

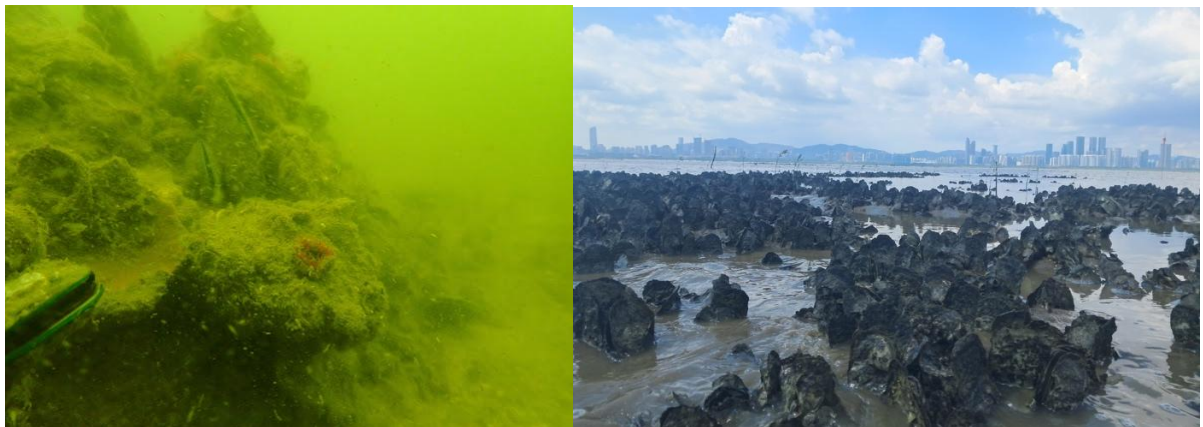
How much could oyster restoration improve nutrient cycling? Assessment of oyster habitat denitrification capacity in Hong Kong's Western waters

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1. Executive Summary

The coastal areas of the Greater Bay Area, including Hong Kong, are densely populated and thus experience environmental pressures (coastal development, overharvest, pollution) that reduce the function of marine ecosystems. Excess nitrogen pollution can lead to eutrophication and harmful algae blooms that negatively impact water quality, marine organisms and habitats, and make seafood and water unsafe for humans (Glibert et al., 2005).

Bivalve shellfish such as oysters can remove anthropogenic-derived nitrogen from coastal ecosystems by assimilating nitrogen into tissue and shell as well as by excreting bio-deposits, which then become buried (Carmichael et al., 2012; Kellogg et al., 2014). Suspension feeding bivalves can reduce turbidity and particulate organic nitrogen by removing phytoplankton from the water column when they feed, which can improve water conditions for seagrasses and other marine life (Newell, 2004). Over the past two decades, research has shown that oyster reefs enhance benthic denitrification (the conversion of biologically available dissolved nitrogen [N] to inert di-nitrogen [N₂] gas) (Newell et al., 2002, 2005). However, denitrification rates vary with environmental conditions, both spatially and temporally, and the rates of denitrification by oyster reefs relative to the surrounding mudflats has not been quantified in many regions (Ray et al., 2021). Therefore, this project set out to measure denitrification rates in different types of oyster habitats around Hong Kong as the first assessment of the current capacity for these habitats to reduce nitrogen pollution and to assess their potential for enhancing denitrification with future oyster reef restoration

Over the course of this project (01/02/2024 - 31/1/26) five seasons of field sampling at three intertidal and four subtidal oyster habitat sites throughout Hong Kong were completed. Within each sampling period, samples of oyster habitat and sediment cores were incubated with seawater collected at the site to measure denitrification rates of oyster habitats compared to sediments and seawater. In the first season the N₂/Ar method was used to measure denitrification rates as described in Kellogg et al. 2013. Due to issues with long sample transport required to process samples on the equipment (located in Maryland, USA) the first season of sampling did not yield results due to contaminated samples. Therefore, all seasonal samples were repeated using the ¹⁵N isotope pairing technique (¹⁵N IPT) with analytical equipment in China, in which the incubation chambers are spiked with ¹⁵N labeled NaNO₃ and the denitrification rate calculated by comparing the ²⁹N:³⁰N ratio in the final sample (Nielsen, 1992). This method yielded high-quality results and all four sampling seasons were completed.

In general, the denitrification rates were orders of magnitude higher for the oyster habitat (which includes sediment) than the bare sediment alone, but this effect varied by location and intertidal versus subtidal habitats. Denitrification rates were also generally higher in summer than winter, likely because of the higher water temperatures, but, again, the magnitude of this difference varied by location.

Summer temperatures ranged from 27.7 - 29.6 °C, the highest temperatures throughout any of the sampling. Therefore, we hypothesized that summer denitrification rates would be the highest due to the high temperatures and increased microbial activity (as the main driver of denitrification). Overall, the data support this hypothesis. The highest natural (¹⁴NO₃⁻) denitrification rates were recorded in summer in oyster habitats at the intertidal site in Tung Chung (1875.2 μmol hr⁻¹ m⁻²) and the subtidal site Mong Tung Wan (1803.3 μmol hr⁻¹ m⁻²). These rates are 82.4 times and 19.8 times the denitrification rates of adjacent sediment, respectively.

On average denitrification rates in spring were higher than in winter, which is to be expected given that the microbial activity would increase as the temperature increases; water

temperature increased by 7.4 °C on average from winter to spring sampling. In spring the highest natural ($^{14}\text{NO}_3^-$) denitrification rates were recorded in the intertidal site at Pak Nai ($525.7 \mu\text{mol hr}^{-1} \text{m}^{-2}$) and the subtidal site at Sunshine Island ($137.156 \mu\text{mol hr}^{-1} \text{m}^{-2}$), although these rates were only 1.02 and 1.4 times the adjacent bare sediment habitat, respectively. In autumn, the highest natural ($^{14}\text{NO}_3^-$) denitrification rates were recorded in the intertidal sites of Pak Nai ($108.8 \mu\text{mol hr}^{-1} \text{m}^{-2}$) and Tung Chung ($85.0 \mu\text{mol hr}^{-1} \text{m}^{-2}$) and these were 11.97 times and 1.23 times higher than the rates measured from adjacent sediments, respectively. Winter had the lowest denitrification rates, on average, although during this season two intertidal sites still exhibited high denitrification rates; Pak Nai ($362.6 \mu\text{mol hr}^{-1} \text{m}^{-2}$) and Sha Kiu Tsuen ($224.3 \mu\text{mol hr}^{-1} \text{m}^{-2}$) which were 163.1 times and 54.5 times higher than rates measured in the adjacent sediment, respectively.

DNA barcoding of oysters revealed that the sampled intertidal oyster habitats are mostly formed by *Magallana hongkongensis* (48.1 %), *M. sikamea* (37.03 %), *M. ariakensis* (5.55 %), *M. angulata* (3.70 %), and *Saccostrea malabonensis* (3.70 %). However, these intertidal habitats were not exclusively oysters they also comprised a mixed assemblage of bivalves including *Barbatia* sp., *Brachidontes* sp., though oysters were the main habitat-forming species.

The subtidal sites were primarily populated by *Dendostrea crenulifera* (62.2%), *M. angulata* (22.6 %), *M. sikamea* (1.8 %), *Ostrea stentina* (11.3 %), and *Saccostrea echinata* (1.88 %). The mixed subtidal habitats also had many associated bivalves living in the structure created by the oysters, commonly including *Perna viridis*, and *Chama* sp.

Overall, this project demonstrates that both subtidal and intertidal oyster habitats have the ability to process nitrate in marine environments at rates which are orders of magnitude faster than adjacent sediments and provides some of the first evidence for the potential benefits of oyster reef restoration in amplifying the removal of nitrogen pollution in the coastal waters of Hong Kong.