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Flow cytometric enumeration of bacterial in the coral surface mucus layer

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Abstract. The direct counts of bacteria inhabiting coral mucus were performed by flow cytometry testing four fluorescent dyes (SYBR[®]Green I, HCS, TOPRO[®]3, SYTO[®]62) with three different scleractinian species. Results obtained with SYTO62 were the most reliable based on the comparison with standardized epifluorescence counts and the resolution of cytograms.

Introduction. Flow cytometry (FCM) has been long used to count marine planktonic bacteria (Gasol et al., 2000; Müller and Nebe-von-Caron, 2010), however no accurate protocols have yet been adapted for epibiotic cells, including those found in coral mucus secretion. Until then, vain attempts to perform automated counts of fluorescently labelled bacteria from coral mucus were mostly explained by the strong autofluorescence and viscosity of this biogel (Brown and Bythell, 2005; Heredia et al., 2008). The only reliable enumerations of mucosal cells were obtained from epifluorescence microscopy-based protocols (EFM); though this typically requires a preliminary step of mucus dissolution with the trypsin enzyme (Garren and Azam, 2010) or a solution of potassium citrate (Nguyen et al., 2015). Additionally, such EFM protocols are relatively expensive, fastidious, time-consuming and often lead to highly variable values which are strongly operator-dependent. In this methodological study, we

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