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Investigating the in-situ bacterial production of aquatic fluorescent organic matter using a freshwater laboratory model

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This research explores the *in-situ* bacterial production of aquatic fluorescent organic matter (AFOM) under controlled laboratory conditions. Whilst fluorescence techniques have long been used to monitor AFOM distribution, origin and dynamics within aquatic systems, the extent to which AFOM characteristics are defined by microbial processing in surface freshwaters has largely been overlooked. Current convention champions the assumption that humic-like (Peak C) and protein-like (Peak T) fluorescence signatures are exclusively derived from terrestrial (allochthonous) or microbial (autochthonous) origins respectively, with Peak T having been directly correlated with microbial enumeration. Under intensifying anthropogenic perturbations and changing catchment characteristics, the complexities associated with bacterial-organic matter (OM) interactions in freshwater systems are increasing, challenging our understanding as to the origin and fate of aquatic OM. To what extent the observed AFOM in freshwater systems is defined by bacterial processing and how such processing may be influenced by nutrient availability are key knowledge gaps that need to be addressed. Previous research has observed the *in-situ* bacterial production of humic-like compounds in a laboratory model system with a high-nutrient and high-carbon content synthetic growth medium. This work describes a non-fluorescing, simulated freshwater matrix which is low in both nutrient and organic carbon concentrations. Using this model, growth curve incubation experiments have been undertaken over a 48-hour period with a monoculture laboratory strain of *Pseudomonas aeruginosa*. Microbiological and fluorescence analyses undertaken at regular time intervals demonstrate the bacterial production of humic-like OM (Peak C) under oligotrophic (after 8hrs) and simulated high-nutrient conditions (after 6hrs). These findings, albeit under laboratory conditions, are important as they show that this fluorescence region, currently viewed as allochthonous in origin, can also represent labile OM generated *in-situ* by bacteria and, furthermore, that this bacterial production increases as a function of nutrient loading. In addition, the data quantitatively demonstrates that fluorescence intensities increase independently of cell density. These results challenge the assumption that humic-like AFOM is exclusively terrestrial in origin and suggest that bacteria may “engineer”

OM *in-situ* that gives rise to these fluorescence characteristics as a function of metabolism. Importantly, nutrient availability is a key driver of metabolic activity, outlining the potential for the use of fluorescence as a marker for stream metabolism as opposed to a measure of bacterial numbers. Further development of the laboratory model via the utilisation of environmentally-sourced bacterial communities is required. Ultimately, this laboratory model will inform field studies that look to improve our understanding of how microbial communities respond to catchment stressors, and how these responses influence AFOM fluorescence signatures and ultimately the origin and fate of OM in freshwater systems.