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Growth rates of *Synechococcus* types with different phycoerythrin composition estimated by dual-laser flow cytometry in relationship to the light environment in the Uwa Sea

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Abstract

In the Uwa Sea, *Synechococcus* types with low- and high-phycourobilin (PUB) to phycoerythrobilin (PEB) ratio co-occurred throughout a year. To clarify the effects of light quality and quantity on the two types of *Synechococcus*, we measured the growth rates of two pigment types of *Synechococcus* cells with in situ incubation experiments. Incubations were conducted at 2, 10, and 20 m depth between May and October 2002. *Synechococcus* were divided into high- and low-PUB types using a dual-laser flow cytometer. Two indexes were used to evaluate the light environment: one was the relative light intensity (RLI) to that at the surface, and the other was the ratio of the light intensities of blue (490–500 nm) to green (540–550 nm). At 2 m depth, where the relative light intensities were above 20%, the growth rates of the low-PUB type were generally slightly higher than those of the high-PUB type. In contrast, at 10 and 20 m depth, the type that grew faster did not depend on the combination of light intensity and quality. Available light in the deeper layer of the Uwa Sea ranged from 490 to 550 nm. The range covers absorbance maxima of both PUB (ca. 495 nm) and PEB (545 nm). For this reason, light quality may not have caused one type to grow faster. These results explain the co-occurrence of two pigment types of *Synechococcus* in coastal waters.

Keywords: *Synechococcus*; Growth rate; Light quality and intensity; Pigment type; Coastal sea

1. Introduction

Picophytoplankton, especially cyanobacteria of the genus *Synechococcus*, makes globally important contributions to marine primary production (Agawin et al., 2000). Many studies on the abundance of *Synechococcus* in marine environments have revealed that the distribution extends from coastal sea to open ocean (Wood et al., 1985; Olson et al., 1985, 1988, 1990; Li and Wood, 1988; Campbell and Vaulot, 1993; Li, 1995; Partensky et al., 1996; Brown et al., 1999; Zubkov et al., 2000; DuRand et al., 2001; Blanchot et al., 2001; Collier and Palenik, 2003; Lohrenz et al., 2003). It has been established that *Synechococcus* is genetically diverse (Honda et al., 1999; Fuller et al., 2003). There is also a variation in pigmentation among *Synechococcus* with phycobiliproteins (phycoerythrin, phycoerythrocyanin, phycocyanins, and allophycocyanins) as light-harvesting pigments. Most marine *Synechococcus* in oligo- and mesotrophic environments are phycoerythrin-rich types (Wood et al., 1998; Neveux et al., 1999). Phycoerythrin in the cell contains both PUB

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(phycourobilin) and PEB (phycoerythrobilin) or PEB alone.

PUB and PEB have absorption maxima at ca. 495 and 545 nm, respectively (Ong et al., 1984). Since the ratio of PUB to PEB of phycoerythrin is closely related to a light-harvesting property, this is one of the important phenotypes for *Synechococcus* though the phenotype is independent of the phylotype (Fuller et al., 2003). The ratio of orange fluorescence intensity at 495 nm to that at 545 nm (Ex495:Ex545) has been used to identify the relative content of PUB and PEB of phycoerythrin in cultured *Synechococcus* (Toledo et al., 1999; Palenik, 2001) and natural seawater (Lantoine and Nexeux, 1997; Wood et al., 1998, 1999). Ex495:Ex545 is highly variable from 0 to ca. 2.0 depending on the strain.

Ex495:Ex545 of phycoerythrin in oligogtrophic oceans was ca 2.0, which is almost the highest value ever measured (Lantoine and Nexeux, 1997). Similar results were obtained in the Arabian Sea (Campbell et al., 1998). Olson et al. (1990) showed that high-PUB-type cells predominated among *Synechococcus* in the open ocean of both the Pacific and Atlantic Oceans by flow cytometry. These results indicate that *Synechococcus* in the open ocean has a high PUB:PEB ratio.

In contrast, Ex495:Ex545 is low (0.4–1.0) in more eutrophic, coastal waters. In addition, the co-occurrence of a different PUB:PEB ratio of cells was found by flow cytometry in coastal seas of the North Atlantic and Pacific Oceans (Olson et al., 1990), the Arabian Sea (Campbell et al., 1998; Liu et al., 1998), and Southern California (Collier and Palenik, 2003). Katano et al. (2004) also reported two types of pigment of *Synechococcus* in the Uwa Sea, Japan. *Synechococcus* in the coastal environment is more complex in terms of the PUB:PEB ratio than that in the open ocean and is probably affected by underwater light.

Glover et al. (1986) examined the effects of light quality on the photosynthesis and growth of *Synechococcus* strains. *Synechococcus* strain WH7803, containing PUB, was able to use a low flux of blue light more efficiently for both photosynthesis and growth than *Synechococcus* WH7805, which lacks PUB. Wood (1985) demonstrated a high photosynthesis rate by high-PUB-type *Synechococcus* strains in the deeper layer of the Pacific Ocean, where blue light dominated. The prevailing light regime below 15 m in open oceans, where primary production is relatively low compared to that in an upwelling region and in the coastal sea, is blue and has wavelengths of 440–490 nm (Kirk, 1994). In contrast, the dominant light regime in the coastal seas is generally green and has wavelengths of 550–600 nm.

The present study investigates possible effects of light quality and intensity on the growth rate of the two types of phycoerythrin pigment of *Synechococcus* populations in the Uwa Sea. We enumerated the abundance of these two types of *Synechococcus* in the Uwa Sea separately, using a dual-beam flow cytometer. We conducted in situ incubations five times from May to October to estimate the growth rates of two types of pigment separately from the changes in cell densities during incubation. The relationship between the growth rates of *Synechococcus* subpopulations and the light environment is discussed.

2. Materials and methods

2.1. Sampling

Water samples were collected with a Van-Dorn water sampler at 10 m depth in the Uwa Sea on 22 May, 12 July, 2 August, 11 September and 9 October 2002. The study site was described in Hashimoto and Nakano (2003) and Katano et al. (2004). The water temperature profile was measured vertically with a CTD profiler (Arec Electronics, Japan). Light intensity at 0, 2, 10 and 20 m depth was measured with a spectroradiometer (LI-1800, Li-Cor, USA) at each wavelength between 320 and 700 nm. The relative intensities (relative light intensity, RLI) of photosynthetic active radiation (400–700 nm) at 2, 10, and 20 m depth to that at 0 m depth were calculated. Water samples were stored in 10-L polyethylene bottles and filtered through a 2-μm Nuclepore filter (Whatman, USA). Water samples for the counting of *Synechococcus* cells were fixed with glutaraldehyde at a final concentration of 0.1% and stored in liquid nitrogen.

2.2. Incubations

In situ incubation experiments were conducted to clarify the effects of light environment (intensity and quality) on the growth rates of *Synechococcus*. The samples collected from 10 m depth were filtered through a 0.2 μm Gelman culture capsule filter and a 2.0 μm Nuclepore filter. In the <2.0 μm filtrate, most of *Synechococcus* grazers were probably removed. In addition, to reduce grazing pressure of remaining grazers (<2 μm in size) on *Synechococcus*, the filtrates were mixed with ratios of 2.0 μm filtrate: 0.2 μm filtrate = 1:9 and then stored in 300 ml polycarbonate bottles. Thus, in our experiments, the removal of grazers and the