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Baseline

Trophic and growth baseline of dominant subtidal gastropods in contrasting subtropical marine environments

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ABSTRACT

Using $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$ isotopes, the trophic relationship and growth estimation were analyzed in gastropods *Nassarius siquijorensis*, *Murex trapa* and *Turritella bacillum* and their potential food sources and predators in summer and winter from estuarine and oceanic environments in subtropical Hong Kong. Results of $\delta^{13}C$ and $\delta^{15}N$ values and isotopic mixing model revealed *N. siquijorensis* and *M. trapa* were one trophic level higher than *T. bacillum*, in which its main food source was particulate organic matter (POM) whereas *N. siquijorensis* largely consumed POM and polychaetes and *M. trapa* also preyed on other gastropods. Crabs were the major predator of gastropods. Organisms collected from oceanic waters were more ^{13}C enriched than from estuarine waters, reflecting different carbon food sources from marine or terrestrial origin. The $\delta^{18}O$ profile from shell carbonate suggested these gastropods were one to two years old. *T. bacillum* exhibited faster summer growth than the other two species.

Marine subtidal gastropods are diverse and abundant components of many benthic communities. In addition to their high biodiversity, these animals embrace a broad array of feeding modes, including algal grazing, suspension and deposit feeding, grazing upon sedentary organisms, parasitism and predation (Taylor et al., 1980). Hence, subtidal gastropods may be found at several trophic levels in marine communities. In many shallow-water, tropical and subtropical areas, predatory gastropods are particularly dominant. However, little is known about their contributions to ecosystem functioning and interactions with other benthic fauna (Urra et al., 2012). Studies on changes in gastropod assemblages are also important to further understanding of the trophodynamics in subtidal habitats, but current reports on such field-based studies are limited to seagrass bed (Arroyo et al., 2006; Rueda et al., 2009), salt marsh (Wang et al., 2014), intertidal bay (Riera, 2010) and coastal lake (Miranda and Perissinotto, 2012). In similar, highly productive tropical and subtropical waters (Ferguson and Eyre, 2010; Moreno-Ostos et al., 2011), the contribution of gastropods to trophic transfer and how changes in their assemblages affect community structure within soft-bottom marine ecosystems are virtually unknown. Excluding trophic relationships, field-based observations on the growth of subtidal gastropods are also limited (Alfaro and Carpenter, 1999; Chavanich and Harris, 2002). The lack of comprehensive growth data on dominant gastropod populations precludes our understanding of how these diverse animals respond to seasonal changes and/or biotic factors, such as predation pressure, especially in tropical and subtropical ecosystems, where inter- and intra-competition among different species can be intense. Since gastropods serve as important food sources for other marine species, including crabs and fish, the lack of such growth data may also preclude an accurate estimation in the trophic modelling of ecosystem production for resource management purposes (Ortiz and Wolff, 2002; Okey et al., 2004).

Traditionally, researchers adopt gut content and morphometric analyses to study of the feeding modes and growth of marine organisms, including gastropods (Ismail et al., 2000; Malaquias et al., 2004; Zamora-Silva and Malaquias, 2016). Over the last decade, stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) have been increasingly used as biomarkers to analyze the trophic position of marine organisms, so as to better understand how they interact within the food web of an ecosystem (Post, 2002; del Rio et al., 2009; Won et al., 2013). This is based on the premise that carbon stable isotope ratios tend to increase by approximately 1‰ per trophic level (Peterson and Fry, 1987), and thus can be more representative of the food web source (primary producer) and used to trace the contributions of different primary producers (e.g., phytoplankton, kelp, seagrass) and carbon sources (e.g., marine versus

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terrestrial) to food webs. On the other hand, nitrogen isotope ratios become enriched by ~3‰ at successive trophic levels (Minagawa and Wada, 1984), and can be applied to determine predator-prey relationships. Apart from deciphering trophic relationships in different habitats (Zabala et al., 2013; Piñón-Gimate et al., 2016), recent such studies on gastropods also revealed feeding specialization and resource partitioning among different genera of a family (Fedosov et al., 2014), diet preference of omnivory instead of carnivory (Casey et al., 2016) and dietary niche overlap between alien and native origins of the same species (Miranda and Perissinotto, 2012).

In addition to $^{13}C/^{12}C$ and $^{15}N/^{14}N$, $^{18}O/^{16}O$ isotope has been applied as an indirect method for assessment of age and growth rate of organisms with shell made of calcium carbonate (Allmon et al., 1994). Owing to the temperature-dependent fractionation of ^{18}O and ^{16}O during calcification (Epstein et al., 1953), oxygen isotope values obtained from such shell structure serve as a proxy for temperature and can be applied to determine growth and climate records in paleontological studies (Kobashi and Grossman, 2003; Scourse et al., 2006; Leng and Lewis, 2016). The examination of internal growth band patterns and the application of $^{18}O/^{16}O$ isotope analysis of shell aragonite have also provided accurate measurements of incremental growth and estimates of ultimate longevity of the gastropods under study (Schöne et al., 2007; Arrighetti et al., 2011). Such data are particularly useful to reflect the changes in hydrographic conditions, especially temperature, where the organisms thrive (Dettman et al., 2004; Mannino et al., 2008), in which a favourable environment may induce faster growth and possibly longer lifespan, and can be employed in fisheries management (Gurney et al., 2005).

In subtropical Hong Kong, the influence of the Pearl River's discharges on the west and oceanic waters from the South China Sea on the east has resulted in contrasting marine environments (Morton and Morton, 1983). As the second largest river in China, the Pearl River delivers some 336 km^3 of freshwater outflow annually, 80% of which in the flood season from April to September (Zhang et al., 2007). Such runoff is also laden with various pollutants from untreated wastewater discharged by domestic and industrial sources in the catchment area (Cheung et al., 2003; Chau, 2006), resulting in the formation of a hypoxic “dead zone” in the estuarine waters (Chen et al., 2013) and affecting the general environs and ecology of the west coast of Hong Kong (Richardson et al., 2000). In contrast, the South China Sea brings in saline and oxygen enriched water to the east coast of Hong Kong directly, coupled with occasional tropical storms in the wet, summer months (Morton and Morton, 1983). As a result, these waters are characterized by distinct subtidal communities (Shin et al., 2004; Wang et al., 2017), including different gastropod assemblages (Leung and Morton, 2003). To our knowledge, apart from literature on biodiversity, there are only few reports on trophic studies using gut content analysis (Taylor, 1980, 1982; Taylor and Morton, 1996) and no growth investigations on subtidal gastropods in Hong Kong waters. The aim of the present study was to establish preliminary baseline information on trophic position and growth pattern of dominant gastropod species in different environmental regimes using stable isotope analyses.

Routine monitoring of subtidal benthic organisms has been conducted by the Environmental Protection Department, Hong Kong Special Administrative Region Government (CPSL, 2006, 2010; CPSL, 2013). Based on these reports, three numerically dominant gastropod species were selected for the present study, including a filter feeder *Turritella bacillum*, an omnivore *Nassarius siquijorensis*, and a carnivore *Murex trapa*. Samples of these gastropods and their potential food sources and predators (primarily crustaceans) were collected from Mirs Bay on the east and Urmston Road on the west coast of Hong Kong in both wet, summer and dry, winter seasons (Fig. 1). Mirs Bay is characterized by stable dissolved oxygen (6–8 mg/L) and salinity (mostly around 30–35‰) and low suspended solids (mostly below 5 mg/L), whereas Urmston Road experiences larger fluctuations in these parameters, particularly with salinity lowering to 15‰ in summer and suspended solids reaching over 10 mg/L occasionally (Fig. 2). In each study area, sampling was conducted at three locations using a pair of beam trawls of 2.5 m width and 15 mm cod-end mesh. The trawls were deployed and hauled for 5 min, bottom time, in one fixed direction and in the opposite direction for another 5 min at each location. Specimens of gastropods and crustaceans collected from the trawl returns were pooled for each study area, and species were later identified and enumerated in the laboratory. In each study area, at least triplicate samples of primary food sources were also collected, including particulate organic matter (POM) and sediment organic matter (SOM). For POM, samples were collected by filtering 20 L of near-bottom seawater through a 125 μm mesh (to remove zooplankton), followed by a precombusted (450 °C for 3 h) GF/C filter paper (0.7 μm pore size). The residue on the filter papers was rinsed with distilled, double deionized water to remove the salt adsorbed on the particle surface. For SOM, samples of the top 1 cm of surface sediment collected from a 0.1 m^2 van Veen grab were obtained using a spatula. For polychaetes, ten replicate grab samples were collected in each study area. Sediments were gently washed through a 0.5 mm screen and polychaete specimens were handpicked, placed in vials and kept in an icebox on board, and later identified in the laboratory.

All collected animals were starved for 12 h in 0.7 μm GF/F filtered seawater to clear gut content, as necessary (Gao et al., 2006). For gastropods, the shells were removed and the whole of the soft tissues were used for analysis. For other organisms, such crabs and polychaetes, the whole animals were used. Specimens were pooled if individual biomass was too small for subsequent analyses. Samples of SOM, POM and animal tissues were treated with 1.2 N isotonic HCl to remove inorganic carbonates (Kang et al., 2003). Then, the samples were rinsed with distilled, double deionized water to remove the acid. All samples were freeze-dried, ground with a mortar and pestle, and kept frozen at -20 °C prior to isotopic analysis.

A representative live specimen for each selected gastropod species obtained from Mirs Bay and Urmston Road in each sampling event was used for analysis of the periodicity of shell growth. Individual shell height (from apex to anterior tip of siphon canal) was measured and number of whorls was recorded. The outer surface of each specimen was cleaned ultrasonically for 5 min to remove extraneous materials. Samples of shell carbonate material of approximately 0.5 mg were obtained from each whorl of the specimen, as far as practicable, using a small dental burr (diameter $<$ 0.5 mm) mounted on a hand-held drill. Each sample of powdered carbonate was then washed in 15% hydrogen peroxide (H_2O_2) for 3 h to remove organic contaminants. The H_2O_2 was later pipetted off, and the samples were flushed with methanol (99.9%) and dried.

^{13}C and ^{15}N isotopic ratios of food sources and consumers were measured on the same samples and determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, University of California, Davis, USA. Results of isotopic ratios were expressed in standard δ-unit notation, which is defined as follows:

$$\delta X = [(R_{sample}/R_{reference}) - 1] \times 1000‰$$

where X is ^{13}C or ^{15}N, and R is either the ^{13}C:^{12}C ratio for carbon or the ^{15}N:^{14}N ratio for nitrogen. These values were reported relative to the Vienna-PeeDee Belemnite (V-PDB) standard for C and to air N_2 for N. For shell growth analysis, samples were measured via a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a Gas Bench with a CombiPAL autosampler at the Stable Isotope Laboratory, Department of Geological and Atmospheric Sciences, Iowa State University, USA. Data were expressed in standard