

Hemichordata: Enteropneusta (Acorn worm) bioturbation: Maintaining and facilitating the balance of coral reef biogeochemical cycles

K.K. Takagi¹, I.S.S. Mchenga¹, M. Tsuchiya²

1) Univ. of the Ryukyus, Grad. School of Eng. and Sci., 1 Senbaru, Nishihara, Okinawa, 901-0213, Japan

2) Univ. of the Ryukyus, Faculty of Science, 1 Senbaru, Nishihara, Okinawa, 901-0213, Japan

Abstract. In Bise, Okinawa, Japan, acorn worms inhabit the sandy beach, seagrass and coral/seagrass environments. As such, we assert that its bioturbation behavior aids in “biopurifying” and impoverishing the coral reef ecosystem in regards to nutrients and organic matter. Through the use of fatty acid (FA) biomarkers, C:N, and nutrient analysis, we analyzed tissues of two acorn worm species (*Ptychodera* sp. and *Schizocardium* sp.) and sediments from areas inhabited by, not inhabited by, and fecal casts of the acorn worms. Seawater samples from inhabited and not inhabited areas were analyzed for total nitrates and ammonium concentrations. FA biomarkers of acorn worm tissues indicate significant differences in diatom and plant matter consumption by the two species. In addition, acorn worms assimilate ‘reactive’ organic matter in the beach habitat. A significant decrease in the beach sediment overall nitrate concentration indicates acorn worm presence mitigates the release of nitrates into the water column and sediments. As the overall FA contribution and TOC contents changed little in the seagrass and coral/seagrass environments, the acorn worm demonstrates the ability to “biopurify” by subtracting “nutrient rich” organic matter, thus facilitating the maintenance of the biogeochemical balance within the coral reef ecosystem.

Key words: acorn worms, biogeochemical cycles, bioturbation, coral reef, fatty acids

Introduction

Maintaining energetic balance is essential for ecosystem function. In addition, as sediments represent the largest physiographic zone, the metabolism of benthic processes tends to dominate the coral reef ecosystem (Rasheed et al. 2002; Gattuso et al. 1998). Thus, bioturbators, which modify the sediments and directly impact benthic processes, subsequently affect the immediate, local, and ecosystem level bioenergetic cycles (Reise 1985; Tenore 1988).

In addition, as sandy beaches have been shown to act as “environmental purification systems” in coral reefs (Tsuchiya et al. 1999), and few species occupy the entire tidal gradient (Reise 1985), it is possible that deposit feeding bioturbators such as the acorn worm (Hemichordate: Enteropneusta) function as biological purifiers in the coral reef ecosystem. Thus, by assessing the role of the acorn worm, it is possible to better understand the overall impact of an organism on the biogeochemical cycles within this system.

In this study, the interactions between bioturbation and the biogeochemical cycles within the coral reef ecosystem are examined using the acorn worm.

Materials and Methods

This study was conducted in Bise, in the northern part of Okinawa Island, Japan (127° 52' 46" N, 127° 52' 43" E) from autumn 2006 to autumn 2007. The intertidal zone is characterized by beach, seagrass, and mixed coral/seagrass, habitats which are exposed during low tide. The dominant coral species is *Montipora digitata*. In addition, the dominant seagrass species is *Thalassia hemprichii*. Based on evidence of fecal cast production, acorn worms were observed at densities of up to 24 individuals m⁻².

Schizocardium sp. and *Ptychodera* sp. tissue samples were collected during spring and fall 2007. Sediment and water samples were collected during low tide from the beach (BE), seagrass (SG) and coral/seagrass (CS) habitats, during fall (October-November) 2006. In each habitat, the top 0.0-0.5cm of sediment was sampled with a small plastic spoon. Samples were collected from: surface sediments uninhabited (UN) by the acorn worm, surface sediments next to the fecal cast (NX, <10cm diameter from the fecal casts), and fecal casts (FC, entire fecal casts were collected). All samples were placed in plastic bags and immediately put on ice. Sediments were stored under -40°C until analysis.

Water samples, were collected from two areas within each habitat: uninhabited (UN) and inhabited (HB) by the acorn worm. 250mL of water were collected just above the sediments in the same areas. To prevent the alteration of seawater nutrients due to oxygen presence, the polyethylene bottles were opened, capped underwater and immediately put in ice and transported to the laboratory. Samples were then frozen (at -20°C) until analysis. Analysis was conducted within 24 hours of sampling.

Prior to analysis, all sediment and tissue samples were freeze-dried (FRD-51, IWAKI, Japan). Total Organic Carbon (TOC) and Total Nitrogen (TN) analysis sample preparation was carried out following the methods of Yamamuro and Kayanne (1995) for sediment samples. Analysis was conducted using a Shimadzu high sensitivity CN analyzer (Sumigraph NC-80).

Lipid and fatty acid (FA) analyses were conducted following a modified version of Bligh and Dyer (1959) as detailed by Meziane and Tsuchiya (2000). The FA biomarkers utilized for organic matter (OM) source identification are listed in Table 1.

Seawater and extracted sediment samples were analyzed for nitrate, nitrite and ammonium

Table 1. List of the FA markers used and associated OM sources identified.

Fatty acid (s)	Signature organism (OM source)
15:0 and 17:0 (<i>iso</i> and <i>anteiso</i>), 18:1 ω 7	Bacteria
18:2 ω 6, 18:3 ω 6, 18:3 ω 3	Green macroalgae
20:5 ω 3	Diatoms
18:4 ω 3, 22:6 ω 3	Dinoflagellates
24:0, 26:0, 28:0, 30:0, 32:0	Vascular plants

concentrations ($\text{NO}_2^- + \text{NO}_3^- - \text{N}$ and $\text{NH}_4^+ - \text{N}$, respectively) using an automatic water analyzer (QuAAatro, Bran + Luebbe). Sediment nutrients were analyzed following the procedure outlined by Mulvaney (1996).

Data analysis was conducted using SPSS software (Standard version 11.0.1 for Windows, SPSS Inc., US). Results were considered significant if $p < 0.05$.

Results

Tissue analysis of *Ptychodera* sp. and *Schizocardium* sp. revealed significantly higher SAFAs (saturated fatty acids) and BrFAs (branched fatty acids) in *Ptychodera* sp. tissues. *Schizocardium* sp. tissue showed a significantly higher assimilation of ω 3 and ω 6 EFAs (essential fatty acids) (fig. 1A). Comparison of the biomarker composition of each species

indicated similarities in the food resources, yet significant differences in the proportions assimilated. *Ptychodera* sp. assimilated a higher proportion of vascular plants while *Schizocardium* sp. assimilated a higher proportion of diatoms (fig. 1B).

The total organic carbon contents did not vary significantly within the beach and coral/seagrass habitat areas. In the seagrass habitat, the total organic matter (g m^{-1}) content was significantly greater in fecal casts of acorn worms than in areas uninhabited by acorn worms (ANOVA, $F = 5.3$, $df = 2, 8$, $p < 0.05$). Although not significant, the total amount of FAMES tended to decrease in the fecal casts of the beach and coral/ seagrass habitats (data not shown).

In all habitats and sediment areas, saturated fatty acids (SAFAs) contributed most abundantly (between 70.1% and 45.3%) followed by monounsaturated fatty acids (MUFAs), (23-38.7%, fig. 2). Palmitic acid, 16:0 was consistently the most abundant individual FA (30.7-56.5%), followed by 16:1 ω 9 (18.0-30.6%).

In the beach habitat, the MUFAs (ANOVA, $F = 5.6$, $df = 2, 8$, $p < 0.05$), polyunsaturated fatty acids (PUFAs) (ANOVA, $F = 40.5$, $df=2, 8$, $p < 0.001$), and essential fatty acids (EFAs) (ANOVA, $F = 50.7$, $df = 2, 8$, $p < 0.001$) showed a significantly lower percent contribution to fecal cast sediments in comparison to uninhabited and next to fecal cast sediments (fig. 2A). SAFAs however, showed a significantly greater contribution (ANOVA, $F = 46.8$, $df = 2, 8$, $p < 0.001$).

The seagrass habitat however, only showed significantly lower contributions of PUFAs and EFAs in fecal cast sediments (fig. 2B ANOVA, $F = 11.2$, $df = 2, 6$, $p < 0.01$ and $F = 11.3$, $df = 2, 6$, $p < 0.01$, respectively). In contrast, the MUFAs, PUFAs and EFAs of fecal casts in the coral/seagrass habitat did not change significantly. Instead, the branched fatty acids (BrFAs) and long-chain fatty acids (LCFAs) showed significantly lower contributions (ANOVA, $F = 18.3$, $df = 2, 6$, $p < 0.01$ and $F = 6.5$, $df = 2, 6$, $p < 0.05$, respectively) while MUFAs showed a significantly greater contribution (ANOVA, $F = 11.1$, $df = 2, 6$, $p < 0.05$) in comparison to uninhabited sediments (fig. 2C).

However, in assessing the FA biomarkers (data not shown), the beach area showed a significantly lower contribution of green macroalgae and the diatom marker 20:5 ω 3 (eicosapentaenoic acid) in the fecal cast sediments (ANOVA, $F = 69.8$, $df = 2, 8$, $p < 0.001$ and $F = 56.6$, $df = 2, 8$, $p < 0.001$, respectively).

Although bacterial FAs did not show any differences in percent contribution to the FAMES composition overall, the individual FA, 15:0a showed a significantly greater contribution in the fecal cast sediments (ANOVA, $F = 30.7$, $df = 2, 8$, $p < 0.01$). In contrast, the bacteria markers within fecal cast sediments of the seagrass habitat showed a

significantly lower contribution in comparison to uninhabited and next to fecal cast sediments (ANOVA, $F = 37.6$, $df = 2, 6$, $p < 0.001$). Green macroalgae and vascular plant markers also showed significantly lower contributions in fecal casts as compared to next to fecal cast and uninhabited areas respectively (ANOVA, $F = 6.7$ $df = 2, 6$, $p < 0.05$ and $F = 15.4$, $df = 2, 6$, $p < 0.01$).

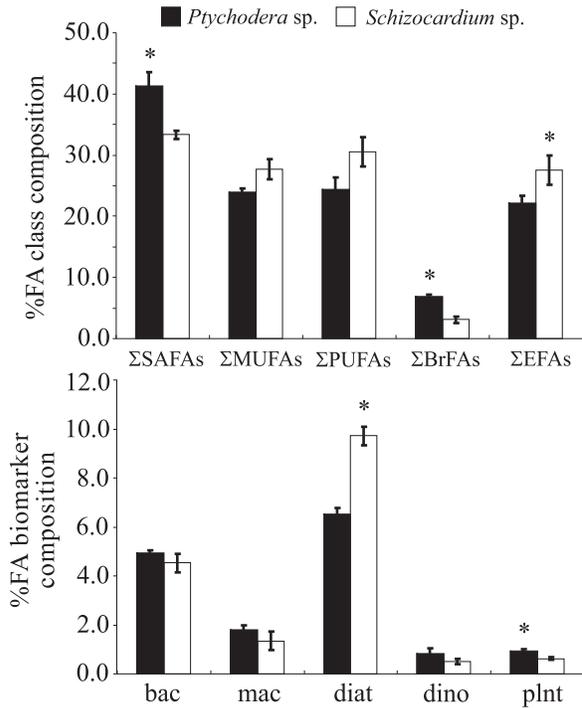


Figure 1. Fatty acid class (A) and biomarker (B) composition comparison between *Ptychodera sp.* and *Schizocardium sp.* tissues. Biomarkers are: bacteria (bac), macroalgae (mac), diatoms (diat), dinoflagellates (dino), and vascular plants (plnt). Values are averages \pm SE. * indicates a significant difference, Mann-Whitney, $p < 0.05$.

The decreases in FA markers within the coral/seagrass habitat were not consistent with either the beach or seagrass habitats. Similar to the seagrass habitat, the bacteria markers showed a significantly lower contribution in fecal cast sediments (ANOVA, $F = 46.1$, $df = 2, 6$, $p < 0.001$). In addition, the comparison to the other two sediment areas vascular plant markers also showed a significantly lower percent contribution to fecal cast sediments in comparison to uninhabited areas (ANOVA, $F = 11.1$, $df = 2, 6$, $p < 0.05$). However, although the dinoflagellate markers made up less than 1% of the overall FAMES composition in each area, a significantly lower contribution was also seen in comparison to uninhabited sediments (ANOVA, $F = 8.8$, $df = 2, 6$, $p < 0.05$).

$\text{NO}_2^- + \text{NO}_3^- - \text{N}$ (hereafter referred to as NO_3^- or nitrate) and $\text{NH}_4^+ - \text{N}$ (ammonium) concentrations in

seawater just above sediments uninhabited by the acorn worm and just above fecal casts of the acorn worm did not vary significantly in all habitats. However, nitrate concentrations tended to be higher than ammonium in all areas ($0.05\text{--}3.05\mu\text{mol l}^{-1}$ and $0.09\text{--}0.68\mu\text{mol l}^{-1}$, respectively). Nitrate tended to show a lower concentration in the seawater above fecal casts in all habitats whereas ammonium concentrations were slightly greater only in the coral/seagrass habitat.

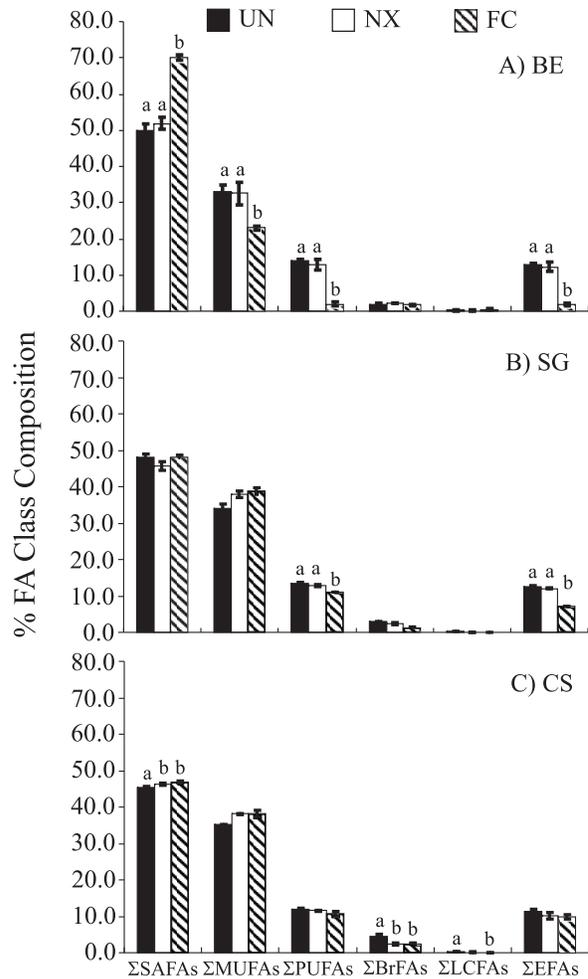


Figure 2. Habitat comparison of the percent FA class composition in the beach, seagrass, and coral/seagrass habitats between uninhabited (UN), next to fecal cast (NX) and fecal cast (FC) sediments. Values are means \pm SE, lower case letters indicate significance between areas (ANOVA, $p < 0.05$).

In contrast, in regards to sediment nutrient content, the beach habitat showed a significantly greater concentration of ammonium than nitrate in fecal cast sediment samples (fig. 3). Here, the nitrate concentration significantly decreased in a step-wise manner from uninhabited to next to fecal cast and fecal cast sediments (fig. 3A, ANOVA, $F = 279.8$, $df = 2, 6$, $p < 0.001$) and the ammonium concentration

was significantly greater in fecal cast sediments (fig. 3B, ANOVA, $F = 483.6$, $df = 2, 6$, $p < 0.001$). In the seagrass habitat, a decreasing trend is seen in the both the nitrate and ammonium concentrations. However, the concentrations in the coral/ seagrass habitat did not vary significantly between areas.

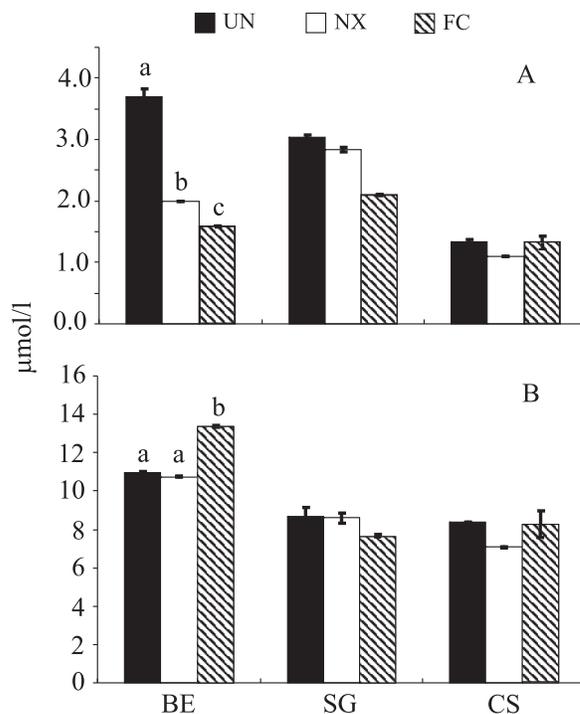


Figure 3. Area comparison of the sediment (A) nitrate and (B) ammonium concentrations in the sediments uninhabited by (UN), next the fecal casts (NX) and the fecal casts (FC) of acorn worms in the beach (BE), seagrass (SG) and coral/seagrass (CS) habitats.

The total nitrogen (TN), total organic carbon (TOC), carbon: nitrogen ratio (C:N) and total organic matter (TOM) contents in the sediment areas of the beach, seagrass and coral/seagrass did not vary significantly within all habitats.

Discussion

Tissue analysis of *Ptychodera* sp. and *Schizocardium* sp. reveals that both species primarily assimilate diatoms, followed by bacteria and macroalgae. However, significant differences in the proportions of diatoms and plants assimilated into the tissues indicates that, on a fine-scale, the impact of an acorn worm on the surrounding environment is species specific.

However, on a large-scale, the impact on the organic matter and nutrient cycles may be similar. The overall FAMES and significant changes in OM composition exemplify the ability of the acorn worm to assimilate and biologically purify (clean) its surrounding environment, also referred to as

biopurification. Similar to earthworms (Lavelle 1988), acorn worms are dependent on external biotic and abiotic parameters. This is confirmed in the inconsistent patterns of FAMES biomarker composition (i.e. food resources) between the beach, seagrass, and coral/seagrass habitats. However, regardless of the variations due to habitat, the biopurification role of the acorn worm can still be elucidated in the overall “quality” of OM as a result of acorn worm presence. As lipids include essential fatty acids which are utilized by all organisms to maintain bodily function, the absence of EFAs in the fecal cast sediments indicates successful assimilation by the acorn worm. Consequently, the sediment processed by the acorn worm is “stripped,” not “enriched” of useful FAs, and the sediment is of less value to other OM consumers. This exemplifies biopurification.

In regards to dietary fatty acids, organisms in the marine food web have shown preferential assimilation/degradation of PUFAs, resulting in a relative increase of SAFAs (Grossi et al. 2006; Mfilinge et al. 2003). In concurrence with this, the proportional SAFA contribution was significantly higher in fecal cast sediments while PUFAs were significantly lower in the beach habitat. However, the same pattern was not seen in the seagrass and coral/seagrass habitats. Therefore, it is possible that the degradation of PUFAs is due to the preferential utilization by bacteria, rather than the direct assimilation by the acorn worm (Mfilinge et al. 2003). This is also supported by the increased contribution of MUFAs in areas near the fecal cast and fecal casts in the seagrass and coral/seagrass habitats which could instead, indicate an increase in microbial re-colonization on the fecal casts. Therefore, an indirect effect of acorn worm presence on its surrounding habitats likely includes the consequential impact on microbial utilization of nutrients in the sediments and subsequent release and/or uptake in the water column.

In addition, due to the anoxic nature of sediment, nitrate concentrations are expected to be high and ammonium low (Kogure and Wada 2005). This is exemplified by the results from each habitat. However, as the beach environment lacks the dynamics and respiration from corals, plants and other substrates, the significant decrease in nitrates and significant increase in ammonium in the fecal cast sediments clearly indicates the effects of the acorn worm presence. As such, the microbial ammonification activity in the beach habitat seems more enhanced in fecal cast sediments than in the sediment areas in the seagrass and coral/seagrass habitats.

Kogure and Wada (2005) pointed out that, although bioturbation enhances the exchange of water and

oxygen between the water column and sediment, the fecal casts of the bioturbators often remain mainly as reduced environments. Thus, if the acorn worm fecal casts contain micro-anoxic environments then the release of nitrate back into the water column would be limited by the denitrification processes occurring in reduced fecal cast environments. This indirect effect of the acorn worm presence is seen in the decreased concentrations of nitrates in the water column just above the fecal casts in the beach and seagrass habitats. Thus, perhaps a greater part of the bioturbated nitrogen in acorn worm habitats remains within the fecal casts and sediments while a lesser part is oxidized and returned to the water column.

Tenore (1988) stated that the microbial recycling of fecal material (coprophagy) allows further degradation of egested materials making re-ingestion by other organisms possible. This would result in the net increase in nitrogen and nutritional content of detritus. However, as TN concentrations remained relatively the same throughout each area, and the FA compositions showed an overall decrease in EFAs, the acorn worm fecal casts do not increase the sediment nutritional content and therefore do not encourage re-ingestion by subsequent trophic levels. Yet maintenance of the oligotrophic environment is enhanced.

The impact of bioturbators within an ecosystem is two-fold. In the case of the acorn worm, there is a direct and immediate impact on its environment based on its burrowing behavior, sediment ingestion and subsequent production of fecal casts. This “cleansing” effect is its biopurification ability. However, it also has an indirect, chronic impact on its environment based on the degradation of fecal casts over time as well as the changes in microbial dynamics as a result of acorn worm activity and presence. In addition, as acorn worms and most deposit feeders are dependent on their external, abiotic environments for resources, their subsequent influence on the environment will change depending on the season and organic matter inputs. Thus, to further understand the role of the acorn worm in the coral reef ecosystem, it is important to consider how the chronic presence of the acorn worm impacts the surrounding habitat. Studies concerning seasonal changes, acorn worm fecal cast degradation over time, microbial activity as a result of acorn worm presence, and changes in organic matter and nutrient dynamics at depth in the sediment should be conducted to further clarify the direct and indirect

impacts of this bioturbator in the coral reef environment.

Acknowledgements

The authors wish to thank the Ministry of Education, Culture, Sports, Science and Technology, Japan (Monbukagakusho), for a scholarship grant during this study and the University of the Ryukyus 21st Century COE (Center of Excellence) for financial support. Many thanks are given to Dr. Prosper L. Mfilinge, Dr. T. Meziane, Ms. K. Adulyanukosol, and Ms. Yu Tamura for their assistance and advice in FA analysis.

References

- Bligh EG, Dyer WJ, 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37(8):911-917
- Dobbs FC, Guckert JB, 1988. Microbial food resources of the macrofaunal – deposit feeder *Ptychodera bahamensis* (Hemichordata: Enteropneusta). *Mar Ecol Progr Ser* 45:127 – 136
- Gattuso JP, Frankignoulle M, Wollast R, 1998. Carbon and carbonate metabolism in coastal aquatic ecosystems. *Annu Rev Ecol Syst* 29:405-434
- Grossi V, Cuny P, Caradec S, et al., 2006. Impact of feeding by *Arenicola marina* (L.) and ageing of fecal material on fatty acid distribution and bacterial community structure in marine sediments: an experimental approach. *J Exp Mar Biol Ecol* 336:54-64
- Kogure K, Wada M, 2005. Impacts of Macrobenthic bioturbation in marine sediment on bacterial metabolic activity. *Microbes and Environments*. 20(4):191 – 199
- Lavelle P. 1988. Earthworm activities and the soil system. *Biology and Fertility of Soils*. 6:237-251.
- 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. *Mar Ecol Prog Ser* 200:49-57
- Mfilinge PL, Meziane T, Bachok Z, et al., 2003. Fatty acids in decomposing mangrove leaves: microbial activity, decay and nutritional quality. *Mar Ecol Prog Ser* 265:97-105
- Mulvaney RL, 1996. Nitrogen – Inorganic Forms. In: Sparks DL, editor. *Methods of Soil Analysis*. Part 3. Wisconsin: American Society of Agronomy-Soil science Society of America: p1123-1184
- Rasheed M, Badran MI, Richter C, et al., 2002. Effect of reef framework and bottom sediment on nutrient enrichment in a coral reef of the Gulf of Aquaba, Red Sea. *Mar Ecol Prog Ser* 239:277-285
- Reise K. 1985. In: *Ecological Studies*, Billings WD, Lange OL, Remmert H (eds) *Tidal Flat Ecology: An experimental approach to species interactions*. Germany: Springer-Verlag. Vol. 54
- Tenore KR, 1988. Nitrogen in benthic food chains. In: Blackburn TH, Sørensen J (eds) *Nitrogen cycling in coastal marine environments*. Essex: John Wiley and Sons Ltd, p191-206
- Tsuchiya M, Kise M, Tanaka M, et al. 1999. The role of sandy beaches as environmental purification systems in coral reefs. *Proceedings of the 2nd International workshop on Oceanography and Fisheries in the East China Sea*. “East China Sea.” 2:113-124
- Yamamuro M, Kayanne H, 1995. Rapid direct determination of organic carbon and nitrogen in carbonate-bearing sediments with a Yanaco MT-5 CHN analyzer. *Limnol Oceanogr* 40(5):1001 – 1005