish culture on meiofauna in the Bay of Piran. PhD thesis, University of Ljubljana, Ljubljana, Slovenia

Grego M, De Troch M, Forte J, Malej A (2009) Main meiofauna taxa as an indicator for assessing the spatial and seasonal impact of fish farming. Marine Pollution Bulletin 58:1178-1186

Grego M, Riedel B, Stachowitsch M, De Troch M (2014) Meiofauna winners and losers of coastal hypoxia: case study harpacticoid copepods. Biogeosciences 11:281-292

Grenz C, Hermin M-N, Baudinet D, Daumas R (1990) In situ biochemical and bacterial variation of sediments enriched with mussel biodeposits. Hydrobiologia 207:153-160

Guo X, Luo Y (2016) Scallops and scallop aquaculture in China. In: Shumway SE, Parsons GJ (eds) Scallops, 3E. Elsevier Science, Oxford, p 937–952

Ha TTP, van Dijk H, Visser L (2014) Impacts of changes in mangrove forest management practices on forest accessibility and livelihood: a case study in mangrove-shrimp farming system in Ca Mau Province, Mekong Delta, Vietnam. Land Use Policy 36:89-101

Hamoutene D (2014) Sediment sulphides and redox potential associated with spatial coverage of Beggiatoa spp. at finfish aquaculture sites in Newfoundland, Canada. ICES Journal of Marine Science 71:1153-1157

Han Q, Wang Y, Zhang Y, Keesing J, Liu D (2013) Effects of intensive scallop mariculture on macrobenthic assemblages in Sishili Bay, the northern Yellow Sea of China. Hydrobiologia 718:1-15

Hargrave BT (1976) The central role of invertebrate faeces in sediment decomposition. In: Anderson JM, Macfadyen A (eds) The role of terrestrial and aquatic organisms in decomposition processes. Blackwell Scientific, Oxford, p 301–321

Hargrave BT, Holmer M, Newcombe CP (2008) Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. Marine Pollution Bulletin 56:810-824

Hargrave BT, Phillips GA, Doucette LI, White MJ, Milligan TG, Wildish DJ, Cranston RE (1997) Assessing Benthic Impacts of Organic Enrichment from Marine Aquaculture. Water, Air, and Soil Pollution 99:641-650

Hargreaves JA (1998) Nitrogen biogeochemistry of aquaculture ponds1. Aquaculture 166:181-212

Harris PT, Baker EK (2012) Why map benthic habitats? In: Harris PT, Baker EK (eds) Seafloor geomorphology as benthic habitat, Elsevier, p 3-22

Harris RP (1972) Seasonal Changes in the Meiofauna Population of an Intertidal Sand Beach. Journal of the Marine Biological Association of the United Kingdom 52:389-403

Hartstein ND, Rowden AA (2004) Effect of biodeposits from mussel culture on macroinvertebrate assemblages at sites of different hydrodynamic regime. Marine Environmental Research

Hartstein ND, Stevens CL (2005) Deposition beneath long-line mussel farms. Aquacultural engineering 33:192-213

Hartwich M, Martin-Creuzburg D, Wacker A (2013) Seasonal changes in the accumulation of polyunsaturated fatty acids in zooplankton. Journal of Plankton Research 35:121-134

Hasan MR (2012) Transition from low-value fish to compound feeds in marine cage farming in Asia. FAO Fisheries and Aquaculture Technical Paper:I

Hatcher A, Grant J, Schofield B (1994) Effects of suspended mussel culture (*Mytilus* spp.) on sedimentation, benthic respiration and sediment nutrient dynamics in a coastal bay. Marine Ecology Progress Series 115:219-235

Hatje V, de Souza MM, Ribeiro LF, Eça GF, Barros F (2016) Detection of environmental impacts of shrimp farming through multiple lines of evidence. Environmental pollution 219:672-684

Hawkins C, Norris R (2000) Effects of taxonomic resolution and use of subsets of the fauna on the performance of RIVPACS-type models. In: Wright JF, Sutcliffe DW, Furse MT (eds) Assessing the biological quality of fresh waters: RIVPACS and other techniques. Proceedings of an International Workshop held in Oxford, UK, on 16-18 September 1997. Freshwater Biological Association (FBA), Ambleside, UK, p 217-228

Hayashi K (1996) Taxonomy and Distribution. In: Kittaka J, Takashima F, Kanazwa A (eds) Aquaculture of Crustaceans. Kouseishakouseikaku, Tokyo, Japan, p 1-32

Heino J, Soininen J (2007) Are higher taxa adequate surrogates for species-level assemblage patterns and species richness in stream organisms? Biological Conservation 137:78-89

Heip C, Vincx M, Vranken G (1985) The ecology of marine nematodes. Oceanography and Marine Biology Annual Review 23:399-389

Hena MA, Hishamuddin O, Misri K (2011) Benthic meiofaunal predation and composition in the tiger shrimp *Penaeus monodon* culture ponds, Malaysia. Advances in Environmental Biology:605-612

Hicks G (1983) The ecology of marine meiobenthic harpacticoid copepods. Oceanography and Marine Biology, An Annual Review 21:67-175

Hicks GRF, Coull BC (1983) The ecology of marine harpacticoid copepods. Oceanography and Marine Biology Annual Review 21:67-175

Higgins RP, Thiel H (1988) Introduction to the study of meiofauna. Smithsonian Institution Press, Washington DC

Hill JE (2009) Risk analysis for non-native species in aquaculture. SRAC Publication 4304, U.S. Department of Agriculture, Southern Regional Aquaculture Center, Stoneville, Mississippi

Høj L, Bourne DG, Hall MR (2009) Localization, abundance and community structure of bacteria associated with *Artemia*: Effects of nauplii enrichment and antimicrobial treatment. Aquaculture 293:278-285

Holmer M (2009) Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. Aquaculture Environment Interactions 1:57-70

Holmer M, Black K, Duarte CM, Marbà N, Karakassis I (2007) Aquaculture in the Ecosystem. 326 p, Springer Netherlands

Holmer M, Wildish D, Hargrave B (2005) Organic Enrichment from Marine Finfish Aquaculture and Effects on Sediment Biogeochemical Processes. In: Hargrave BT (ed) Environmental Effects of Marine Finfish Aquaculture. Springer Berlin Heidelberg, Berlin, Heidelberg

Holt EA, Miller SW (2011) Bioindicators: using organisms to measure environmental impacts. Nature Education Knowledge 3(10):8

Hooper DU, Chapin F, Ewel J, Hector A, Inchausti P, Lavorel S, Lawton J, Lodge D, Loreau M, Naeem S (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological monographs 75:3-35

Hopkins JS, Hamilton RD, Sandier PA, Browdy CL, Stokes AD (1993) Effect of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. Journal of the World Aquaculture Society 24:304-320

Hoshika A, Sarker MJ, Ishida S, Mishima Y, Takai N (2006) Food web analysis of an eelgrass (*Zostera marina* L.) meadow and neighbouring sites in Mitsukuchi Bay (Seto Inland Sea, Japan) using carbon and nitrogen stable isotope ratios. Aquatic Botany 85:191-197

Höss S, Williams PL (2009) Ecotoxicity testing with nematodes. In: Wilson MJ, Kakouli-Duarte T (eds) Nematodes as Environmental indicators, CABI, UK, p 208-224

Hu B, Li G, Li J, Bi J, Zhao J, Bu R (2013) Spatial distribution and ecotoxicological risk assessment of heavy metals in surface sediments of the southern Bohai Bay, China. Environmental Science and Pollution Research 20:4099-4110

Hu Z, Lee JW, Chandran K, Kim S, Sharma K, Khanal SK (2014) Influence of carbohydrate addition on nitrogen transformations and greenhouse gas emissions of intensive aquaculture system. Science of The Total Environment 470-471:193-200

Huang L, Huang X, Song X, Tan Y, Wang H (2003) Frequent occurrence areas of red tide and its ecological characteristics in Chinese coastal waters. Ecological Science 22:252-256 (in Chinese with English abstract)

Huang Q, Olenin S, Sun S, De Troch M (2018) Impact of farming non-indigenous scallop

Argopecten irradians on the benthic ecosystem functioning: a case-study in Laizhou Bay, China. Aquaculture Environment Interactions 10: 227–241

Hui L, Jianguang F, Jianxin Z, Shuanglin D, Fang W, Xingming L, Jihong Z, Yan L, Lichao W, Weiwei J (2004) Study on limiting nutrients and phytoplankton at long-line-culture areas in Laizhou Bay and Sanggou Bay, northeastern China. Aquatic Conservation: Marine and Freshwater Ecosystems 14:551-574

Hulings NC, Gray JS (1971) A manual for the study of meiofauna. Smithsonian Contributions to Zoology, 78: 1-83

Huys R, Gee JM, Moore CG, Hamond R (1996) Marine and brackish water harpacticoid copepods (Part 1). Keys and notes for identification of the species. In: Bames RSK, Crothers JH (eds) Synopses of the British fauna, new series 51, Field Studies Council, Shrewsbury

Irisarri J, Fernández-Reiriz M-J, De Troch M, Labarta U (2014) Fatty acids as tracers of trophic interactions between seston, mussels and biodeposits in a coastal embayment of mussel rafts in the proximity of fish cages. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 172-173:105-115

Iverson SJ (2009) Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Lipids in Aquatic Ecosystems. Springer, New York, p 281–308

Jansen HM, Strand Ø, Verdegem M, Smaal A (2012) Accumulation, release and turnover of nutrients (CNP-Si) by the blue mussel *Mytilus edulis* under oligotrophic conditions. Journal of Experimental Marine Biology and Ecology 416:185-195

Jaramillo E, Bertran C, Bravo A (1992) Mussel biodeposition in an estuary in southern Chile. Marine Ecology Progress Series 82:85-94

Jensen P (1987) Feeding ecology of free-living aquatic nematodes. Marine Ecology Progress Series 35:187-196

Jia J, Chen J (2001) Sea farming and sea ranching in China. Food and Agriculture Organization of the United Nations (FAO) Fisheries Technical Paper 418, Rome, 75 p

Jin X (2008) Biological assessment of ecologically important areas for fish and invertebrate taxonomic groups of the Yellow Sea Ecoregion. Biological Assessment Report of the Yellow Sea Ecoregion, p 77-91

Jin X, Shan X, Li X, Wang J, Cui Y, Zuo T (2013) Long-term changes in the fishery ecosystem structure of Laizhou Bay, China. Science China Earth Sciences 56:366-374

Joseph MM, Kumar CR, Kumar TG, Renjith K, Chandramohanakumar N (2008) Biogeochemistry of surficial sediments in the intertidal systems of a tropical environment. Chemistry and Ecology 24:247-258

Kalantzi I, Karakassis I (2006) Benthic impacts of fish farming: meta-analysis of community and geochemical data. Marine Pollution Bulletin 52:484-493

Kan W-J, Zhang Q-F, Hu Y-Z, Shi H-M, Bo W-J (2010) Preliminary analysis on the state of organic pollution and eutrophication in Bohai Bay. Marine Science Bulletin 2:012

Karakassis I, Tsapakis M, Hatziyanni E, Papadopoulou K-N, Plaiti W (2000) Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. ICES Journal of Marine Science 57:1462-1471

Kaspar H, Gillespie P, Boyer I, MacKenzie A (1985) Effects of mussel aquaculture on the nitrogen cycle and benthic communities in Kenepuru Sound, Marlborough Sounds, New Zealand. Marine biology 85:127-136

Kautsky N, Evans S (1987) Role of biodeposition by *Mytilus edulis* in the circulation of matter and nutrients in a Baltic coastal ecosystem. Marine Ecology Progress Series 38:201-212

Kautsky N, Rönnbäck P, Tedengren M, Troell M (2000) Ecosystem perspectives on management of disease in shrimp pond farming. Aquaculture 191:145-161

Keeley NB, Forrest BM, Crawford C, Macleod CK (2012) Exploiting salmon farm benthic enrichment gradients to evaluate the regional performance of biotic indices and environmental indicators. Ecological Indicators 23:453-466

Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446:1–22

Kelly M (1998) Use of the trophic diatom index to monitor eutrophication in rivers. Water Research 32:236-242

Kennedy AD, Jacoby CA (1999) Biological indicators of marine environmental health: meiofauna – a neglected benthic component? Environmental Monitoring and Assessment 54:47-68

Kon K, Kawakubo N, Aoki J-I, Tongnunui P, Hayashizaki K-I, Kurokura H (2009) Effect of shrimp farming organic waste on food availability for deposit feeder crabs in a mangrove estuary, based on stable isotope analysis. Fisheries Science 75:715-722

Kristensen E (2000) Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. In: Liebezeit G, Dittmann S, Kröncke I (eds) Life at Interfaces and Under Extreme Conditions: Proceedings of the 33rd European Marine Biology Symposium, Wilhelmshaven, Germany, 7-11 September 1998. Hydrobiologia, 426: 1-24

Kristensen E (2001) Impact of polychaetes (*Nereis spp.* and *Arenicola marina*) on carbon biogeochemistry in coastal marine sediments Presented during the ACS Division of Geochemistry symposium 'Biogeochemical Consequences of Dynamic Interactions Between Benthic Fauna, Microbes and Aquatic Sediments', San Diego, April 2001. Geochemical Transactions 2:92-103

Kritzer JP, DeLucia M-B, Greene E, Shumway C, Topolski MF, Thomas-Blate J, Chiarella LA, Davy KB, Smith K (2016) The importance of benthic habitats for coastal fisheries. BioScience 66:274-284

Kuebbing SE, Maynard DS, Bradford MA (2018) Linking functional diversity and ecosystem processes: a framework for using functional diversity metrics to predict the ecosystem impact of functionally unique species. Journal of Ecology 106:687-698

Kumar P, Jetani K, Yusuzai S, Sayani A, Dar SA, Rather MA (2012) Effect of sediment and water quality parameters on the productivity of coastal shrimp farm. Advances in Applied Science Research 3:2033-2041

La Rosa T, Mirto S, Mazzola A, Danovaro R (2001) Differential responses of benthic microbes and meiofauna to fish-farm disturbance in coastal sediments. Environmenal Pollution 112:427-434

Lambshead P (1986) Sub-catastrophic sewage and industrial waste contamination as revealed by marine nematode faunal analysis. Marine Ecology Progress Series 29:247-260

Landry MR (2002) Integrating classical and microbial food web concepts: evolving views from the open-ocean tropical Pacific. Hydrobiologia 480:29-39

Lavens P, Sorgeloos P (2000) The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. Aquaculture 181:397-403

Lebreton B, Richard P, Galois R, Radenac G, Brahmia A, Colli G, Grouazel M, André C, Guillou G, Blanchard GF (2012) Food sources used by sediment meiofauna in an intertidal *Zostera noltii* seagrass bed: a seasonal stable isotope study. Marine Biology 159:1537-1550

Leduc D, Probert PK (2009) The effect of bacterivorous nematodes on detritus incorporation by macrofaunal detritivores: A study using stable isotope and fatty acid analyses. Journal of Experimental Marine Biology and Ecology 371:130-139

Leduc D, Probert PK, Duncan A (2009) A multi-method approach for identifying meiofaunal trophic connections. Marine Ecology Progress Series 383:95-111

Lee C-S (2010) Introduced species and aquaculture. Bulletin of Fisheries Research Agency p 69-78

Lee H, Gerdes D, Vanhove S, Vincx M (2001) Meiofauna response to iceberg disturbance on the Antarctic continental shelf at Kapp Norvegia (Weddell Sea). Polar Biology 24:926-933

Lee M, Correa J, Castilla J (2001) An assessment of the potential use of the nematode to copepod ratio in the monitoring of metals pollution. The Chanaral case. Marine Pollution Bulletin 42:696-701

Lefebvre S, Marín Leal JC, Dubois S, Orvain F, Blin J-L, Bataillé M-P, Ourry A, Galois R (2009) Seasonal dynamics of trophic relationships among co-occurring suspension-feeders in two shellfish culture dominated ecosystems. Estuarine, Coastal and Shelf Science 82:415-425

Legeżyńska J, Kędra M, Walkusz W (2014) Identifying trophic relationships within the high Arctic benthic community: how much can fatty acids tell? Marine Biology 161:821-836

Levinton JS (1995) Marine biology: function, biodiversity, ecology. Oxford University Press, New York, p 515

Li S, Li F, Zhang Y, Liu Y, Xu Z, Zheng L (2014) Secondary productivity of macrobenthos in Laizhou Bay, East China. Chinese Journal of Ecology 33:190-197 (in Chinese)

Li X, Li J, Wang Y, Fu L, Fu Y, Li B, Jiao B (2011) Aquaculture industry in China: current state, challenges, and outlook. Reviews in Fisheries Science 19:187-200

Li Y, Hu Y, Chen S (2013) Distribution and influence factors of nutrients in the North Yellow Sea in summer and autumn. China Environmental Science 33:1-060 (in Chinese with English abstract)

Li Y, Jiang L, Wang R (2014) Layered farming for *Marsupenaeus japonicus* Bate. Chinese Journal of Oceanology and Limnology 32:549-553

Li Y, Veilleux DJ, Wikfors GH (2009) Particle removal by Northern bay scallops *Argopecten irradians irradians* in a semi-natural setting: Application of a flow-cytometric technique. Aquaculture 296:237-245

Liao C, Peng R, Luo Y, Zhou X, Wu X, Fang C, Chen J, Li B (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytologist 177:706-714

Lin Y, Gao Z, Zhan A (2015) Introduction and use of non-native species for aquaculture in China: status, risks and management solutions. Reviews in Aquaculture 7:28-58

Liu H, Fang J, Zhu J, Dong S, Wang F, Liang X, Zhang J, Lian Y, Wang L, Jiang W (2004) Study on limiting nutrients and phytoplankton at long-line-culture areas in Laizhou Bay and Sanggou Bay, northeastern China. Aquatic Conservation: Marine and Freshwater Ecosystems 14:551-574

Liu L (2008) Checklist of marine biota of China Seas. Science Press, Academia Sinica, Beijing, p 1267

Liu SM, Zhang J, Li DJ (2004) Phosphorus cycling in sediments of the Bohai and Yellow Seas. Estuarine, Coastal and Shelf Science 59:209-218

Liu Y, Yang X, Jin Y (2011) Distribution and inter-annual variation of nutrients in Laizhou Bay. Progress in Fishery Science 32:1-5 (in Chinese with English abstract)

Lodge D, Williams S, MacIsaac H, Hayes K, Leung B, Reichard S, Mack R, Moyle P, Smith M, Andow D, Carlton J, McMichael A (2006) Biological invasions: recommendations for US policy and management. Ecological Applications 16:2035-2054

Lohrer AM, Thrush SF, Gibbs MM (2004) Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431:1092

Loreau M, Mazancourt C (2013) Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. Ecology letters 16:106-115

Losi V, Ferrero TJ, Moreno M, Gaozza L, Rovere A, Firpo M, Marques JC, Albertelli G (2013) The use of nematodes in assessing ecological conditions in shallow waters surrounding a Mediterranean harbour facility. Estuarine, Coastal and Shelf Science 130:209-221

Lubchenco J (1998) Entering the century of the environment: a new social contract for science. Science 279:491-497

Lytle JS, Lytle TF, Ogle JT (1990) Polyunsaturated fatty acid profiles as a comparative tool in assessing maturation diets of *Penaeus vannamei*. Aquaculture 89:287-299

Ma C (2015) Whiteleg shrimp *Litopenaeus vannamei*, China. In: Monterey Bay Aquarium Seafood Watch

MAC (Ministry of Agriculture of China, Bureau of Aquatic Products) (2013) China fishery annual statistics, 1986-2013. MAC Bureau of Aquatic Products, Beijing, China (in Chinese)

MacKenzie Jr CL (2008) History of the bay scallop, *Argopecten irradians*, fisheries and habitats in eastern North America, Massachusetts through northeastern Mexico. Marine Fisheries Review 70:1-5

Majdi N, Tackx M, Traunspurger W, Buffan-Dubau E (2012) Feeding of biofilm-dwelling nematodes examined using HPLC-analysis of gut pigment contents. Hydrobiologia 680:219-232

Maldonado C, Cuzon G, Guzmán E, Brito R, Soto L, Arena L, Gaxiola G (2009) Effect of an herbivorous diet on energy balance of *Litopenaeus vannamei* at selected ontogenetic stages. Aquaculture 296:123-128

Mangion M, Borg JA, Schembri PJ, Sanchez ‐ Jerez P (2017) Assessment of benthic biological indicators for evaluating the environmental impact of tuna farming. Aquaculture Research 48:5797-5811

Mansour MP, Volkman JK, Jackson AE, Blackburn SI (1999) The fatty acid and sterol composition of five marine dinoflagellates. Journal of Phycology 35:710-720

Mao TY, Dai MX, Peng ST, Li GL (2009) Temporal-spatial variation trend analysis of heavy metals (Cu, Zn, Pb, Cd, Hg) in Bohai Bay in 10 years. Journal of Tianjin University Science and Technology 42:817-825 (in Chinese with English abstract)

Mao Y, Yang H, Zhou Y, Ye N, Fang J (2009) Potential of the seaweed *Gracilaria lemaneiformis* for integrated multi-trophic aquaculture with scallop Chlamys farreri in North China. Journal of Applied Phycology 21:649

Mare MF (1942) A study of a marine benthic community with special reference to the micro-organisms. Journal of the Marine Biological Association of the United Kingdom 25:517-554

Maria TF, Vanaverbeke J, Esteves AM, De Troch M, Vanreusel A (2012) The importance of biological interactions for the vertical distribution of nematodes in a temperate ultra-dissipative sandy beach. Estuarine, Coastal and Shelf Science 97:114–126

Marić M, De Troch M, Occhipinti-Ambrogi A, Olenin S (2016) Trophic interactions between indigenous and non-indigenous species in Lampedusa Island, Mediterranean Sea. Marine Environmental Research 120:182-190

Marins R, Paula Filho F, Eschrique S, Lacerda L (2011) Anthropogenic sources and distribution of phosphorus in sediments from the Jaguaribe River estuary, NE, Brazil. Brazilian Journal of Biology 71:673-678

Martin J-LM, Veran Y, Guelorget O, Pham D (1998) Shrimp rearing: stocking density, growth, impact on sediment, waste output and their relationships studied through the nitrogen budget in rearing ponds. Aquaculture 164:135-149

Martin J-LM, Veran Y, Guelorget O, Pham D (1998) Shrimp rearing: stocking density, growth, impact on sediment, waste output and their relationships studied through the nitrogen budget in rearing ponds. Aquaculture 164:135-149

Martinez-Cordova LR, Porchas-Cornejo MA, Villarreal-Colemnares H, Calderon-Perez JA, Naranjo-Paramo J (1998) Evaluation of three feeding strategies on the culture of white shrimp *Penaeus vannamei* boone 1931 in low water exchange ponds. Aquacultural Engineering 17:21-28

Martinez-Garcia E, Carlsson MS, Sanchez-Jerez P, Sánchez-Lizaso JL, Sanz-Lazaro C, Holmer M (2015) Effect of sediment grain size and bioturbation on decomposition of organic matter from aquaculture. Biogeochemistry 125:133-148

Mascart T, De Troch M, Remy F, Michel LN, Lepoint G (2018) Seasonal dependence on seagrass detritus and trophic niche partitioning in four copepod eco-morphotypes. Food Webs 16:e00086

Mascart T, Lepoint G, De Troch M (2013) Meiofauna and harpacticoid copepods in different habitats of a Mediterranean seagrass meadow. Journal of the Marine Biological Association of the United Kingdom 93:1557-1566

Masser MP (1988) What is cage culture? SRAC Publication 160, U.S. Department of Agriculture, Southern Regional Aquaculture Center, Mississippi

Mazzola A, Mirto S, Danovaro R (1999) Initial fish-farm impact on meiofaunal assemblages in coastal sediments of the Western Mediterranean. Marine Pollution Bulletin 38:1126-1133

Mazzola A, Mirto S, La Rosa T, Fabiano M, Danovaro R (2000) Fish-farming effects on benthic community structure in coastal sediments: analysis of meiofaunal recovery. ICES Journal of Marine Science 57:1454-1461

McArdle JF, McKiernan F, Foley H, Jones DH (1991) The current status of Bonamia disease in Ireland. Aquaculture 93:273-278

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378-390

McIntyre A (1969) Ecology of marine meiobenthos. Biological Reviews 44:245-288

McKindsey CW, Archambault P, Callier MD, Olivier F (2011) Influence of suspended and off-bottom mussel culture on the sea bottom and benthic habitats: a review[1]. Canadian Journal of Zoology 89:622-646

Mcleod E, Chmura GL, Bouillon S, Salm R, Björk M, Duarte CM, Lovelock CE, Schlesinger WH, Silliman BR (2011) A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. Frontiers in Ecology and the Environment 9:552-560

Mermillod-Blondin F, Rosenberg R (2006) Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquatic Sciences 68:434-442

Mevenkamp L, Ong EZ, Van Colen C, Vanreusel A, Guilini K (2018) Combined, short-term exposure to reduced seawater pH and elevated temperature induces community shifts in an intertidal meiobenthic assemblage. Marine Environmental Research 133:32-44

Meyer FP (1991) Aquaculture disease and health management. Journal of Animal Science 69:4201-4208

Meziane T, Tsuchiya M (2000) Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Marine Ecology Progress Series:49-57

Miao W (2005) Status of aquaculture of *Penaeus vannamei* in China. In: Regional technical consultation on the aquaculture of *P. vannamei* and other exotic shrimps in southeast Asia, Manila, Philippines. SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, p 84-91

Miller DC, Norkko A, Pilditch CA (2002) Influence of diet on dispersal of horse mussel *Atrina zelandica* biodeposits. Marine Ecology Progress Series 242:153-167

Miller LM, Kapuscinski AR, Senanan W (2002) A biosafety approach to addressing risks posed by aquaculture Escapees. In: Gupta MV, Bartley DM, and Acosta BO (eds) Use of genetically improved and alien species for aquaculture and conservation of aquatic biodiversity in Africa, WorldFish Center, Penang, Malaysia, p 56-65

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between δ^{15}N and animal age. Geochimica et Cosmochimica Acta 48:1135-1140

Minchin D, Gollasch S, Cohen AN, Hewitt CL, Olenin S (2009) Characterizing vectors of marine invasion. In: Rilov G, Crooks JA (eds) Biological invasions in marine ecosystems. Ecological Studies 204. Springer, Berlin, p 153–172

Ministry of Agriculture of China (MOA) (2012-2017) China Fishery Statistics Yearbook of the Ministry of Agriculture of the People's Republic of China. China Ocean Press, Beijing

Miron G, Landry T, Archambault P, Frenette B (2005) Effects of mussel culture husbandry practices on various benthic characteristics. Aquaculture 250:138-154

Mirto S, Arigò C, Genovese L, Pusceddu A, Gambi C, Danovaro R (2014) Nematode assemblage response to fish-farm impact in vegetated (*Posidonia oceanica*) and non-vegetated habitats. Aquaculture Environmental Interactions 5:17-28

Mirto S, Bianchelli S, Gambi C, Krzelj M, Pusceddu A, Scopa M, Holmer M, Danovaro R (2010) Fish-farm impact on metazoan meiofauna in the Mediterranean Sea: analysis of regional vs. habitat effects. Marine Environmental Research 69:38-47

Mirto S, Danovaro R, Mazzola A (2000) Microbial and meiofaunal response to intensive mussel-farm biodeposition in coastal sediments of the western Mediterranean. Marine Pollution Bulletin 40:244-252

Mirto S, Gristina M, Sinopoli M, Maricchiolo G, Genovese L, Vizzini S, Mazzola A (2012) Meiofauna as an indicator for assessing the impact of fish farming at an exposed marine site. Ecological Indicators 18:468-476

Mirto S, La Rosa T, Gambi C, Danovaro R, Mazzola A (2002) Nematode community response to fish-farm impact in the western Mediterranean. Environmental Pollution116:203-214

Moens T, Bouillon S, Gallucci F (2005) Dual stable isotope abundances unravel trophic position of estuarine nematodes. Journal of the Marine Biological Association of the United Kingdom 85:1401-1407

Moens T, Luyten C, Middelburg JJ, Herman PM, Vincx M (2002) Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. Marine Ecology Progress Series 234:127-137

Moens T, Vincx M (1997) Observations on the feeding ecology of estuarine nematodes. Journal of the Marine Biological Association of the United Kingdom 77:211–227

Mohanty R (2000) Performance evaluation of different water exchange patterns on growth and survival of *Penaeus monodon* (Fabricius). International Journal of Tropical Agriculture 18:63-68

Monroig Ó, Tocher DR, Navarro JC (2013) Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. Marine Drugs 11:3998-4018

Montagna PA, Blanchard GF, Dinet A (1995) Effect of production and biomass of intertidal microphytobenthos on meiofaunal grazing rates. Journal of Experimental Marine Biology and Ecology 185:149-165

Moore JC, Berlow EL, Coleman DC, Ruiter PC, Dong Q, Hastings A, Johnson NC, McCann KS, Melville K, Morin PJ (2004) Detritus, trophic dynamics and biodiversity. Ecology Letters 7:584-600

Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11:470-480

Moreno M, Ferrero T, Gallizia I, Vezzulli L, Albertelli G, Fabiano M (2008) An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuarine, Coastal and Shelf Science 77:565-576

Moriarty DJ (1997) The role of microorganisms in aquaculture ponds. Aquaculture 151:333-349

Moriarty DJW (1986) Bacterial productivity in ponds used for culture of penaeid prawns. Microbial Ecology 12:259-269

Mu F-H, Huys R (2004) Canuellidae (Copepoda, Harpacticoida) from the Bohai Sea, China. Journal of Natural History 38:1-36

Mu F-H, Somerfield P, Warwick R, Zhang Z (2002) Large-scale spatial patterns in the community structure of benthic harpacticoid copepods in the Bohai Sea, China. Raffles Bulletin of Zoology 50:17-26

Nair K, Anger K (1979) Life cycle of *Corophium insidiosum* (Crustacea, Amphipoda) in laboratory culture. Helgoländer Wissenschaftliche Meeresuntersuchungen 32:279

Nanton DA, Castell JD (1999) The effects of temperature and dietary fatty acids on the fatty acid composition of harpacticoid copepods, for use as a live food for marine fish larvae. Aquaculture 175:167-181

Napolitano GE (1999) Fatty acids as trophic and chemical markers in freshwater ecosystems. In: Arts MT, Wainman BC (eds) Lipids in Freshwater Ecosystems. Springer, New York, NY, p 21-44

Nascimento F, Näslund J, Elmgren R (2013) Meiofauna enhances organic matter mineralization in soft sediment ecosystems. Limnology and Oceanography 57:338-346

Navarro J, Thompson R (1997) Biodeposition by the horse mussel *Modiolus modiolus* (Dillwyn) during the spring diatom bloom. Journal of Experimental Marine Biology and Ecology 209:1-13

Naylor RL, Goldburg RJ, Mooney H, Beveridge M, Clay J, Folke C, Kautsky N, Lubchenco J, Primavera J, Williams M (1998) Nature's subsidies to shrimp and salmon Farming. Science 282:883

Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MC, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. Nature 405:1017-1024

Naylor RL, Williams SL, Strong DR (2001) Aquaculture--A gateway for exotic species. Science 294:1655-1656

Nelson MM, Mooney BD, Nichols PD, Phleger CF (2001) Lipids of Antarctic Ocean amphipods: food chain interactions and the occurrence of novel biomarkers. Marine Chemistry 73:53-64

Netto SA, Valgas I (2010) The response of nematode assemblages to intensive mussel farming in coastal sediments (Southern Brazil). Environmental Monitoring and Assessment 162:81-93

Nevejan N, De Schryver P, Wille M, Dierckens K, Baruah K, Van Stappen G (2018) Bacteria as food in aquaculture: do they make a difference? Reviews in Aquaculture 10:180-212

New MB (1987) Feed and feeding of fish and shrimp: a manual on the preparation and presentation of compound feeds for shrimp and fish in aquaculture. Aquaculture Development and Coordination Rogramme ADCP/REP/87/26, Food and Agriculture Organization of the United Nations, Rome, 275 p

Newell RI (2004) Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. Journal of Shellfish Research 23:51-62

Newell RI, Cornwell JC, Owens MS (2002) Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: a laboratory study. Limnology and Oceanography 47:1367-1379

Newton J (2010) Stable isotopes as tools in ecological research. In: Encyclopedia of Life Sciences. John Wiley & Sons Ltd, Chichester

Nixon SW (1995) Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41:199-219

Noodt W (1971) Ecology of the Copepoda. Smithsonian Contributions to Zoology 76:102

Nunes A, Gesteira T, Goddard S (1997) Food ingestion and assimilation by the Southern brown shrimp *Penaeus subtilis* under semi-intensive culture in NE Brazil. Aquaculture 149:121-136

Nyssen F, Brey T, Dauby P, Graeve M (2005) Trophic position of Antarctic amphipods—enhanced analysis by a 2-dimensional biomarker assay. Marine Ecology Progress Series 300:135-145

Odum EP (1959) Fundamentals of ecology, Vol. WB Saunders company, Philadelphia & London

Odum HT (1996) Environmental accounting: emergy and environmental decision making. John Wiley & Sons, New York, US

Ólafsson E, Modig H, van de Bund WJ (1999) Species specific uptake of radio-labelled phytodetritus by benthic meiofauna from the Baltic Sea. Marine Ecology Progress Series:63-72

Olenin S, Alemany F, Cardoso AC, Gollasch S, Goulletquer P, Lehtiniemi M, McCollin T, Minchin D, Miossec L, Occhipinti Ambrogi A, Ojaveer H, Rose K, Jensen MS, Wallentinus I, Aleksandrov B (2010) Marine Strategy Framework Directive - Task Group 2 Non-indigenous Species, Vol. Office for Official Publications of the European Communities, Luxembourg

Olenin S, Minchin D (2011) Biological introductions to the systems: macroorganisms. In: Wolanski E and McLusky DS (eds) Treatise on estuarine and coastal science, Vol 8, Waltham: Academic Press, p 149–183

Olenin S, Minchin D, Daunys D (2007) Assessment of biopollution in aquatic ecosystems. Marine Pollution Bulletin 55:379-394

Orlova MI, Telesh IV, Berezina NA, Antsulevich AE, Maximov AA, Litvinchuk LF (2006) Effects of nonindigenous species on diversity and community functioning in the eastern Gulf of Finland (Baltic Sea). Helgoland Marine Research 60:98-105

Orvain F, Galois R, Barnard C, Sylvestre A, Blanchard G, Sauriau P-G (2003) Carbohydrate production in relation to microphytobenthic biofilm development: an integrated approach in a tidal mesocosm. Microbial Ecology 45:237-251

Ouraji H, Esmaeili Fereidoni A, Shayegan M (2011) Comparison of fatty acid composition between farmed and wild Indian white shrimps, *Fenneropenaeus indicus*. Food and Nutrition Sciences, 2:824-829

Padilla DK, McCann MJ, Shumway SE (2011) Marine invaders and bivalve aquaculture: sources, impacts, and consequences. In: Shumway SE (ed) Shellfish aquaculture and the environment, Blackwell Pblishing, Danvers, MA, p 395-424

Paez-Osuna F (2001) The Environmental Impact of Shrimp Aquaculture: Causes, Effects, and Mitigating Alternatives. Environmental Management 28:131-140

Pan K, Wang W-X (2012) Trace metal contamination in estuarine and coastal environments in China. Science of The Total Environment 421-422:3-16

Papageorgiou N, Kalantzi I, Karakassis I (2010) Effects of fish farming on the biological and geochemical properties of muddy and sandy sediments in the Mediterranean Sea. Marine Environmental Research 69:326-336

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PloS one 5:e9672

Patrona LD, Bianchelli S, Beliaeff B, Pusceddu A (2012) Meiobenthos in earthen ponds used for semi-intensive shrimp farming (New Caledonia, South Pacific). Chemistry and Ecology 28:506-523

Pearson T, Black K (2000) The environmental impacts of marine fish cage culture. In: Black KD (ed) Environmental impacts of aquaculture. Sheffield Academic Press, Sheffield, p 1-31

Pearson T, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology, An Annual Review 16:229-311

Pearson T, Rosenberg R Feast and famine: structuring factors in marine benthic communities. In: Gee JHR and Giller PS (eds), The 27th Symposium of the British Ecological Society, Aberystwyth 1986, pp 373-395. Blackwell Scientific Publications, Oxford

Pérez J, Alfonsi C, Nirchio M, Muñoz C, A Gómez J (2003) The introduction of exotic species in aquaculture: A solution or part of the problem? Interciencia 28:234-238

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293-320

Peterson BJ, Heck KL (1999) The potential for suspension feeding bivalves to increase seagrass productivity. Journal of Experimental Marine Biology and Ecology 240:37-52

Pheloung P, Williams P, Halloy S (1999) A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. Journal of environmental management 57:239-251

Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261-269

Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology 92:823-835

Phillips DL, Newsome SD, Gregg JW (2005) Combining sources in stable isotope mixing models: alternative methods. Oecologia 144:520-527

Pike J, Bernhard JM, Moreton SG, Butler IB (2001) Microbioirrigation of marine sediments in dysoxic environments: Implications for early sediment fabric formation and diagenetic processes. Geology 29:923-926

Pillay TVR (2008) Aquaculture and the environment, 2nd edn. Blackwell, London

Pine HJ, Jescovitch LN, Roy LA, Boyd CE, Davis DA, Brown TW (2018) Growth of Pacific white shrimp *Litopenaeus vannamei* cultured on inland low-salinity pond soils. North American Journal of Aquaculture 80:273–277

Piroddi C, Teixeira H, Lynam CP, Smith C, Alvarez MC, Mazik K, Andonegi E, Churilova T, Tedesco L, Chifflet M, Chust G, Galparsoro I, Garcia AC, Kämäri M, Kryvenko O, Lassalle G, Neville S, Niquil N, Papadopoulou N, Rossberg AG, Suslin V, Uyarra MC (2015) Using ecological models to assess ecosystem status in support of the European Marine Strategy Framework Directive. Ecological Indicators 58:175-191

Polidoro BA, Carpenter KE, Collins L, Duke NC, Ellison AM, Ellison JC, Farnsworth EJ, Fernando ES, Kathiresan K, Koedam NE (2010) The loss of species: mangrove extinction risk and geographic areas of global concern. PloS One 5:e10095

Porchas-Cornejo M, Martinez-Cordova L, Ramos-Trujillo L, Hernandez-Lopez J, Martinez-Porchas M, Mendoza-Cano F (2011) Effect of promoted natural feed on the production, nutritional, and immunological parameters of *Litopenaeus vannamei* (Boone, 1931) semi-intensively farmed. Aquaculture Nutrition 17:622–628.

Porchas-Cornejo M, Martinez-Porchas M, Martinez-Cordova LR, Ramos-Trujillo L, Barraza-Guardado R (2012) Consumption of natural and artificial foods by shrimp (*Litopenaeus vannamei*) reared in ponds with and without enhancement of natural productivity. The Israeli journal of aquaculture-Bamidgeh IJA 64.2012, 709(7):1–7

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Power ME (1992) Habitat heterogeneity and the functional signifiance of fish in river food webs. Ecology 73:1675-1688

Power ME (1992) Habitat heterogeneity and the functional significance of fish in river food webs.. Ecology 73:733-746

Primavera JH (2006) Overcoming the impacts of aquaculture on the coastal zone. Ocean & Coastal Management 49:531-545

Primavera, JH (1998) Tropical shrimp farming and its sustainability. In: de Silva S. (ed) Tropical Mariculture. Academic Press, London, p 257–289.

Pusceddu A, Danovaro R (2007) Indicators of trophic state and environmental quality of marine coastal environments: a benthic perspective. In. Proc In proceedings of the Symposim 'Mediterranean Coastal Monitoring: Problems and Tools'. CNR Press, Sassari, Italy, p 1-8

Pusceddu A, Dell'Anno A, Fabiano M, Danovaro R (2009) Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status. Marine Ecology Progress Series 375:41-52

Pusceddu A, Della Patrona L, Beliaeff B (2011) Trophic status of earthen ponds used for semi-intensive shrimp (*Litopenaeus stylirostris*, Stimpson, 1874) farming in New Caledonia (Pacific Ocean). Marine Environmental Research 72:160-171

Pusceddu A, Fraschetti S, Mirto S, Holmer M, Danovaro R (2007a) Effects of intensive mariculture on sediment biochemistry. Ecological Applications 17:1366-1378

Pusceddu A, Gambi C, Manini E, Danovaro R (2007b) Trophic state, ecosystem efficiency and biodiversity of transitional aquatic ecosystems: analysis of environmental quality based on different benthic indicators. Chemistry and Ecology 23:505-515

Qin Y, Hou J, Deng M, Liu Q, Wu C, Ji Y, He X (2016) Bacterial abundance and diversity in pond water supplied with different feeds. Scientific Reports 6:35232

Quigley DT, Herdson D, Flannery K (2013) Occurrence of the kuruma prawn *Marsupenaeus japonicus* (Spence Bate, 1888) in the Celtic Sea, English Channel, and north-west France. BioInvasions Records 2:51-55

Raffaelli D (1981) Monitoring with meiofauna—a reply to Coull, Hicks and Wells (1981) and additional data. Marine Pollution Bulletin 12:381-382

Raffaelli D, Mason C (1981) Pollution monitoring with meiofauna, using the ratio of nematodes to copepods. Marine Pollution Bulletin 12:158-163

Redmond KJ, Magnesen T, Hansen PK, Strand Ø, Meier S (2010) Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). Aquaculture 298:202-210

Reuss N, Poulsen L (2002) Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland. Marine Biology 141:423-434

Reymond H, Lagardère JP (1990) Feeding rhythms and food of *Penaeus japonicus* Bate (Crustacea, penaeidae) in salt marsh ponds; role of halophilic entomofauna. Aquaculture 84:125-143

Ribeiro LF, Eça GF, Barros F, Hatje V (2016) Impacts of shrimp farming cultivation cycles on macrobenthic assemblages and chemistry of sediments. Environmental Pollution 211:307-315

Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6:93-107

Riera R, Sanchez-Jerez P, Rodríguez M, Monterroso Ó, Ramos E (2012) Long-term monitoring of fish farms: application of Nematode/Copepod index to oligotrophic conditions. Marine Pollution Bulletin 64:844-850

Rilov G, Crooks J (2009) Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives. Springer, Berlin, Heidelberg

Rodríguez C, Bécares E, Fernández-Aláez M, Fernández-Aláez C (2005) Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. Biological Invasions 7:75

Rosenberg R (2001) Marine benthic faunal successional stages and related sedimentary activity. Scientia Marina 65:107-119

Rothlisberg PC (1998) Aspects of penaeid biology and ecology of relevance to aquaculture: a review1. Aquaculture 164:49-65

Rozan TF, Taillefert M, Trouwborst RE, Glazer BT, Ma S, Herszage J, Valdes LM, Price KS, Luther III GW (2002) Iron-sulfur-phosphorus cycling in the sediments of a shallow coastal bay: implications for sediment nutrient release and benthic macroalgal blooms. Limnology and Oceanography 47:1346-1354

Rubright JS, Harrell JL, Holcomb HW, Parker JC (1981) Responses of planktonic and benthic communities to fertilizer and feed applications in shrimp mariculture ponds. Journal of the World Mariculture Society 12:281-299

Ruesink JL, Lenihan HS, Trimble AC, Heiman KW, Micheli F, Byers JE, Kay MC (2005) Introduction of non-native oysters: ecosystem effects and restoration implications. Annual Review of Ecology, Evolution, and Systematics 36:643-689

Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. American Zoologist 37:621-632

Rzeznik-Orignac J, Boucher G, Fichet D, Richard P (2008) Stable isotope analysis of food source and trophic position of intertidal nematodes and copepods. Marine Ecology Progress Series 359:145-150

Sanders R, Caron DA, Berninger UG (1992) Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Marine Ecology Progress Series 86:1-14

Sandulli R, De Nicola M (1991) Responses of meiobenthic communities along a gradient of sewage pollution. Marine Pollution Bulletin 22:463-467

Sanz-Lázaro C, Sanchez-Jerez P (2017) Mussels do not directly assimilate fish farm wastes: shifting the rationale of integrated multi-trophic aquaculture to a broader scale. Journal of Environmental Economics and Management 201:82–88

Sarà G (2007) A meta-analysis on the ecological effects of aquaculture on the water column: Dissolved nutrients. Marine Environmental Research 63:390-408

Saumena (2015) Indonesia becomes second-largest vannamei producer in the world. www.undercurrentnews.com/2015/02/24/indonesia-becomes-second-largest-vannamei-producer-in-the-world (accessed 24 Feb 2015)

Schlechtriem C, Arts M, Zellmer I (2006) Effect of temperature on the fatty acid composition and temporal trajectories of fatty acids in fasting Daphnia pulex (Crustacea, Cladocera). Lipids 41:397-400

Schlechtriem C, Ricci M, Focken U, Becker K (2004) Mass produced nematodes Panagrellus redivivus as live food for rearing carp larvae: preliminary results. Aquaculture Research 35:547-551

Schnick RA (2001) International harmonization of antimicrobial sensitivity determination for aquaculture drugs. Aquaculture 196:277-288

Scholz B, Liebezeit G (2012) Microphytobenthic dynamics in a Wadden Sea intertidal flat–Part II: Seasonal and spatial variability of non-diatom community components in relation to abiotic parameters. European Journal of Phycology 47:120-137

Schratzberger M, Gee J, Rees H, Boyd S, Wall C (2000) The structure and taxonomic composition of sublittoral meiofauna assemblages as an indicator of the status of marine environments. Journal of the Marine Biological Association of the United Kingdom 80:969-980

Schroeder GL (1983) Stable isotope ratios as naturally occurring tracers in the aquaculture food web. Aquaculture 30:203-210

Schwamborn R, Criales MM (2000) Feeding strategy and daily ration of juvenile pink shrimp (*Farfantepenaeus duorarum*) in a South Florida seagrass bed. Marine Biology 137:139-147

Sdrigotti E, Umani SF (2002) Effects of suspended mussel culture on microphytobenthos community in the Gulf of Trieste (North Adriatic Sea). Atti Associazione Italiana

Oceanologia Limnologia 15:11-23

Seifried S, Dürbaum J (2000) First clear case of carnivory in marine Copepoda Harpacticoida. Journal of Natural History 34:1595-1618

Semprucci F, Balsamo M, Sandulli R (2016) Assessment of the ecological quality (EcoQ) of the Venice lagoon using the structure and biodiversity of the meiofaunal assemblages. Ecological Indicators 67:451-457

Senanan W, Panutrakul S, Barnette P, Manthachitra V, Chavanich S, Kapuscinski A, Tangkrock-Olan N, Intacharoen P, Viyakarn V, Wongwiwatanawute C (2010) Ecological risk assessment of an alien aquatic species: a case study of *Litopenaeus vannamei* (Pacific Whiteleg Shrimp) aquaculture in the Bangpakong River, Thailand. Tropical Deltas and Coastal Zones: Food Production, Communities and the Environment at the Land–water Interface, 9, p 64-79

Senarath U, Visvanathan C (2001) Environmental issues in brackish water shrimp aquaculture in Sri Lanka. Environmental Management 27:335-348

Shan Z, Zheng Z, Xing H, Liu X, Liu X, Liu Y (2000) Study on eutrophication in Laizhou Bay of Bohai. Transactions of Oceanology and Limnology 2:41-46 (in Chinese)

Shelton WL, Rothbard S (2006) Exotic species in global aquaculture – A review. The Israeli Journal of Aquaculture – Bamidgeh 58:3-28

Shiells G, Anderson K (1985) Pollution monitoring using the Nematode/Copepod Ratio a practical application. Marine Pollution Bulletin 16:62-68

Shumway SE (1990) A review of the effects of algal blooms on shellfish and aquaculture. Journal of the World Aquaculture Society 21:65-104

Shumway SE, Davis C, Downey R, Karney R, Kraeuter J, Parsons J, Rheault R, Wikfors G (2003) Shellfish aquaculture–in praise of sustainable economies and environments. World aquaculture 34:8-10

Silva FB, Silva JF, Bezerra RS, Santos PJ (2017) Are biochemical composition parameters of sediment good tools for assessing the environmental quality of estuarine areas in tropical systems? Journal of the Marine Biological Association of the United Kingdom 1-10

Silva SSD, Nguyen TT, Turchini GM, Amarasinghe US, Abery NW (2009) Alien species in aquaculture and biodiversity: a paradox in food production. AMBIO: A Journal of the Human Environment 38:24-28

Slater MJ, Jeffs AG, Carton AG (2009) The use of the waste from green-lipped mussels as a food source for juvenile sea cucumber, *Australostichopus mollis*. Aquaculture 292:219-224

Smetacek V (1984) The Supply of Food to the Benthos. In: Fasham MJR (ed) Flows of Energy and Materials in Marine Ecosystems: Theory and Practice. Springer US, Boston, MA

Smith PT (1998) Effect of removing accumulated sediments on the bacteriology of ponds used to culture *Penaeus monodon*. Asian Fisheries Science 10, 355–370.

Smith RJ, Hobson KA, Koopman HN, Lavigne DM (1996) Distinguishing between populations of fresh-and salt-water harbour seals (*Phoca vitulina*) using stable-isotope ratios and fatty acid profiles. Canadian Journal of Fisheries and Aquatic Sciences 53:272-279

Smol N, Willems KA, Govaere JC, Sandee A (1994) Composition, distribution and biomass of meiobenthos in the Oosterschelde estuary (SW Netherlands). Hydrobiologia 282(283):197–217

Sofia G (2010) Caution is needed when applying Margalef diversity index. Ecological Indicators 10:550-551

Sousa R, Gutiérrez JL, Aldridge DC (2009) Non-indigenous invasive bivalves as ecosystem engineers. Biological Invasions 11:2367-2385

Spaargaren DH (1998) Cultivation of Tiger Prawns, *Penaeus monodon* Fabricius, 1798 (Decapoda, Natantia) in Hainan, P. R. China. Crustaceana 71:144-157

State Oceanic Administration (SOA) (2006-2009) Bulletin of Marine Environmental Quality of China. Ocean Press, Beijing, China (in Chinese)

Stefanou P, Tsirtsis G, Karydis M (2000) Nutrient scaling for assessing eutrophication: The development of a simulated normal distribution. Ecological Applications 10:303-309

Stenton-Dozey J, Probyn T, Busby A (2001) Impact of mussel (*Mytilus galloprovincialis*) raft-culture on benthic macrofauna, in situ oxygen uptake, and nutrient fluxes in Saldanha Bay, South Africa. Canadian Journal of Fisheries and Aquatic Sciences 58:1021-1031

Stenton-Dozey J, Probyn T, Busby A (2001) Impact of mussel (*Mytilus galloprovincialis*) raft-culture on benthic macrofauna, in situ oxygen uptake, and nutrient fluxes in Saldanha Bay, South Africa. Canadian Journal of Fisheries and Aquatic Sciences 58:1021-1031

Stevens CJ, Deibel D, Parrish CC (2004) Copepod omnivory in the North Water Polynya (Baffin Bay) during autumn: spatial patterns in lipid composition. Deep Sea Research Part I: Oceanographic Research Papers 51:1637-1658

Stevens CJ, Deibel D, Parrish CC (2004) Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment with Calanus glacialis Jaschnov. Journal of Experimental Marine Biology and Ecology 303:135-156

Stevenson NJ (1997) Disused shrimp ponds: Options for redevelopment of mangroves. Coastal Management 25:425-435

Steyaert M, Vanaverbeke J, Vanreusel A, Barranguet C, Lucas C, Vincx M (2003) The importance of fine-scale, vertical profiles in characterising nematode community structure. Estuarine, Coastal and Shelf Science 58:353-366

Stillwell W, Wassall SR (2003) Docosahexaenoic acid: membrane properties of a unique fatty acid. Chemistry and Physics of Lipids 126:1-27

Stock BC, Semmens BX (2013) MixSIAR GUI user manual, version 1.0. Accessible online at: http://conserver iugo-cafe org/user/brice semmens/MixSIAR

Støttrup JG, Jensen J (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. Journal of Experimental Marine Biology and Ecology 141:87-105

Su Y, Ma S, Tian X, Dong S (2009) An experimental study on nitrogen, phosphorus and carbon budgets in intensive pond of shrimp *Fenneropenaeus chinensis*. South China Fisheries Science 5:54-58

Suárez JA, Gaxiola G, Mendoza R, Cadavid S, Garcia G, Alanis G, Suárez A, Faillace J, Cuzon G (2009) Substitution of fish meal with plant protein sources and energy budget for white shrimp *Litopenaeus vannamei* (Boone, 1931). Aquaculture 289:118-123

Subasinghe R (2017) Regional review on status and trends in aquaculture development in Asia-Pacific – 2010. Aquaculture Service, FAO Fisheries and Aquaculture Department, and Network of Aquaculture Centres in Asia-Pacific, Food and Agriculture Organization of the United Nations, Rome, 89 p

Suter II GW (2001) Applicability of indicator monitoring to ecological risk assessment. Ecological Indicators 1:101-112

Sutherland T, Levings C, Petersen S, Poon P, Piercey B (2007) The use of meiofauna as an indicator of benthic organic enrichment associated with salmonid aquaculture. Marine Pollution Bulletin 54:1249-1261

Svensson CJ, Hyndes GA, Lavery PS (2010) An efficient method for collecting large samples of live copepods free from detritus. Marine and Freshwater Research 61:621-624

Swedmark B (1964) The interstitial fauna of marine sand. Biological Reviews 39:1-42

Tacon A, Cody J, Conquest L, Divakaran S, Forster I, Decamp O (2002) Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. Aquaculture Nutrition 8:121-137

Tacon A, Metian M (2013) Fish Matters: importance of aquatic foods in human nutrition and global food supply. Reviews in Fisheries Science 21:22-38

Tang D, Kawamura H, Oh IS, Baker J (2006) Satellite evidence of harmful algal blooms and related oceanographic features in the Bohai Sea during autumn 1998. Advances in Space Research 37:681-689

Tang Q, Han D, Mao Y, Zhang W, Shan X (2016) Species composition, non-fed rate and trophic level of Chinese aquaculture. Fishery Sciences of China 23:729-758 (in Chinese)

Thompson FL, Abreu PC, Cavalli R (1999) The use of microorganisms as food source for *Penaeus paulensis* larvae. Aquaculture 174:139-153

Thompson FL, Abreu PC, Wasielesky W (2002) Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203:263-278

To V (2016) Optimization of feed management for Pacific White shrimp (*Litopenaeus vannamei*). MS thesis, Auburn University, US

Tsani S, Koundouri P (2018) A methodological note for the development of integrated aquaculture production models. Frontiers in Marine Science 4:1-13

Vafeiadou AM, Materatski P, Adão H, De Troch M, Moens T (2014) Resource utilization and trophic position of nematodes and harpacticoid copepods in and adjacent to *Zostera noltii* beds. Biogeosciences 11:4001

Van Oevelen D, Soetaert K, Middelburg JJ, Herman PM, Moodley L, Hamels I, Moens T, Heip CH

(2006) Carbon flows through a benthic food web: integrating biomass, isotope and tracer data. Journal of Marine Research 64:453-482

Vanderploeg HA, Liebig JR, Gluck AA (1996) Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena polymorpha*): the importance of size and polyunsaturated fatty acid content. Journal of Great Lakes Research 22:36-45

Vanreusel A, Vincx M, Bett BJ, Rice AL (1995) Nematode biomass spectra at two abyssal sites in the NE Atlantic with a contrasting food supply. Internationale Revue der gesamten Hydrobiologie und Hydrographie 80:287-296

Venturini N, Pita AL, Brugnoli E, García-Rodríguez F, Burone L, Kandratavicius N, Hutton M, Muniz P (2012) Benthic trophic status of sediments in a metropolitan area (Rio de la Plata estuary): linkages with natural and human pressures. Estuarine, Coastal and Shelf Science 112:139-152

Vezzulli L, Chelossi E, Riccardi G, Fabiano M (2002) Bacterial community structure and activity in fish farm sediments of the Ligurian sea (Western Mediterranean). Aquaculture International 10:123-141

Vezzulli L, Marrale D, Moreno M, Fabiano M (2003) Sediment organic matter and meiofauna community response to long-term fish-farm impact in the Ligurian Sea (Western Mediterranean). Chemistry and Ecology 19:431-440

Vezzulli L, Moreno M, Marin V, Pezzati E, Bartoli M, Fabiano M (2008) Organic waste impact of capture-based Atlantic bluefin tuna aquaculture at an exposed site in the Mediterranean Sea. Estuarine, Coastal and Shelf Science 78:369-384

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters 14:702-708

Vismann B (1996) Sulfide species and total sulfide toxicity in the shrimp Crangon crangon. Journal of Experimental Marine Biology and Ecology 204:141-154

Viso A-C, Marty J-C (1993) Fatty acids from 28 marine microalgae. Phytochemistry 34:1521-1533

Vizzini S, Mazzola A (2012) Tracking multiple pathways of waste from a northern bluefin tuna farm in a marine-coastal area. Marine Environmental Research 77:103-111

Volk EC, Wissmar RC, Simenstad CA, Eggers DM (1984) Relationship between otolith microstructure and the growth of juvenile chum salmon (*Oncorhynchus keta*) under different prey rations. Canadian Journal of Fisheries and Aquatic Sciences 41:126-133

Wai TC, Leung KM, Wu RS, Shin PK, Cheung SG, Li X, Lee JH (2011) Stable isotopes as a useful tool for revealing the environmental fate and trophic effect of open-sea-cage fish farm wastes on marine benthic organisms with different feeding guilds. Marine Pollution Bulletin 63:77-85

Walker PJ, Mohan C (2009) Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies. Reviews in Aquaculture 1:125-154

Wang C, Wang R, Yan Q, Chen H, Yan J (2009) Effects of temperature on survival, growth and development of amphipod(*Corophium acherusicum*). Marine Environmental Science 28:138-141 (in Chinese with English abstract)

Wang F (2015) Feeding ecology of two megagrazers in two typical bays. MSc thesis, University of Chinese Academy of Sciences, Beijing

Wang F, Zhou Y, Wang P, Xu S, Liu P (2018) Potential role of scallops *Argopecten irradians* in deposition of particulate nutrient and trace elements in a eutrophic estuary, northern China. Aquaculture Environment Interactions 10:135-147

Wang L, Gao Q, Shi F, Yang C, Qiu L, Zhang H, Zhou Z, Song L (2013) The comparative study of immunity between two scallop species *Chlamys farreri* and *Argopecten irradians*. Invertebrate Survival Journal 10:141-150

Wang Y (2001) China P.R.: a review of national aquaculture development. In: Subasinghe RP, Bueno P, Phillips MJ, Hough C, McGladdery SE, Arthur JR (eds) Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000, NACA, Bangkok and FAO, Rome, p 307-316

Wartenberg R, Feng L, Wu J, Mak Y, Chan L, Telfer T, Lam P (2017) The impacts of suspended mariculture on coastal zones in China and the scope for Integrated Multi-Trophic Aquaculture. Ecosystem Health and Sustainability 3: 1-20

Warwick R (1988) The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. Marine Pollution Bulletin 19:259-268

Watanabe T, Ohta M, Kitajima C, Fujita S (1982) Improvement of dietary value of brine shrimp *Artemia salina* for fish larvae by feeding them on ω3 highly unsaturated fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 48:1775-1782

Wei H, Sun J, Moll A, Zhao L (2004) Phytoplankton dynamics in the Bohai Sea—observations and modelling. Journal of Marine Systems 44:233-251

Weise AM, Cromey CJ, Callier MD, Archambault P, Chamberlain J, McKindsey CW (2009) Shellfish-DEPOMOD: Modelling the biodeposition from suspended shellfish aquaculture and assessing benthic effects. Aquaculture 288:239-253

Werbrouck E, Van Gansbeke D, Vanreusel A, De Troch M (2016) Temperature affects the use of storage fatty acids as energy source in a benthic copepod (*Platychelipus littoralis*, Harpacticoida). PLOS ONE 11:e0151779

Werry J, Lee S (2005) Grapsid crabs mediate link between mangrove litter production and estuarine planktonic food chains. Marine Ecology Progress Series 293:165-176

Westoby M (1984) The self-thinning rule. Advances in Ecological Research 14:167-225

Weston DP (1990) Quantitative examination of macrobenthic community changes along an organic enrichment gradient. Marine Ecology Progress Series 61:233-244

Wetzel MA, Fleeger JW, Powers SP (2001) Effects of hypoxia and anoxia on meiofauna: a review with new data from the Gulf of Mexico. In: Rabalais NN, Turner RE (eds) Coastal hypoxia: consequences for living resources and ecosystems. American Geophysical

Union, Washington DC, p 165-184

White CA, Bannister RJ, Dworjanyn SA, Husa V, Nichols PD, Kutti T, Dempster T (2017) Consumption of aquaculture waste affects the fatty acid metabolism of a benthic invertebrate. Science of The Total Environment 586:1170-1181

Wilding TA (2012) Changes in sedimentary redox associated with mussel (*Mytilus edulis* L.) farms on the West-Coast of Scotland. PloS One 7:e45159

Wildish D, Hargrave B, Pohle G (2001) Cost-effective monitoring of organic enrichment resulting from salmon mariculture. ICES Journal of Marine Science 58:469-476

Williamson M (1996) Biological invasions, p 244, Springer Netherlands

Wiyoto W, Sukenda S, Harris E, Nirmala K, Djokosetiyanto D, Ekasari J (2017) The effects of sediment redox potential and stocking density on Pacific white shrimp *Litopenaeus vannamei* production performance and white spot syndrome virus resistance. Aquaculture Research 48:2741-2751

Wotton RS, Malmqvist B (2001) Feces in aquatic ecosystems. BioScience 51:537-544

Wu H (2012) Pollution of nutrients and heavy metals in mariculture ponds and adjacent mangrove wetlands of China. PhD thesis, Xiamen University, Xiamen, China

Wu H, Peng R, Yang Y, He L, Wang W, Zheng T, Lin G (2014) Mariculture pond influence on mangrove areas in south China: Significantly larger nitrogen and phosphorus loadings from sediment wash-out than from tidal water exchange. Aquaculture 426-427:204-212

Wu Q, Li Z, Dai F, Chen R, Wang J, Shan X, Jin X (2016) Taxonomic diversity of crustaceans in Yellow-Bohai Sea. Biodiversity Science 24:1306-1314 (in Chinese)

Wu Z, Yu Z, Song X, Yuan Y, Cao X, Liang Y (2013) Application of an integrated methodology for eutrophication assessment: a case study in the Bohai Sea. Chinese Journal of Oceanology and Limnology 31:1064-1078

Wyban J, Sweeney JN (1991) Intensive shrimp production technology: the Oceanic Institute shrimp manual. Oceanic Institute, Honolulu, Hawaii, 158 p

Xiao J, Ford SE, Yang H, Zhang G, Zhang F, Guo X (2005) Studies on mass summer mortality of cultured zhikong scallops *(Chlamys farreri* Jones et Preston) in China. Aquaculture 250:602-615

Xie B, Yu K (2007) Shrimp farming in China: Operating characteristics, environmental impact and perspectives. Ocean and Coastal Management 50:538-550

Yakupitiyage A, Bhujel RC (2005) Role of exotic species in aquaculture: problems and prospects in Indochina. In: Bartley DM, Bhujel RC, Fung-Smith S, Olin PG, Phillips MJ (eds) International Mechanisms for the control and responsible use of alien species in aquaculture ecosystems. Report of an Ad Hoc Expert Consultation, 27-30 August 2003, Xishuangbanna, China, FAO, 195 p

Yang H, Wang J, Zhou Y, Zhang T, Wang P, He Y, Zhang F (2000) Comparison of efficiencies of different culture systems in the shallow sea along Yantai. Journal of Fisheries of China 24:140-145

Yang J (2001) The trophic levels and diets of invertebrates in Bohai Sea. Modern Fisheries Information 16:8-16

Yang P, Lai DY, Jin B, Bastviken D, Tan L, Tong C (2017) Dynamics of dissolved nutrients in the aquaculture shrimp ponds of the Min River estuary, China: Concentrations, fluxes and environmental loads. Science of the Total Environment 603:256-267

Yang Q, Tan B, Dong X, Chi S, Liu H (2015) Effect of replacing fish meal with extruded soybean meal on growth, feed utilization and apparent nutrient digestibility of juvenile white shrimp (*Litopenaeus vannamei*). Journal of Ocean University of China 14:865-872

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep Sea Research and Oceanographic Abstracts 10:221-231

Yokoyama H, Abo K, Ishihi Y (2006) Quantifying aquaculture-derived organic matter in the sediment in and around a coastal fish farm using stable carbon and nitrogen isotope ratios. Aquaculture 254:411-425

Yokoyama H, Higano J, Adachi K, Ishihi Y, Yamada Y (2002) Evaluation of shrimp polyculture system in Thailand based on stable carbon and nitrogen isotope ratios. Fisheries Science 68:745-750

Yu Q (2014) A study on structure, characteristics and perfermances of bay scallop industry in China. PhD thesis, Ocean University of China, Qingdao, China

Yuan X, Yang H, Zhou Y, Mao Y, Zhang T, Liu Y (2006) The influence of diets containing dried bivalve feces and/or powdered algae on growth and energy distribution in sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). Aquaculture 256:457-467

Yue YR, Liu YJ, Tian LX, Gan L, Yang HJ, Liang GY (2012) Effects of replacing fish meal with soybean meal and peanut meal on growth, feed utilization and haemolymph indexes for juvenile white shrimp *Litopenaeus vannamei*, Boone. Aquaculture Research 43:1687-1696

Zaiko A, Daunys D, Olenin S (2009) Habitat engineering by the invasive zebra mussel *Dreissena polymorpha* (Pallas) in a boreal coastal lagoon: impact on biodiversity. Helgoland Marine Research 63:85-94

Zanden M, Rasmussen JB (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnology and oceanography 46:2061-2066

Zhan A, Ni P, Xiong W, Chen Y, Lin Y, Huang X, Yang Y, Gao Y (2017) Biological Invasions in Aquatic Ecosystems in China. In Wan F, Jiang M and Zhan A (eds) Biological Invasions and Its Management in China. Springer, Dordrecht. p 67-96

Zhang F, He Y, Yang H (2000) The integrated effect and outcome of introducing *Argopecten irradians* to China. Engineering Science 2:30-35

Zhao J, Chen JF (2001) Research on aquacultural hydro-environment of Eastern Laizhou Bay. Marine Fisheries Research 22:62–67 (in Chinese with English abstract)

Zhao Q, Tian J, Zhao S, Wu Z (2004) Winter and summer chlorophyll *a* and nutrient distribution and characteristics in the Bohai Sea. Marine Science 28:34-39 (in Chinese)

Zhao Y, Qin H, Lai S, Wang Y, Li Y (2016) Penaeid shrimp farming along the coastal area in Shandong Province. Chinese Fishery Quality and Standards 6:7-12 (in Chinese)

Zhong D, Wang F, Dong S, Li L (2015) Impact of *Litopenaeus vannamei* bioturbation on nitrogen dynamics and benthic fluxes at the sediment–water interface in pond aquaculture. Aquaculture International 23:967-980

Zhong Y, Ma S (2012) The population dynamic of amphipods in *Penaeus japonicus* ponds. Periodical of Ocean University of China 42:59-66 (in Chinese)

Zhou Y, Yang H, Hu H, Liu Y, Mao Y, Zhou H, Xu X, Zhang F (2006) Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. Aquaculture 252:264-276

Zhou Y, Yang H, Zhang T, Liu S, Zhang S, Liu Q, Xiang J, Zhang F (2006) Influence of filtering and biodeposition by the cultured scallop *Chlamys farreri* on benthic-pelagic coupling in a eutrophic bay in China. Marine Ecology Progress Series 317:127-141

Zhou Y-B (1994) Biomass and Population Dynamics of Neanthes Japonica in Shrimp Ponds. Journal of Dalian Fisheries University 9:12-20 (in Chinese with English abstract)

Zhuang W, Gao X, Zhang Y, Xing Q, Tosi L, Qin S (2014) Geochemical characteristics of phosphorus in surface sediments of two major Chinese mariculture areas: the Laizhou Bay and the coastal waters of the Zhangzi Island. Marine Pollution Bulletin 83:343-351

Zou J, Dong L, Qin B (1985) Preliminary studies on eutrophication and red tide problems in Bohai Bay. Hydrobiologia 127:27-30

Zupo V, Lumare F, Bisignano V (1998) Feeding of *Penaeus japonicus* Bate (Decapoda: Penaeidae) in pond cultures: size descriptors and food selection. Journal of Natural History 32:1813-1824