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## The dynamics of attached and free-living bacterial population in tropical coastal waters

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**Abstract.** We investigated the dynamics of attached and free-living bacterial abundance over a period of 18 months in tropical coastal waters of Malaysia. We measured the abundance at both oligotrophic coastal water (Port Dickson) and eutrophic estuary (Klang), and hypothesised that attached bacteria are predominant in eutrophic waters. We found that bacterial abundance was higher at Klang than Port Dickson (Student's *t*-test: t = 4.87, d.f. = 19, P < 0.001). Attached bacteria also formed a large fraction of the total bacteria at Klang ( $75\% \pm 13$  s.d.) relative to Port Dickson ( $56\% \pm 22$ ), and showed preference for chlorophyll-*a*-based particles rather than total suspended solids. The bacterial community structure was clearly different between the two stations but was similar between the attached and free-living bacterial population. Our results showed the importance of attached bacteria in eutrophic water where they could play a major role in carbon and nutrient cycling.

Additional keywords: 16S rRNA PCR-DGGE, LIVE/DEAD bacterial staining, Straits of Malacca.

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## Introduction

Bacteria are ubiquitous, abundant, diverse, and represent a large pool of genetic and physiological diversity in aquatic habitats. Bacteria are also the main consumers of dissolved organic matter and, subsequently, play a major role in carbon and nutrient cycling in aquatic ecosystems (Azam *et al.* 1994; DeLong and Karl 2005). In an aquatic environment, bacteria are separated into two main groups, namely, attached and freeliving bacteria (Murrell *et al.* 1999). These two groups are not independent communities but can be considered interacting as shifts between attached and free-living modes that occur among them (Riemann and Winding 2001; Grossart *et al.* 2003). Bacterial attachment, detachment, and release of free-living progeny when attached (Stocker *et al.* 2008), are processes that contribute to the dynamic nature of the mode-shifts.

Generally, the proportion of attached bacteria in an aquatic environment increases with eutrophication, and can range from <5% in oligotrophic to >60% in eutrophic ecosystems (Bano *et al.* 1997; Crump *et al.* 1998; Garneau *et al.* 2009). One major reason is that eutrophic waters contain more organic-rich particles that are 'hotspots' for attached bacteria (Azam *et al.*  1994). However, not all particle-rich environments have high attached bacterial abundance because free-living bacteria can also contribute to recycling of particulate organic matter (Lapoussière *et al.* 2011). Attached bacteria are larger and more active than free-living bacteria (Acinas *et al.* 1999; Lee *et al.* 2001; Mével *et al.* 2008; Lapoussière *et al.* 2011), although their abundance over total bacteria vary among different locations and seasons (Iriberri *et al.* 1987; Griffith *et al.* 1994).

There is also ambiguity whether particles can host distinct microbial communities, with some studies being affirmative (Acinas *et al.* 1999; Crump *et al.* 1999; Ghiglione *et al.* 2009) and others contradictory (Hollibaugh *et al.* 2000; Ghiglione *et al.* 2007; Ortega-Retuerta *et al.* 2013). Because of the dynamic nature of the mode-shifts between attached and free-living bacteria, a majority of bacterial groups are ubiquitous. However, niche segregation and, therefore, distinct bacterial populations, are still possible because different metabolic capacities are required when growing on particulate versus dissolved matter. The bacterial community structure in different fractions is well studied in temperate (e.g. Crump *et al.* 2013) and