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Population dynamics of various ribotypes of *Cochlodinium polykrikoides* using novel development of Real-Time PCR assay based on EvaGreen

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Cochlodinium polykrikoides population was composed of three ribotypes which were "East Asian (Korean/Japanese)" ribotype, "Philippines" ribotype and "American/Malaysian" ribotype (Iwataki et al. 2008). In this study, we developed a rapid, simple, sensitive and economic format quantitative real-time polymerase chain reaction assay based on a new DNA-binding dye, EvaGreen to detection and quantification of various ribotypes of *C. polykrikoides*.

Ribotype-specific primers to *C. polykrikoides* were designed to target the Large subunit ribosomal RNA region. Primer specificities were tested via BLAST searches. In addition, specificity was verified using empirical tests, including competitive PCR. The qRT PCR assay analyzing $C_t$valueandthelogofcopynumbershowedasignificantlinearrelationship($r^2 \geq 0.99$). Melting curve analysis has one informative peak. Using qRT-PCR assay, two ribotypes of *C. polykrikoides* (Philippines and East Asian) were detected in Southern Korean coastal water. *C. polykrikoides* of Philippines ribotype was distribute'd from early August to November and has high copy number in summer period (temperature 24~26℃, Salinity 32.4~32.5 psu). On the other hand, *C. polykrikoides* of East Asian ribotype was detected after September and had high abundance from late October to early November (temperature 17.5~18.5℃, Salinity 33.8 psu). NP ratio and pH were important factors on both ribotypes of *C. polykrikoides*. According to result, it is able to provide a clue about *C. polykrikoides* long-term bloom mechanism.