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Food availability on the shore: Linking epilithic and planktonic microalgae to the food ingested by two intertidal gastropods

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ABSTRACT

Research on the interaction of primary producers and consumers is crucial for understanding trophic transfer in intertidal food webs. This study explores the association between epilithic and planktonic microalgae, and gut contents of two targeted intertidal gastropods, the periwinkle *Echinolittorina radiata* (splash zone) and the limpet *Cellana toreuma* (mid-intertidal zone). With the application of gut fluorescence technique and metabarcoding, this study investigates the quantity and composition of two different sources of microalgae (epilithic and planktonic) and the food ingested by the gastropods. The results suggest the following findings: 1) The planktonic microalgae have higher compositional similarity to the gut contents of grazing gastropods. 2) Increased gut pigment content in *C. toreuma* is observed with increasing abundance of epilithic and planktonic microalgae. However, there was no such pattern observed for *E. radiata*. This difference could be attributed to potentially divergent foraging behaviours of the two species that inhabit different shore heights.

1. Introduction

The transfer of food (energy) in the intertidal food chain is a long-standing interest of intertidal ecologists. The intertidal rocky shores are one of the key interfaces of the ocean, atmosphere and terrestrial environments, which have long served as a natural laboratory for examining ecological patterns and other biological processes (Decho, 2000; Helmuth et al., 2006). Grazing gastropods in this region are exposed to daily emersion in air and immersion in seawater. Thus, microalgal food can come from both epilithic species and planktonic microalgae deposited during the tide.

Epilithic microalgae provide a food resource for herbivorous grazers and can be regarded as a major fraction of the biomass produced and directly consumed *in situ* on rocky shores (Hawkins et al., 1992; Thompson et al., 2000, 2004; Van Colen et al., 2014). The abundance of epilithic microalgae shows clear temporal and spatial patterns, i.e. increasing during winter and declining during summer, and generally being greater on the lower shore than the upper shore (Underwood and Jernakoff, 1981; Underwood, 1984; Hill and Hawkins, 1991; Jenkins et al., 2001; Thompson et al., 2004, 2005; Murphy and Underwood, 2006). The vital role of microalgal species in the diet of grazing gastropods has been demonstrated extensively (Nicotri, 1977; Medlin, 1980; Raffaelli, 1985; Croudace, 1987; Decho, 2000). Many intertidal grazers are known to forage primarily on diatoms, cyanobacteria, spores and sporelings of macroalgae (Underwood, 1979; Hawkins et al., 1989; Norton et al., 1990; Nagarkar et al., 2004), and macroalgae can also contribute to the diet of grazers (e.g. chitons, Latyshev et al., 2004; abalone, Daume, 2006; limpet, Notman et al., 2016).

There are limited studies of the gut contents of gastropods suggesting that some planktonic algal species could also be possible food resources (Hill and Hawkins, 1991; Sitnikova et al., 2012). Some gastropods can secrete pedal mucus, which has been shown to help bind planktonic microalgae, and the mucus embedded with food particles is reingested by its producers (Davies et al., 1992; Davies and Beckwith, 1999; Kamimura and Tsuchiya, 2004, 2006). Therefore, the importance of planktonic microalgae in the food ingested by the gastropods still needs more attention (but see Hill and Hawkins, 1991; Davies et al., 1992).

The present study, combining gut fluorescence technique and metabarcoding, aims to investigate whether planktonic microalgae are important sources for two intertidal gastropods, the periwinkle *E. radiata* (in the splash zone) and the limpet *C. toreuma* (in the mid-intertidal zone). The gut fluorescence technique is efficient in estimating the amount of ingested photosynthetic material of gastropods (Miranda

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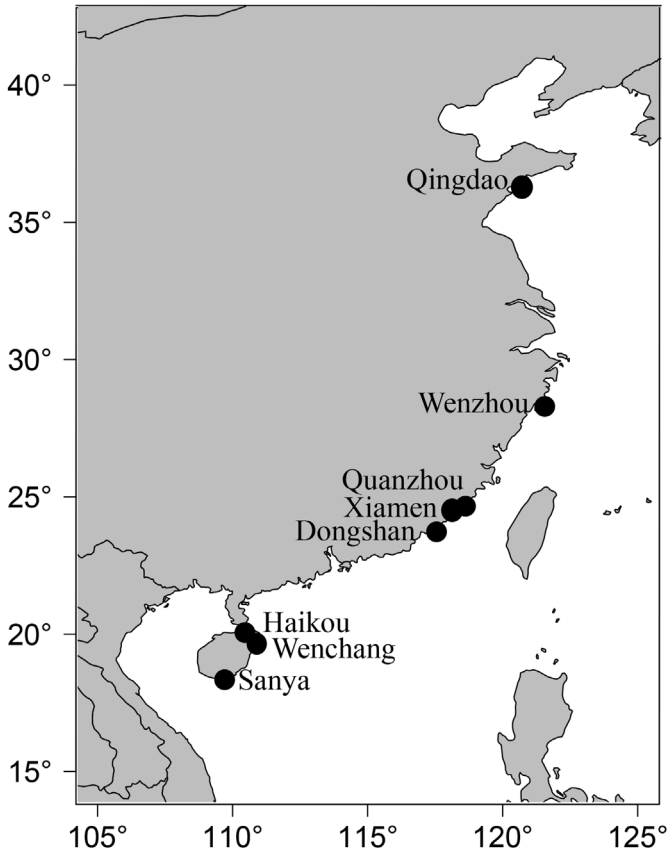

Fig. 1. Map of study sites along the China coastline.
(Note: Samples for metabarcoding analysis were collected from Qingdao, Xiamen and Wenchang only).

et al., 2011; Raw et al., 2016). Metabarcoding method, which is highly valuable for large-scale, high-throughput detection of microalgal diversity (Ebenezer et al., 2012), was applied for determining the contributions of epilithic and planktonic microalgae to the food ingested by intertidal gastropods.

2. Materials and methods

2.1. Study sites and sampling

Samples were collected from 8 sites along China coast, ranging from temperate shores to tropical shores in the period October 2015 to January 2016 (Fig. 1). The sampling sites include Qingdao (36° 07′ N, 120° 36′ E; 22 Oct., 2015), Wenzhou (27°51′ N, 121°10′ E; 9 Jan. 2016), Quanzhou (24°31′ N, 118°35′ E; 15 Nov., 2015), Xiamen (24°25′ N, 118°08′ E; 29 Nov., 2015), Dongshan (23°39′ N, 117°29′ E; 4 Dec., 2015), Haikou (20°02′ N, 110°13′ E; 27 Oct., 2015), Wenchang (19° 38′ N, 110°59′ E; 1 Nov., 2015) and Sanya (18°13′ N, 109°31′E; 29 Nov., 2015). Samples of rock chips, the surrounding seawater and two species of intertidal grazing gastropods, *E. radiata* and *C. toreuma*, were collected at each site. *E. radiata* was not collected at Sanya and Wenchang due to its absence at both sites. All collected samples were put in an icebox, transported back to the State Key Laboratory of Marine Environmental Science, Xiamen University, and stored at −20 °C for further laboratory works.

Rock chips (~1 cm^2) were randomly taken from 3 shore heights (splash zone, high intertidal zone and mid-intertidal zone), using a hammer and chisel on two transects (~30 meters interval) at each site. The places where the periwinkle *E. radiata* and the limpet *C. toreuma* occurred were defined as the splash zone and the mid-intertidal zone, respectively, and the places in between were defined as the high intertidal zone. Rock samples were removed from areas in which barnacles and other macrobiota were absent. Six 0.5-litre seawater samples were collected at each site. They were pre-filtered with a sieve (150 μm) to remove possible zooplankton and vacuum-filtered onto 47 mm diameter 0.22-μm pore size GF/F filters. Three filters were wrapped in aluminum foil for chlorophyll analysis and another three for metabarcoding analysis.

Periwinkles (*E. radiata*) and limpets (*C. toreuma*) were randomly collected from adjacent areas of open rock on the ebbing tide. It could be assumed that they had been actively feeding before collection and were about to become inactive prior to emersion (Williams et al., 2005). After collection, all animals were immediately placed on ice to halt digestion, and then were transported to the laboratory for storage at −20 °C until dissected. Shell height of individual periwinkles and shell length of individual limpets were measured by a Vernier Caliper.

2.2. Chlorophyll concentration quantification

The concentrations of chlorophyll *a* and its derivative phaeophytin *a* were used as estimations for the amount of photosynthetic microalgal standing stock in the seawater, on the rock surfaces and in the guts of the grazing gastropods (Boyd et al., 1980; Dagg and Wyman, 1983; Underwood and Jernakoff, 1984; Conover et al., 1986; Kamermans, 1994; Miranda et al., 2011). For each sampling site, photosynthetic microbial standing stock was quantified from 18 to 20 subsamples of rock chips (~1 cm^2) from each shore height, 10 gut contents of *E. radiata* and 10–11 of *C. toreuma* along with 3 samples of seawater filters. Samples were extracted in 7.0 ml 90% acetone in the dark at −20 °C for 24 h, following Miranda et al. (2011). After extraction, the concentrations of the samples were determined using a Turner Trilogy flourometer (Axler and Owen, 1994; Welschmeyer, 1994). This method ensures the rapid determination of the low amounts of chlorophyll *a* present in the guts as well as its degradation product phaeophytin *a*. To compare the overall difference in gut pigment contents (chlorophyll *a* + phaeophytin *a*) among sites, the measurements were size-standardized for both species to allow suitable comparisons using the mean shell height (SH) for *E. radiata* or shell length (SL) for *C. toreuma* as follows (Raw et al., 2016):

Gut pigment concentration (μg ind^{-1}) = chlorophyll *a* equivalents × (mean SH or SL / individual SH or SL)

2.3. 454 library preparation and sequencing

The analysis of microalgal composition through high-throughput pyrosequencing was carried out at three sites: Qingdao, Xiamen, and Wenchang, representing temperate, subtropical and tropical shores respectively. On each sampling site, 36 subsamples of rock chips (~1 cm^2), 3 gut samples of *C. toreuma*, 3 gut samples of *E. radiata* and 3 samples of seawater filters were used for DNA extraction. All samples were incubated with 0.5 ml CTAB buffer (2% CTAB, 1.4 *M* NaCl, 20 m*M* EDTA pH = 8.0, 100 m*M* Tris-HCl pH = 8.0, 0.2% SDS, 400 μg mL^{-1} proteinase K) at 56 °C for ~24 h for thorough cell lysis, and were extracted using an improved CTAB method as previously described (Zhang and Lin, 2005). Quality and quantity of DNA were checked with a NanoDrop device (ND-2000, ThermoFisher, USA). DNA extraction replicates from each type of samples were pooled for subsequent PCR.

DNA samples were amplified by PCR for multiplexed pyrosequencing. A set of primers was designed by adding a 6-nucleotide barcode to the primer *rbcL* IA/B and *rbcL* ID (Boling et al., 2012). The Form IA/B primer set amplifies a 615-bp *rbcL* fragment from most marine chlorophytes and cyanobacteria (referred as cyanobacteria below for convenience). The Form ID primer set amplifies a 554-bp *rbcL* fragment from a large diversity of marine haptophyte and stramenopile algae (referred to as diatoms below, for convenience). By adopting these two

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