#### Spatial and seasonal variation of water quality in Batan Bay, Philippines

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#### Abstract

Batan Bay and Tinago Lake are shallow embayments connected to each other, located on the north of Panay Island, central Philippines (11.53° - 11.67°N, 122.38° - 122.52°E). Although they had been originally surrounded by dense mangrove forest till the middle of the last century, mangroves have been mostly cleared and converted into fish and shrimp ponds. Recently, shelves and rafts for cultivating oysters and green mussels have become widespread in the shallow areas of the embayments (see Figure as an example). Replantation of mangroves is also ongoing in limited areas of Batan Bay. We are conducting researches there focusing on ecosystem services of mangroves and seagrass meadows, especially in relation to carbon sequestration and aquaculture production. In this presentation, we report preliminary survey results on environmental conditions that may influence growth and survival of cultivated bivalves, such as freshwater inputs and potential food resources. The survey was conducted in both dry season (February 2019) and rainy season (November 2019). Although the salinity gradient across the bay due to freshwater input was evident in both seasons, the oxygen isotope ratio of seawater indicated that evaporation overwhelmed in inner bay sites in the dry season. Concentrations of chlorophyll and suspended particulate organic matter (POM), i.e. potential food source for bivalves, were high in the inner bay area. Carbon stable isotope ratio ( $\delta$ 13C) of dissolved organic carbon (DIC) and POM showed spatial gradient from the bay mouth (high) to inner sites (low), indicating the influence of riverine DIC and POM inputs. However, the  $\delta 13C$  of ovsters (adductor muscle) was consistently higher than POM and showed no clear spatial gradient. The  $\delta 13C$  of oysters was relatively higher for individuals collected from inside or edge of seagrass meadows than those collected in open areas. These results suggest that ovsters assimilate only a specific fraction of POM relatively enriched in 13C (i.e. marine-origin POM) and that seagrass meadows support growth of oysters by providing additional food source (e.g. attached microalgae that are abundant on seagrass blades).

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# INTRODUCTION

Batan Bay and Tinagong Dagat Bay are shallow embayments connected to each other, located on the north of Panay Island, central Philippines. Although the bay was originally surrounded by dense mangrove forest till the 1980's, mangroves have been mostly cleared and converted into fish and shrimp ponds since then (Altamirano et al. 2010 (https://doi.org/10.1007/s11273-010-9190-2)). Such a situation is common to many coastal regions of East/Southeast Asian countries, and consequently, development of aquaculture fisheries and conservation of coastal wetlands are often considered to be mutually exclusive. On the other hand, it has come to be recognized that the coastal wetlands provide various ecosystem functions and services that enhance sustainability of coastal fisheries including aquacultures (Primavera 2006 (https://doi.org/10.1016/j.ocecoaman.2006.06.018)). In fact, integration of sustainable fisheries and ecosystem restoration has been attempted in these countries as a novel approach for coastal management. Integration of bivalve aquacultures and seagrass meadows is a representative example (Hori et al. 2018 (http://dx.doi.org/10.1007/s12562-017-1173-2)). Filter-feeding bivalves and seagrasses are known to interact and benefit each other in several ways, for example:

- Attached microalgae growing on seagrass blades provide nutrient-rich food source for filter-feeders and enhance shellfish production (Kasim and Mukai 2009 (https://doi.org/10.3800/pbr.4.104));
- Antibiotic substances (e.g. zosteric acid) produced and released by seagrasses suppress growth of microorganisms potentially pathogenic to bivalves and humans (Zidorn 2016 (http://dx.doi.org/10.1016/j.phytochem.2016.02.004), Lamb et al. 2017 (http://dx.doi.org/10.1126/science.aal1956));
- Removal of seston by filter-feeding bivalves improve transparency and light environment for seagrasses (Hori et al. 2018 (http://dx.doi.org/10.1007/s12562-017-1173-2)).

Recently, shelves and rafts for cultivating oysters (*Crassostrea iredalei*) and green mussels (*Perna viridis*) have become widespread in the shallow areas of Batan Bay and Tinagong Dagat Bay (Plate 1a,b). However, the recovery of wetlands such as mangroves and seagrass meadows proceeds slowly and cannot catch up with the development of aquaculture structures. Therefore, to promote harmonized and sustainable use of coastal ecosystems, there is an urgent need to demonstrate the advantages of coastal wetlands in enhancing production and ensuring sustainability of coastal fisheries, especially aquacultures (Lagarde et al. 2020 (http://dx.doi.org/10.1007/978-3-030-43484-7\_18)).

With this aim, we are conducting field researches in Batan Bay focusing on ecosystem services of mangroves and seagrass meadows, especially in relation to the carbon and water cycles and aquaculture production. In this presentation, we report the first preliminary results on environmental conditions that may influence the growth and survival of bivalves, and the basic trophic ecology of oysters in the bay.

### STUDY SITES AND METHODS



Figure 1. Location of study site, Batan Bay and Tinagong Dagat Bay, shown with water/sediment sampling sites (red dots) and oyster collection sites (yellow circles).

Batan Bay and Tinagong Dagat Bay are shallow brackish lagoons located at the north of Panay Island, Central Philippines (11.55°– 11.66°N; 122.40°–122.50°E; Fig. 1). Water sampling was conducted in February (**dry season**) and November (**wet season**) of 2019. Oyster samples were collected in November 2019.

Water samples were collected at the water surface. The following environmental and water quality parameters were obtained and used in this study:

- Water temperature, pH, salinity, and chlorophyll *a* (handheld probes)
- $\delta^{18}$ O of water (CRDS)
- $\delta^{13}$ C of dissolved inorganic carbon (DIC) (Gasbench-IRMS)
- Suspended particulate organic carbon (POC), particulate nitrogen (PN), δ<sup>13</sup>C of POC, δ<sup>15</sup>N of PN (POM was collected on GF/F glassfiber filter, decarbonated by HCl fuming, and analyzed by EA-IRMS)

Sediment samples were also collected using a Van Veen grab sampler at the same stations as water samples, and analyzed for:

- Organic carbon (OC) and total nitrogen (TN) contents and stable isotope ratios (EA-IRMS after carbonate removal by HCl)
- Carbonate content (ion chromatography)

Oyster samples were collected at 5 sites (Stations 1~5) from bamboo poles of fish nets. Stations 3 and 4 were associated with **seagrass meadows** (*Enhalus acoroides*). Samples were analyzed for the following items:

- Species (molecular phylogenetic analysis) and shell length
- $\delta^{13}$ C,  $\delta^{15}$ N, and C/N ratio of adductor muscles (EA-IRMS after lipid and carbonate removal)



Plate 1. (a) Oyster shelf made of bamboos, commonly found in shallow areas of Batan Bay. (b) Oyster rafts deployed at relatively deeper parts of the bay. (c) Main dike of a fishpond facing Batan Bay, with an opening (main gate) from which nutrient-rich effluent water is supplied to the bay. (d) Planted mangrove forest on Isla Kapispisan, the most massive mangrove stand currently found around the bay. (e) Seagrass meadow of *Emhalus acoroides* near Station 3. (f) Samples of oysters (*Saccostrea* spp.) collected from seagrass meadows.

#### ENVIRONMENT OF BATAN BAY



Figure 2. Spatial distribution of surface water salinity (a,d), pH (b,e), and chlorophyll *a* (c,f) in Batan Bay, compared between dry season (Feb. 2019, *top*) and wet season (Nov. 2019, *bottom*). Water temperature was 27°–30°C in both seasons.



Figure 3. Relationships between the salinity and the  $\delta^{18}$ O of water (*top*) and the  $\delta^{13}$ C of DIC (*bottom*) in the dry (*left*) and wet (*right*) seasons in the surface water of Batan Bay. Freshwater-endmember  $\delta^{13}$ C<sub>DIC</sub> was evaluated by the conservative mixing model as -16.2% and -12.5% for the dry and wet seasons, respectively.

Normal estuarine salinity gradient was observed from the bay mouth (southeast) to inner bay (northwest) (Fig. 2a,d). Chlorophyll was always much higher in inner bay area than in outer ocean (Fig. 2c,f).

However, the  $\delta^{18}$ O of water showed contrasting longitudinal trends between the dry and wet seasons (Fig. 3a,b). This indicates that evaporation of water plays a significant role in the water cycle of the bay during the dry season. The  $\delta^{13}$ C of DIC showed similar spatial pattern between the dry and wet seasons (Fig. 3c,d). The  $\delta^{13}$ C of DIC seems to be influenced strongly by internal production of CO<sub>2</sub> within the bay.

Preliminary evaluation of nutrient status in the dry season indicated that most part of the bay was not very eutrophic (up to  $10 \,\mu$ M DIN,  $1 \,\mu$ M DIP, and  $30 \,\mu$ M SiO<sub>2</sub> at the most eutrophicated inner bay station).



Figure 4. Spatial distribution of  $\delta^{13}$ C of suspended POM in the dry (a) and wet (b) seasons and sediment organic matter (c) in Batan Bay.



Figure 5. Relationships between the salinity and the concentration (top) and the  $\delta^{13}$ C (*bottom*) of suspended POM in the dry (*left*) and wet (*right*) seasons.

The concentration of POC was higher in inner bay than outer ocean (Fig. 5a,b). The  $\delta^{13}$ C of suspended POM (Fig. 4a,b) was higher at the bay mouth and gradually became more negative towards inner bay area. The endmember  $\delta^{13}$ C values, estimated by formally applying the conservative mixing model, showed little seasonal variation. Marine-endmember  $\delta^{13}$ C (salinity, 35) was -21.4‰ and -21.7‰ in the dry and wet seasons, respectively, while freshwater-endmember  $\delta^{13}$ C was -30.7‰ and -28.8‰, respectively. Concentration of sediment OC was quite high in inner region and Tinagong Dagat Bay (>2000  $\mu$  mol C g<sup>-1</sup>), compared to stations near the bay mouth (<400  $\mu$ mol C g<sup>-1</sup>). Carbonate content of sediment was always <5 wt.%. The  $\delta^{13}$ C of sediment OC was lower than -25‰ except a few sites near seagrass meadows and at the bay mouth (Fig. 4c).

The above data suggested that suspended POM in the bay was composed of mixture of plankton-derived POM ( $\delta^{13}C = -22$  to -21%) and terrestrial and/or mangrove-derived detrital POM (-30 to -28%), with the latter being selectively preserved in the sediment.

### ECOLOGY OF OYSTERS

We found at least 8 phylogenetically different groups of oysters in Batan Bay and Tinagong Dagat Bay, of which 3 groups were dominant:

- Crassostrea iredalei (cultured species)
- Saccostrea cucullata type D
- Saccostrea sp. Lineage F



- Crassostrea iredalei (Station 1)
- Crassostrea iredalei (Station 2)
- Crassostrea iredalei (Station 4)
- Crassostrea iredalei (Station 5)
- Crassostrea zhangjiangensis (Station 4)
- ♦ Saccostrea spp. (Station 2)
- Saccostrea spp. (Station 3)
- Saccostrea spp. (Station 4)
- × Anadara (Station 3)
- × Anadara (Station 4)
- + Umbonium sp. (Station 3)

Figure 6. The δ<sup>13</sup>C-δ<sup>15</sup>N plot of oysters (adductor muscle) collected in Batan Bay and Tinagong Dagat Bay.



Figure 7. Site-specific differences in  $\delta^{13}$ C (*top*),  $\delta^{15}$ N (*middle*), and C/N ratio (*bottom*) of 3 different groups of oysters. Differences were evaluated by 1-way ANOVA.

Average  $\delta^{13}$ C of oysters was typically higher in seagrass-associated stations (3, 4) than in the other stations (1, 2, 5) (Fig. 6). Average  $\delta^{15}$ N was also slightly higher in Stations 3 and 4 than Stations 2 and 5 (Fig. 7). C/N ratio did not vary so much.

Difference between species in  $\delta^{13}$ C,  $\delta^{15}$ N, and C/N was not statistically significant, except that  $\delta^{13}$ C was higher for *C. iredalei* (– 18.0 ± 0.1‰) than *Saccostrea* sp. Lineage F (–18.3 ± 0.2‰) at Station 4. These parameters were not dependent on shell size, except  $\delta^{13}$ C of *Saccostrea* sp. Lineage F at Station 2 that positively correlated with shell length (*p* = 0.038).



Figure 8. Comparison of  $\delta^{13}$ C of oysters, suspended and sediment POC (*top*), and DIC (*bottom*).

The  $\delta^{13}C$  of oysters was consistently higher than that of suspended POM and sediment OM collected at the same sites.

### DISCUSSION

The salinity gradient of Batan Bay estuary greatly differed between the dry season and the wet season (Fig. 2). The  $\delta^{13}$ C of DIC and the  $\delta^{18}$ O of water in inner bay area were also significantly lower in the wet season than in the dry season (Fig. 3). However, the spatial gradient of the  $\delta^{13}$ C of suspended POC was not so different between the dry and wet seasons (Fig. 4).

The  $\delta^{13}$ C of POC seemed to be constrained by mixing of marine-endmember POC (marine and brackish phytoplankton) and freshwater-endmember POC (terrestrial and mangrove detritus and freshwater algae). The  $\delta^{13}$ C of oysters collected at Stations 1, 2, and 5 was similar to the  $\delta^{13}$ C of the marine-endmember POC (Fig. 5). This fact suggests that the oysters in these sites selectively assimilated phytoplankton from the bulk POC they filtered.

However, the oysters collected at Stations 3 and 4 showed significantly higher  $\delta^{13}$ C than the marine-endmember POC (Fig. 6). This fact implies that these oysters depend on not only phytoplankton but some other food source whose  $\delta^{13}$ C is higher than normal phytoplankton. Most likely source is attached microalgae that grow on seagrass blades, because seagrass-attached microalgae usually show higher  $\delta^{13}$ C than phytoplankton due to DIC limitation and are apparently preferred by many bivalves (Morimoto et al. 2017 (http://dx.doi.org/10.1007/s00227-016-3063-z)).

It is supposed that both phytoplankton and attached microalgae in the bay are benefitted by nutrients supplied by fishpond effluents (Plate 1c). As degradation of seagrass meadows proceeds, attached microalgae would lose their habitats, and consequently, more nutrients would be directed to growth of phytoplankton resulting in higher risks of harmful algal blooms. Thus, conservation and restoration of seagrass meadows may have dual merits for aquaculture production of bivalves: first, by providing abundant attached microalgae as good food source for bivalves, and second, by suppressing phytoplankton blooms that can be harmful for bivalves as well as humans.

# NOTES & ACKNOWLEDGMENTS

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### ABSTRACT

Batan Bay and Tinago Lake are shallow embayments connected to each other, located on the north of Panay Island, central Philippines (11.53° - 11.67°N, 122.38° - 122.52°E). Although they had been originally surrounded by dense mangrove forest till the middle of the last century, mangroves have been mostly cleared and converted into fish and shrimp ponds. Recently, shelves and rafts for cultivating oysters and green mussels have become widespread in the shallow areas of the embayments (see Figure as an example). Replantation of mangroves is also ongoing in limited areas of Batan Bay. We are conducting researches there focusing on ecosystem services of mangroves and seagrass meadows, especially in relation to carbon sequestration and aquaculture production. In this presentation, we report preliminary survey results on environmental conditions that may influence growth and survival of cultivated bivalves, such as freshwater inputs and potential food resources. The survey was conducted in both dry season (February 2019) and rainy season (November 2019). Although the salinity gradient across the bay due to freshwater input was evident in both seasons, the oxygen isotope ratio of seawater indicated that evaporation overwhelmed in inner bay sites in the dry season. Concentrations of chlorophyll and suspended particulate organic matter (POM), i.e. potential food source for bivalves, were high in the inner bay area. Carbon stable isotope ratio ( $\delta^{13}$ C) of dissolved organic carbon (DIC) and POM showed spatial gradient from the bay mouth (high) to inner sites (low), indicating the influence of riverine DIC and POM inputs. However, the  $\delta^{13}$ C of oysters (adductor muscle) was consistently higher than POM and showed no clear spatial gradient. The  $\delta^{13}$ C of oysters was relatively higher for individuals collected from inside or edge of seagrass meadows than those collected in open areas. These results suggest that oysters assimilate only a specific fraction of POM relatively enriched in <sup>13</sup>C (i.e. marine-origin POM) and that seagrass meadows support growth of oysters by providing additional food source (e.g. attached microalgae that are abundant on seagrass blades).

#### [In Japanese]

フィリピン中部のパナイ島北側に位置するバタン湾とそれに隣接するティナゴ海は入り組んだ地形の浅い内湾域で、 マングローブを伐採して造成された養魚池で囲まれている。湾内では浅く平穏な立地条件を利用したカキやミドリイ ガイの養殖がさかんに行われ、フィリピン国内有数の産地となっている。発表者等は、バタン湾における二枚貝類の 養殖に対して湾岸に生育するマングローブや海草藻場が有する環境改善機能に着目した研究を行っている。今回の発 表では、二枚貝類の生理生態に関連の深い水質諸量(塩分、クロロフィル濃度、懸濁態有機物濃度など)の湾全体に おける分布の特徴と、カキ類の炭素・窒素安定同位体比から推定される主要餌資源についての予備調査結果を報告す る。現地調査は2019年2月(乾季)と11月(雨季)の2回実施された。塩分は、河川水の流入する湾奥部から湾口部に 向かって上昇する勾配は雨季・乾季ともに見られたが、海水の酸素同位体比の分布からは、乾季には湾奥部で蒸発が 卓越していることが示唆された。クロロフィルや懸濁態有機物の濃度は湾奥部の方が高く、また懸濁態有機炭素の安 定同位体比は湾奥部ほど低くなる勾配が見られた。これに対してカキ類の炭素安定同位体比は懸濁態有機炭素よりも 高く、カキの主要餌資源は海域起源であることが分かる。また湾奥部と湾口近くとの間でカキの炭素同位体比に明瞭 な違いはなく、海草藻場内もしくはその縁辺部で採取された個体において高くなる傾向が認められた。このことから カキ類の栄養生態に対して海草藻場の存在が強い影響を与えている可能性が示唆され、その詳細についてさらに研究 を進めているところである。

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