

EXPLORING THE RECIPROCAL ROLE OF HYDRODYNAMIC FORCING AND MICROBIAL COLONIZATION TO INDUCE BED SEDIMENT PATCHINESS IN BIOSTABILIZATION

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The experimental project presented herein investigated feedback mechanisms in the complex flow-sediment-ecology tripartite relationship arising from the interactions between hydrodynamics, sediment transport and segregation, and microbial colonization and bed stabilization. The experiment was conducted in a groyne field constructed in the Total Environment Simulator located at “The Deep” facility at the University of Hull. Detailed measurements of flow hydrodynamics in the main channel and groyne embayments were obtained using acoustic doppler velocimeter (ADV) probes, while the redistribution and segregation of bed sediments were measured using an array of ultrasonic range sensors (URS), coupled with detailed laser bed scans and high-resolution photographic images. In terms of investigating biogrowth colonization and stabilization of these segregated sediments, a MagPI (Magnetic Particle Induction) device was used to measure biofilm adhesiveness at various bed locations and at various stages during the growth phase. Preliminary results from this study are presented herein.

1. INTRODUCTION

In the aquatic environment, sediment beds exhibit numerous textures, niches or habitats that are vital to support a variety of benthic life; in turn, these provide important ecosystem services such as regulating nutrient fluxes, purifying polluted waters or transferring carbon to higher trophic levels (Gerbersdorf et al. 2011). The pronounced patchiness observed in the substrate composition and structure is most likely a key characteristic for habitat diversity, biological settlement, bed functionality and ecosystem resilience to external forcing (Stal 2010). In this regard, bed patchiness needs consideration at different spatial scales, ranging from macro-scale structural and compositional arrangements within rivers (e.g. pebble clusters, bed forms, bed-load sheets, ribbons, point-bars, pool-riffle sequences, etc.) to micro-scale heterogeneity in bed substrate, biological colonization, biogeochemical fluxes, etc. Research into these sediment-ecology interactions has been termed “eco-hydraulics” or “eco-geomorphology” (Rice et al. 2010) and has, traditionally, been focussed upon these relationships for large-scale biology. This is somewhat surprising, as it is the smaller-scale microbial communities which are the first-colonisers of a disturbed substrate and likely dictate both sediment stability and subsequent colonization by higher order trophic levels (Characklis & Cooksey 1983, Flemming & Wingender 2010).

The composition, distribution and morphology of the substrate is reciprocally dependent on the hydrodynamic regime of the watercourse, with local turbulence characteristics relating to local bed roughness (e.g. Nezu and Nakagawa, 1993; Raudkivi, 1998) and vice versa. Thus, it is one of the most important variables to explain spatial pattern of biofilm growth (Paterson et al., 2000), given that the flow-sediment interplay determines the probability for micro-organisms to strike and attach permanently to individual sediment grains (e.g. Characklis and Cooksey, 1983). In this regard, micro-organisms commonly prefer fine grained sediments that exhibit high surface / volume ratios, surface loadings appropriate to facilitating attachment, typically high nutrient content, and are usually

deposited in low-energy waters (Meyer-Reil, 2005; Stal, 2003). However, rapid microbial colonization is commonplace even in very coarse-grained sediment beds exposed to higher shear velocities (e.g. Battin et al., 2003; Vignaga et al., 2012) and in extreme environments where macrophyte coverage is hampered (e.g. in the intertidal area, deeper midstream area, groyne fields). This has led recent research to seek explanation of preferences in colonisation, including the significant influence of substrate type over microbial settlement in terms of species composition, biomass and architectural appearance (Besemer et al., 2007; 2009; Haynes et al. 2011). Key to this research has been the process of colonization, whereby these microbes organize in multitaxa/species communities that are embedded in and attached to sediment particles by a self-secreted matrix of sticky polymers (biofilm) that act to virtually glue the sediment together; thereby enhancing the community's resistance to hydraulic forcing (e.g. Black et al., 2002; Gerbersdorf et al., 2008; 2009; Lubarsky et al., 2010; Righetti & Lucarelli, 2010). Whilst knowledge of this 'biostabilization' process is steadily improving, there remains a distinct knowledge gap with regards to colonization preference or mechanics towards substrate patchiness.

The development of strong substrate patchiness is especially found in high aspect ratio (width/depth) channels with lateral perturbations to the flow field that might be perpetuated by artificial structures, such as groyne fields. Groyne fields are important man-made structures in rivers, required to maintain navigation during low flow conditions and to serve as floodable reservoirs during higher discharges. Consequently, groyne fields are prevalent in many of the navigable rivers in the EU [there are about 6900 groynes along the middle section of the Elbe River (Schwartz, 2006)]. An important feature of groyne fields is the preferential sedimentation of finer materials within individual embayments due to reduced flow velocities (and hence shear stresses); under most flow conditions, a net import of sediments thus prevails in these regions (Yossef and de Vriend, 2010). Complex flow re-circulations within these groyne fields may also lead to an equally heterogenic pattern of substrate types with mobile coarse sands and fine gravels mainly found in the outer belt while finer sands and silts deposit in concentric circles increasingly towards the middle of the embayment (Schwartz & Kozerski 2004). While, sediment segregation and patchiness is likely to vary under different discharge conditions, it is currently unknown how biofilm growth within different sediment patches will impact on diagenetic processes and, thus, sediment re-mobilization and redistribution (Schwartz, 2006). Hence, groyne fields constitute the perfect fluvial environments within which to investigate these mutual feedback mechanisms between hydrodynamics, sediment transport, streambed morphology and biofilm growth that are largely unexplored at micro-scales.

2. EXPERIMENTAL SET-UP AND METHODOLOGY

The experiment was conducted in the 12 m-long by 6 m-wide by 1.5 m-deep Total Environment Simulator located at "The Deep" facility of the University of Hull. A regular arrangement of impermeable, exposed groynes with plan-form dimensions of 1.5 m-long by 0.44 m-wide was placed along both walls of the channel at a regular spacing of 2.1 m to create a symmetrical groyne field (see Figure 2.1). The channel bed comprised of a bimodal sand-gravel sediment mixture placed to a nominal layer depth of 0.15 m (locally 0.3 m along the leading edge of the groynes, see Figure 2.1). The channel was then filled to a flow depth of 0.15 m with nutrient-rich, filtered water obtained from "The Deep" aquarium, and a uni-directional steady flow was initiated along the channel. The experiment was conducted in three main phases: (1) sediment segregation phase under stepped (increasing) flow conditions; (2) biological growth phase in segregated bed under low flow conditions; and (3) biofilm break-up under high flow conditions.

In all phases, the mean and turbulent flow conditions were monitored in the main channel and within groyne embayments R3 and L3 during the experiment using 6 ADV probes (3 set at fixed z elevations, 3 set at fixed x locations – see Figure 2.2a). On the right side of the flume, a 2D PIV system was also set-up to measure XZ flow fields in embayments R3 and R4. During the initial sediment segregation phase, changes in bed surface elevation across the main channel and embayments L3 and R3 were monitored under the range of different flow conditions tested by an array of 12 ultrasonic range sensor (URS) probes (Figure 2.2a). On completion of the sediment segregation phase, a full topographic laser scan of bed surface was carried out on the drained, segregated sediment bed.

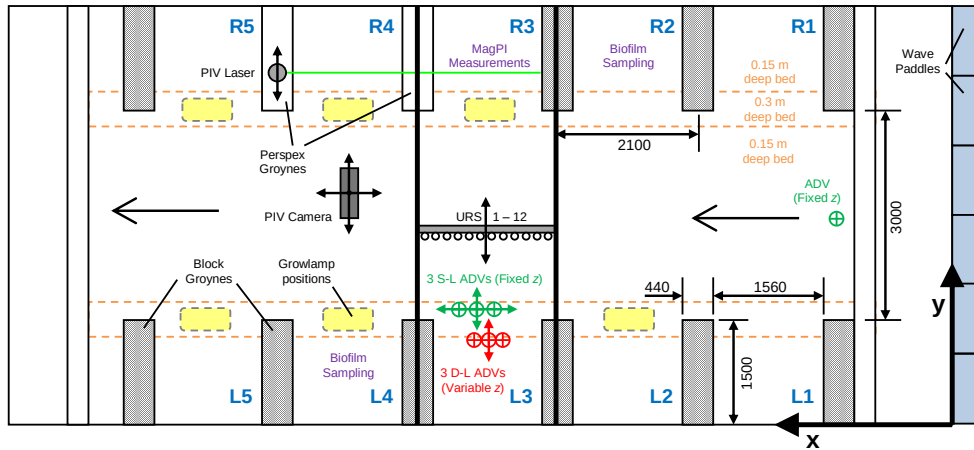


Figure 2.1: Schematic of general experimental layout and measurement locations (plan view)

During the subsequent bio-growth experimental phase, SunMaster FullNova grow-lamps, emitting a full and balanced spectrum of wavelengths close to that of natural sunlight, were positioned above embayments R2 – R5 and L2 – L5 to promote biofilm colonization and development. MagPI (Magnetic Particle Induction) measurements (Larson et al., 2009) were performed at regular intervals during this phase at a number of preselected locations to determine the development of biofilm adhesion. Additionally, biofilm growth was closely monitored by sampling the L4 and R2 embayments to measure chlorophyll (a proxy for microalgal growth), bacterial cell numbers and microbially-produced EPS (extracellular polymeric substances) proteins and sugars. Furthermore, biofilm samples were taken to monitor the microbial community composition under the different segregated bed sediment conditions developed within the groyne embayments.

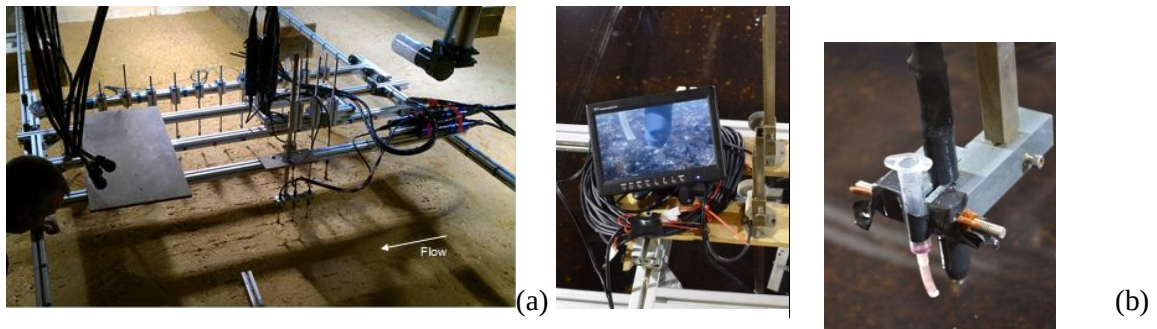


Figure 2.2: (a) ADV and URS probes for velocity and bed elevation measurements; (b) MagPI set-up showing monitor and traverse arrangement and close-up of electromagnet and particle pipette.

The test programme for the experimental study, along with the range of experimental flow conditions tested during each phase is shown in Table 2.1.

Table 2.1: Test programme showing main experimental phases and flow conditions

Phase	Week	Flow depth H [m]	Discharge Q [l/s]	Mean velocity $\bar{u}$ [cm/s]
Initial flume & bed set-up	1	-	-	-
Phase 1: Sediment segregation	2	0.1875	125 180 250	~40 ~50 ~63
Laser bed topography scan	2	-	-	-
Phase 2: Biofilm growth	3 – 7	0.12	34	~10
MagPI adhesion measurements	3 – 6	0.12	34	~10
Collection of bio-samples	3 – 6	0.12	34	~10
Phase 3: Biofilm break-up and floc measurements	8	0.12 – 0.16	50 – 200	~10 – ~55

3. EXPERIMENTAL RESULTS

3.1 Sediment Segregation (Phase 1)

ADV velocity measurements were taken at an array of embayment and main channel measurement locations for the highest flow rate tested ($Q = 250 \text{ l s}^{-1}$, Table 2.1) during the sediment segregation phase of the experiment. The raw ADV datasets obtained at each measurement location were processed using the WinADV post-processing software package and MATLAB, allowing the mean and fluctuating components of the flow velocity and turbulence characteristics to be observed from the main channel into embayment L3. Figure 3.1(a) plots the mean (u,v) velocity vector fields at L3, indicating the presence of the expected flow circulation or gyre forming within the embayment, due to flow separation at the upstream groyne. Here, the colour map shown represents the variation in velocity magnitude $R = (u^2 + v^2)^{1/2}$ between main channel and embayment L3. Figures 3.1(b)-(d) show the root-mean-square velocity fluctuations in the longitudinal u'_{rms} , lateral v'_{rms} and vertical w'_{rms} directions, respectively. These plots indicate the presence of unusual regular patterns of turbulence intensities [particularly for v'_{rms} and w'_{rms} , Figures 3.1(c) and (d)], which possibly result from wake interactions between the closely-spaced ADV probes. However, the plots generally indicate that the highest values of u'_{rms} and v'_{rms} typically occur in the regions of high shear vorticity (i.e. in the wake-generated, flow-separation region downstream of the groyne tip). By contrast, the largest vertical turbulent fluctuations w'_{rms} appear to occur along the upstream face of the downstream groyne [Figure 3.1(d)], most probably due to strong vertical flow circulations driven by the impact of flowing water on this upstream-facing groyne.

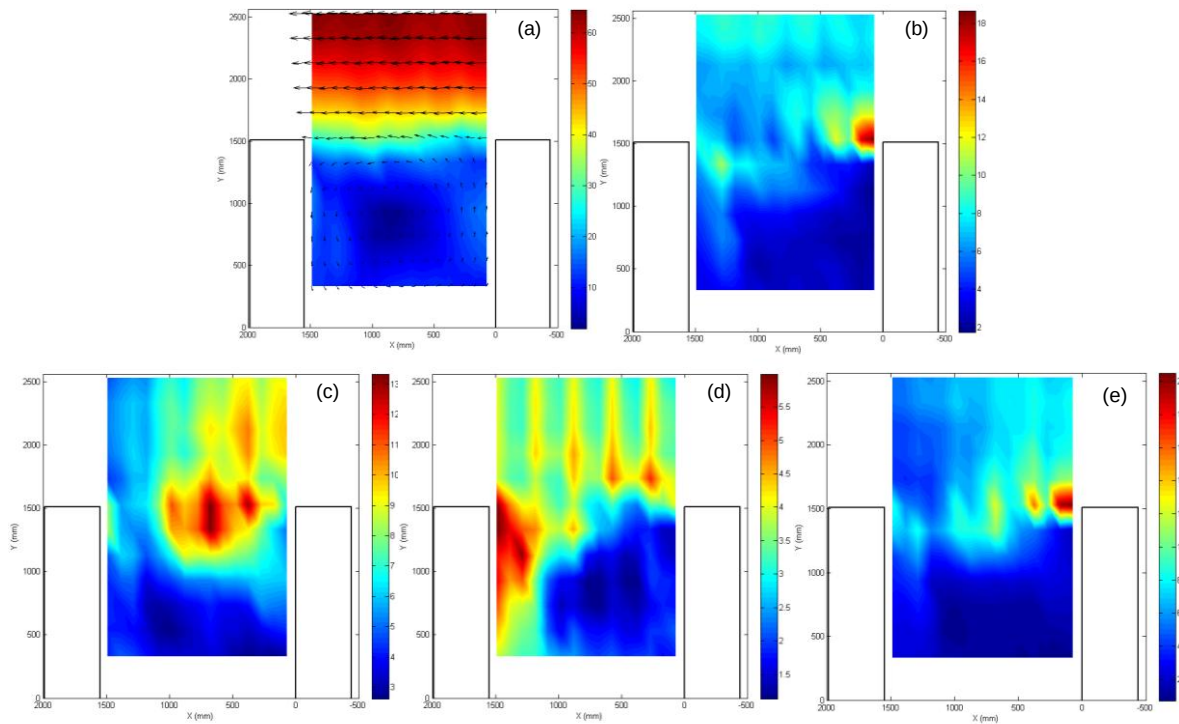


Figure 3.1: (a) (u,v) velocity vector field and colour map of velocity magnitude $R = (u^2 + v^2)^{1/2} \text{ (cm s}^{-1}\text{)}$; colour maps of (b) longitudinal turbulence intensity $u'_{rms} \text{ (cm s}^{-1}\text{)}$, (c) lateral turbulence intensity $v'_{rms} \text{ (cm s}^{-1}\text{)}$, (d) vertical turbulence intensity $w'_{rms} \text{ (cm s}^{-1}\text{)}$, (e) turbulent kinetic energy $\text{TKE} = \frac{1}{2}(u'^2_{rms} + v'^2_{rms} + w'^2_{rms})$. Flow direction from right to left.

Figure 3.2 shows the change in bed elevation Δz , measured by the URS probe array across the flume between embayments L3 and R3 at different times during the sediment segregation phase. The results indicated little bed elevation change occurred during the first two flow increments (i.e. $Q = 125 \text{ l/s}$ and 180 l/s) [$\Delta z = \pm 1.2 \text{ mm}$, Figure 3.2(a)]. However, subsequent bed elevation measurements at the highest flow rate ($Q = 250 \text{ l/s}$) [Figure 3.2(c)] indicated that significant deposition occurs within the embayments (Δz up to $+3.5 \text{ mm}$), with strong, localized erosion observed at the leading edge of the groynes (Δz up to -4.3 mm) and general erosion within the main channel (Δz up to -1.5 mm). Overall,

the erosion and deposition patterns also appeared to be largely symmetric between R3 and L3. This symmetry is also reflected in the observed segregation patchiness of bed sediment grading (e.g. sand; sand-gravel; gravel) and bed form distribution (e.g. sand-ripples) forming within the left and right embayments of the groyne field (e.g. R4 and L4, Figure 3.3).

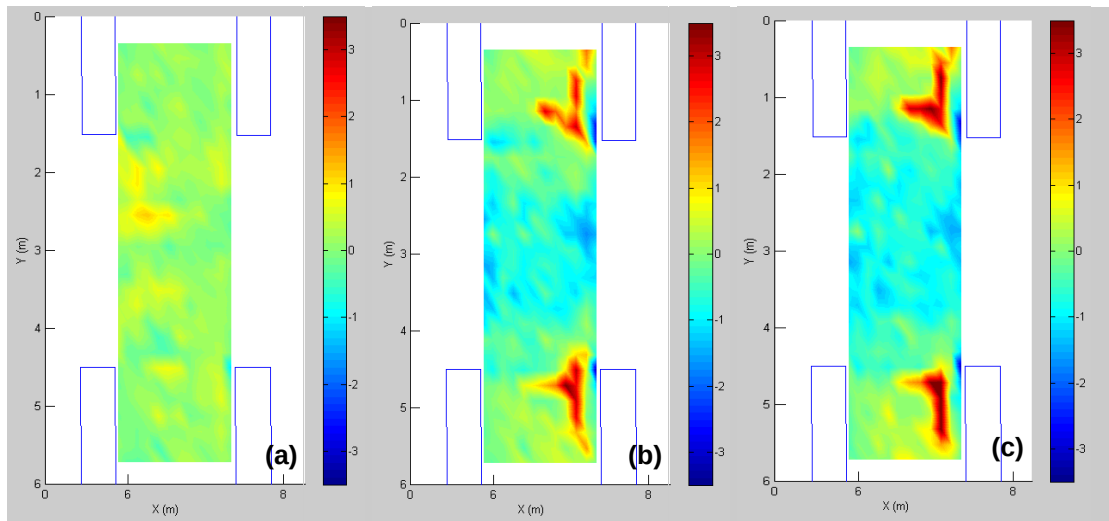


Figure 3.2: Measured URS bed elevation data: (a) after flow rates $Q = 120$ l/s and 180 l/s (02/07/2013 - 03/07/2013); (b) after flow rate $Q = 250$ l/s (04/07/2013, early am); (c) after flow rate $Q = 250$ l/s (04/07/2013, late pm). Colour bar scale shows bed elevation change (mm). Flow from left to right.



Figure 3.3: Symmetrical segregated sediment bed patterns in R4 and L4 embayments.

Direct comparison of the turbulent kinetic energy of the flow $TKE = \frac{1}{2}(u'^2_{rms} + v'^2_{rms} + w'^2_{rms})$ within embayment L3 [Figure 3.1(e)], sediment segregation in L4 (Figure 3.3), and the bed elevation change in L3 [Figure 3.2(c)] reveals that the sediment bed in high turbulent intensity regions (i.e. high TKE) is well scoured with an exposed gravel bed substrate (i.e. at outer edge of embayment and along the upstream groyne face). Conversely, corresponding regions of lower turbulence intensity (i.e. low TKE) typically correspond to regions of sand deposition or sand-gravel mixed substrates (i.e. further into the embayment). The different types of substrate conditions observed within the transition from main channel to embayment are summarised in Figure 3.4 and highlight the mutual dependence of sediment patchiness, with regions of net erosion and deposition, and flow areas of high and low turbulence intensity and TKE.

3.2 Biofilm Development (Phase 2)

The MagPI (Magnetic Particle Induction) device was used to measure biofilm adhesiveness. In this technique, electromagnetic particles are retrieved by an overlying electromagnet from the biofilm surface, the force required to remove these particles being equivalent to the adhesiveness of the biofilm surface; a proxy for sediment stability. Measurements were performed regularly within four fields of one embayment (R3, Figure 2.1), where each field has different substratum characteristics (i.e. *field 1* – a sand dune with measurements both at the luv and lee-side; *field 2* – normal bed height

and sand sediment; *field 4* – a sand/gravel mixture; and *field V* – gravel sediments in the main channel, see Figure 3.4). Approximately 42 MagPI measurements were conducted within *fields 1, 4, V* on each of seven sampling days (i.e. a total of 882 MagPI measurements obtained during the experiment). *Field 2* was measured at a higher resolution with 105 MagPI measurements per sampling day and a total of 735 over the entire experimental duration.

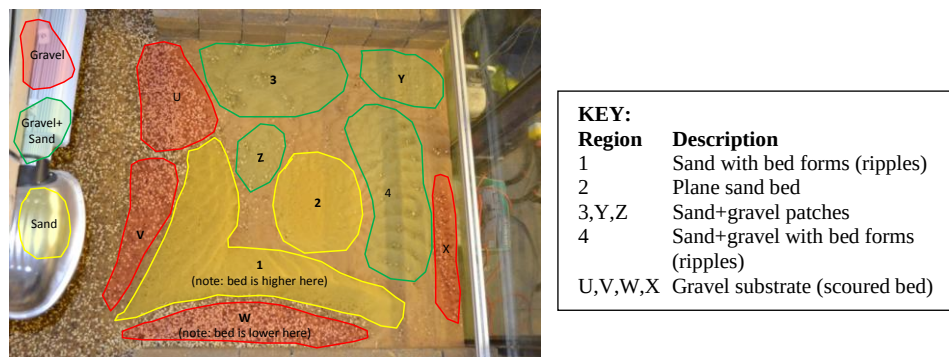


Figure 3.4: Regional characterization of different segregated sediment bed conditions for the MagPI measurements and sediment sampling for biological and chemical analysis.

Parallel to the MagPI measurements, biofilm growth was monitored regularly (twice a week), with investigations focusing on *fields 1, 2 and 4* (see Figure 3.4). Sediment samples were withdrawn (by cut-off 1 cm diameter syringes) from these fields for further biological and chemical analysis; thereby, five sediment mini-cores from each field were pooled. One subsample served for the determination of chlorophyll, as a proxy for microalgal biomass and according to DIN 38 412/16. Another subsample was fixed in 4% paraformaldehyde (final concentration) and ultrasonicated for 15 minutes (Sonopuls UW 3100 ultrasonic probe, Germany) to release bacterial cells from the sediment. The suspended bacterial cells were dyed with 1 µl of 500 µM SYTO 13 (Life Technology, Carlsbad, California, USA) for 15 minutes and counted using a flow cytometer (note: this procedure has still to be carried out). Extracellular polymeric substances (EPS) were extracted from a further subsample through the addition of distilled water, placement for 1 hour on a roller mixer, with the sample subsequently centrifuged to gain the supernatant [for additional details on this procedure, see Gerbersdorf et al. (2008)]. Triplicate samples from this supernatant were used for the photometrical analysis of EPS carbohydrates and proteins, respectively. Further subsamples were deep-frozen and will be used to extract DNA using the Nucleospin Kit for Soil (Macherey and Nagel, Düren, Germany) in order to determine diversity pattern of the microbial community. Therefore, the extracted DNA will be amplified by PCR and DGGE (Denaturing Gradient Gel Electrophoresis) will be performed using the Bio Rad DCode (Bio-Rad Laboratories, Hercules, California, USA) system essentially as described by Muyzer et al. (1993). The last subsample was used for microscopic determination of microalgal species: first, the sample has been fixed in Lugol solution (2%) to be divided in 2 samples: one for direct microscopic evaluation, the other has been prepared to remove all organics and embedded in Naphrax in order to allow the investigation of diatom frustules/determination of diatom species (first impressions obtained in cooperation with Dr. Jane Reed from Hull University; exact evaluation is currently in progress by Dr. Lydia King).

Biofilm development in the first week of phase 2 was probably dominated by bacteria (data not yet analysed) as the microalgal biomass begins to increase only within and after the second week. This confirms the visual observation of a thick, brown (fucoxanthin pigments of diatoms) mat that developed in this time and could be easily seen by eye. However, the steepest increase in microalgal biomass was observed between the last three sampling points (week 3 to 4, Figure 3.5). Over the whole experiment, the ratio between active and dead biomass (> 1 , between *chlorophyll a* and *pheophytin*) indicated a healthy flourishing community. Together, the values measured during the experiment were well within the data range known from pronounced biofilm mats in intertidal flats (e.g. de Brouwer & Stal, 2001).

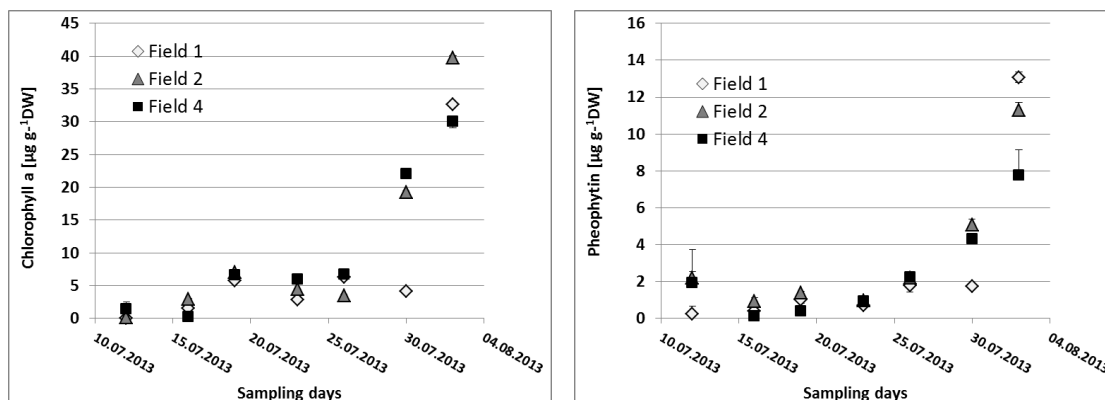


Figure 3.5: Pigment contents within the biofilm plus underlying sediment over the phase 2 duration for (a) chlorophyll a (living microalgal biomass) and (b) pheophytin (dead microalgal biomass). The data series correspond to different fields / substratum types sampled within one embayment.

It was expected that the microalgal community developing within sandy and coarse sediment fields would consist mainly of attached, epipsammic forms with decreasing abundances towards more coarse sediment substrates. However, there were no notable differences in abundances between *fields 1, 2 and 4* (see Figure 3.6) and the algal community consisted mainly of epipelagic forms that usually move between particles. The diversity of algal species was also not nearly as high as would be expected from a microphytobenthic mat. This was presumably due to the high nutrient load in the Deep aquarium water that allowed only the growth of nutrient tolerant species. Diatoms such as *Amphora coffeaeformis*, *Cocconeis spec.*, *Nitzschia apiculata*, *N. palea*, *N. pusilla*, *N. reversa* and *Stauronella spec.* were detected, but all different substrata were dominated by *Nitzschia palea* (Figure 3.6), particularly between the middle and the end of the experiment. The latter species is known from highly eutrophicated waters (as is *N. apiculata*), while *Amphora*, *N. pusilla* and *Stauronella* are typical brackish to saline species, widespread along the European coasts.

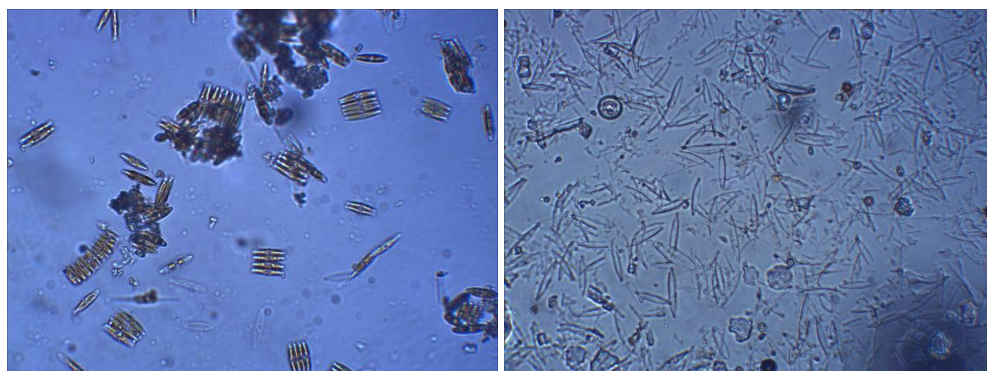


Figure 3.6: Microscopic images (magnification 400) of (a) dominant species *Nitzschia palea* in “live” form, and (b) the Naphrax embedded diatom frustules with the dominant species *Nitzschia palea*. (Courtesy: Dr Jane Reed, University of Hull).

Generally, there was also a significant increase in EPS content over the duration of phase 2 (Figure 3.7) with up to 30 times more proteins and carbohydrates on the last sampling day as was measured on the first, again with the steepest increase observed between weeks 3 and 4. As expected, the sugar content was significantly higher than the protein content (by around 2 – 3 times) and the values detected are again well within the range of the published data on microphytobenthic mats in intertidal areas (e.g. de Brouwer and Stal, 2001).

Overall, the biofilm adhesiveness (measured by MagPI) increased during phase 2 of the experiment (see Figure 3.8). Compared to the 0.3A reference level for abiotic non-biostabilized sediment, adhesiveness was up to 4 times greater over the first 5 days and up to 12 times greater over 3 weeks, with strong morphological implications. After an initial peak (around day 7), the biofilm adhesiveness decreased in all fields before rising significantly again and to reach the highest biostabilization at the

end of phase 3 (i.e. week 3 – 4, Figure 3.8). It is hypothesized that adhesiveness would have continued its development leading to even higher biostabilization effects for a longer duration growth phase.

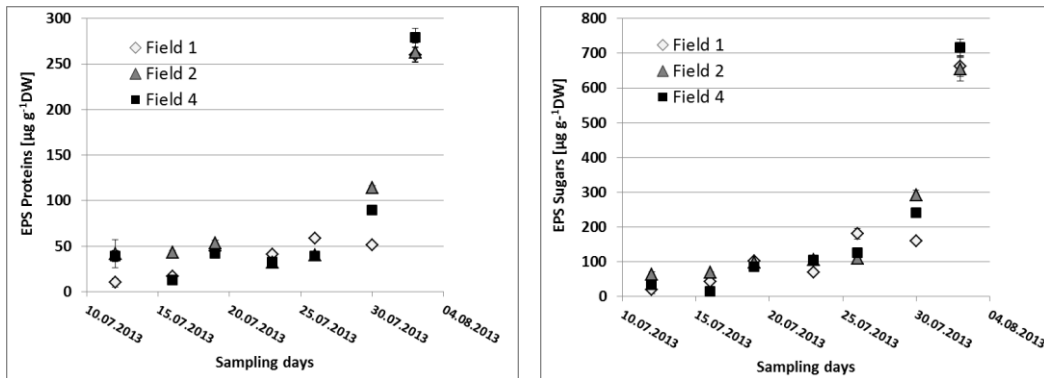


Figure 3.7: EPS content within the biofilm (plus underlying sediment) over phase 2 duration for (a) protein content and (b) polysaccharides content. The data series correspond to different field / substratum types sampled within one embayment.

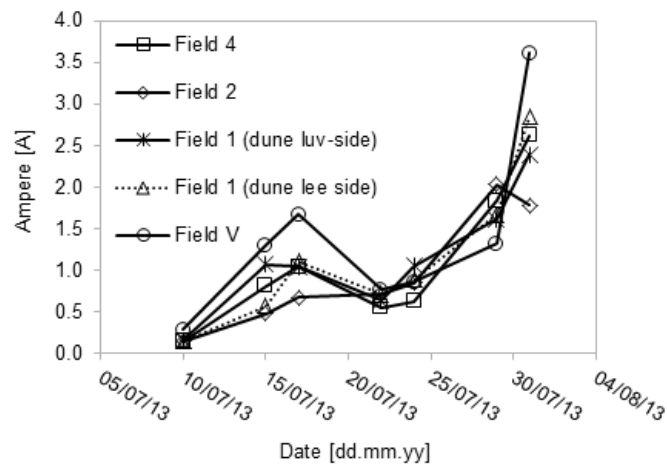


Figure 3.8: Development of adhesiveness measured by MagPI over the duration of phase 2.

4. DISCUSSION

4.1 Statistics and Relations

All single biological and chemical parameters thus far evaluated (note: bacterial cell numbers are not yet included) show significantly different and increasing values over time (all $p < 0.0001$, Kruskal Wallis test). By contrast, these values were shown not to vary significantly between the substrate conditions (i.e. *fields 1, 2 and 4*, Figure 3.4) for all parameters tested. However, there was a tendency demonstrated for *chlorophyll a* values to be highest in *fields 2 and 4* (and lowest in *field 1*), whereas MagPI measured biofilm adhesiveness revealed the opposite trend. This may provide a hint as to the contribution of bacteria to biostabilization. Since EPS values were consistently highest in field 2, where the stabilization values tended to be lower, this also indicates that the quantity of polymeric is not as influential in biostabilization as previously thought. The only significant difference ($p < 0.0001$) was detected for MagPI data between the fields 1, 2 and 4, as opposed to *field V* where the highest values of adhesion were measured. This implies that the greatest biofilm strength is obtained at the main channel / groyne embayment interface, which is exposed to higher hydrodynamic shear stress conditions. Unfortunately, the biological and chemical data for *field V* could not be evaluated.

EPS proteins and sugars were both significantly related ($p < 0.0001$, Pearson) to each other and showed the same temporal development, although the values for sugars were approximately 3 times higher than for proteins, as explained above. Moreover, the microalgae biomass was significantly related to both EPS components, as well as to MagPI measurements on adhesiveness ($p < 0.0001$, Pearson). The MagPI datasets were also significantly related to both EPS proteins and sugars;

indicating interrelations between microbial biomass, their secreted EPS polymers and the adhesiveness of the biofilm.

4.2 Influence of Local Substrate and Hydrodynamics

One of the main aims of the Hydralab experiment was to gain better understanding of the interrelation between local hydrodynamics, sediment substrate type and biofilm growth. Unfortunately, the finer detail of these physical controls on biofilm strength development appears to have been masked, to some extent, by the dominant influence of high nutrient loading from the aquarium water used in the experiment. Overall, however, the strongest biofilms were observed to grow in *field V*, although this could be attributed either to (i) the high flow condition and TKE (see Figure 4.1) at the outer edge of the embayment, or (ii) the coarse gravel substrate in this field. (Note: biological data was not available for equivalent gravel bed regions with lower flows or TKE values, i.e. along upstream face of groyne). For bed areas within the embayment with predominantly sandy substrates, *fields 1* and *4* were shown to have slightly higher biological strength than *field 2* (Figure 3.8), suggesting that lower mean flow velocities [*field 2*, Figure 4.1(a)] typically result in lower biological strengths. However, again as the data focussed on *fields 1*, *2* and *4*, it is difficult to determine whether substrate size was important in biofilm growth and the current analysis of *field V* fails to conclusively determine whether substrate size is more or less influential than local hydrodynamics effect demonstrated in *fields 1*, *2* and *4*. In summary, is it entirely possible that the high nutrient levels, quasi-universal high growth rates and relatively low flow conditions set-up during the growth phase essentially yield a null conclusion for the importance of sediment substrate type within the embayment. It is interesting to note that over the duration of the growth phase the mean velocity magnitude R and turbulent kinetic energy TKE were shown to reduce by 7.6% and 18.9%, respectively, within the embayment due to this biofilm growth.

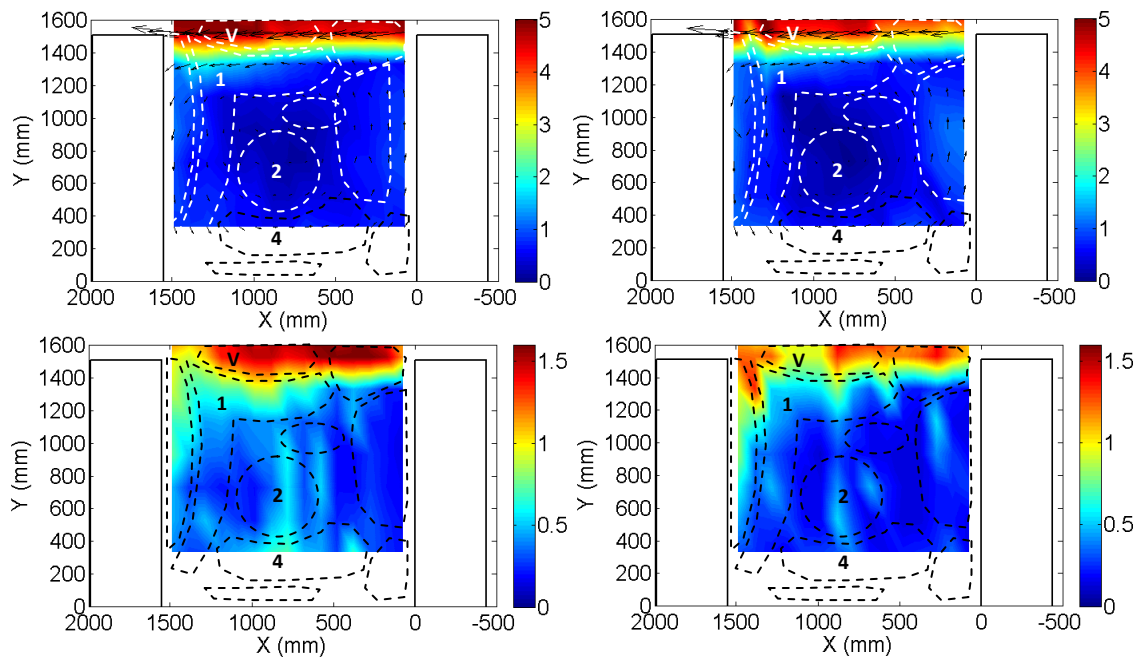


Figure 4.1: ADV measurements with side-looking probes at (i) beginning and (ii) end of biofilm growth phase. Results shown are (a) (U,V) velocity vector field in embayment and velocity magnitude $R = (U^2 + V^2)^{1/2}$ colour map; and (b) turbulent kinetic energy $KE = \frac{1}{2} (u'^2 + v'^2 + w'^2)$ colour map.

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