Surface properties influence marine biofilm rheology, with implications for ship drag

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Supplementary Information

All data supporting this study will be available from the University of Southampton Repository at: https://doi.org/10.5258/SOTON/D2439

Surface topography of marine biofilms

To assess the surface topography of the biofilms grown on different coupons Optical Coherence Tomography (OCT) was implemented. OCT 3D-scans were exported as .oct files into MATLAB. Custom scripts were produced by Stefania Fabbri (1) and are similar to those employed by Blauert (2).

Roughness Coefficient (R_a*)

To calculate surface roughness coefficient (R_a*) the following equation, based on Murga et al., (3) was used:

$$R_a *= \frac{1}{N}\sum_{i=1}^{N}\frac{|L_{F,i}-\bar{L}_F|}{\bar{L}_F} \quad \text{[eq. S1]}$$

Where, i indicates a single A-scan and N represents the total number of A-scans in a B-scan. Simply, the equation is based on biofilm thickness; if a biofilm is relatively homogenous with few variations from the mean thickness, then R_a* will be close to 0. Alternatively, a higher R_a* indicates a heterogenous biofilm with numerous variations from the mean biofilm thickness.

Average thickness (mm)

Average biofilm thickness (mm) was calculated using individual B-scans within a C-scan:

$$\bar{T}_{B-scan} = \frac{1}{N}\sum_{N}^{j=1} T_{A-scan,j} = \frac{1}{N}\sum_{N}^{j=1}(z_b - z_c)_j \quad \text{[eq. S2]}$$

Where, $T_{A-scan,j}$ is biofilm thickness at a single point, j (A-scan) within a B-scan, where N is the total number of A-scans (j's) within the B-scan. Thickness was measured in each A-scan by subtracting coating position, z_c from biofilm position, z_b. Then, overall average biofilm thickness within a C-scan ($\bar{T}_{C-scan}$) could be calculated by taking the average biofilm thickness across all B-scans within the C-scan.

Percent coverage (%)

The percent coverage (%) of the biofilms were measured as follows (1):

$$\text{Coverage}(\%) = \frac{A_b}{A_s} \text{ x } 100 \quad \text{[eq. S3]}$$

Where, A_b is the area covered by a biofilm and A_s is the imaging area (9 mm^2).

Supplementary Figures

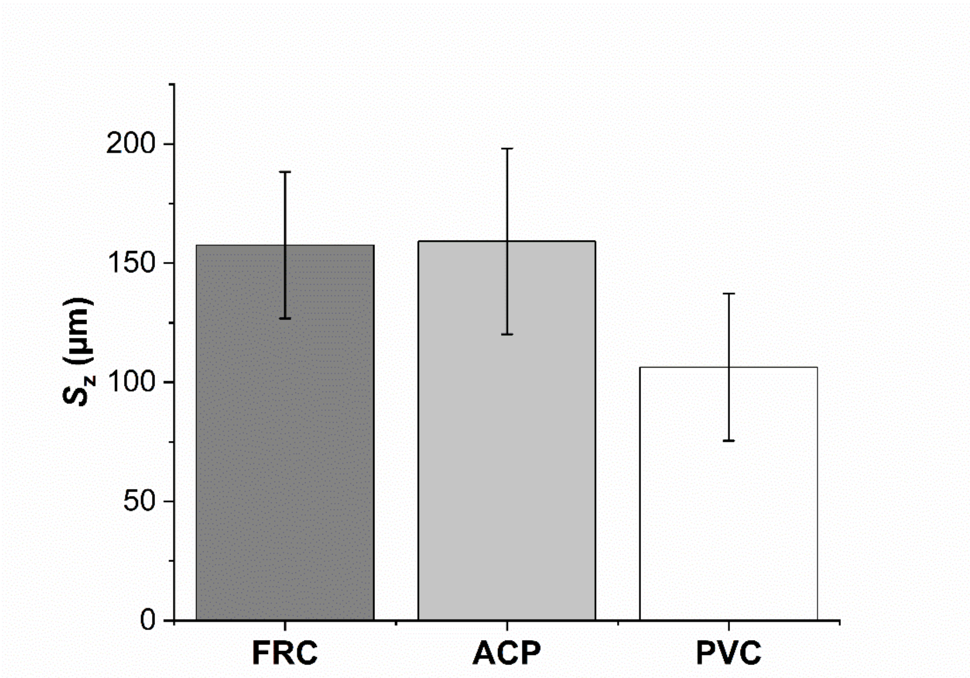

Figure S1. A Blue light interferometer (MikroCAD premium, LMI technologies) was used to confirm that coating surface roughness would not influence the marine biofilm physico-mechanics. Single point scans (20 mm x 27 mm) with a cut-off wavelength of 5 mm were taken of the different surfaces. Mean peak-trough roughness height, S_z (µm) is presented as mean ± SD (n = 4). Statistical analysis confirmed that the FRC, ACP and PVC coupons all had a comparable surface roughness (ANOVA, $P > 0.05$).

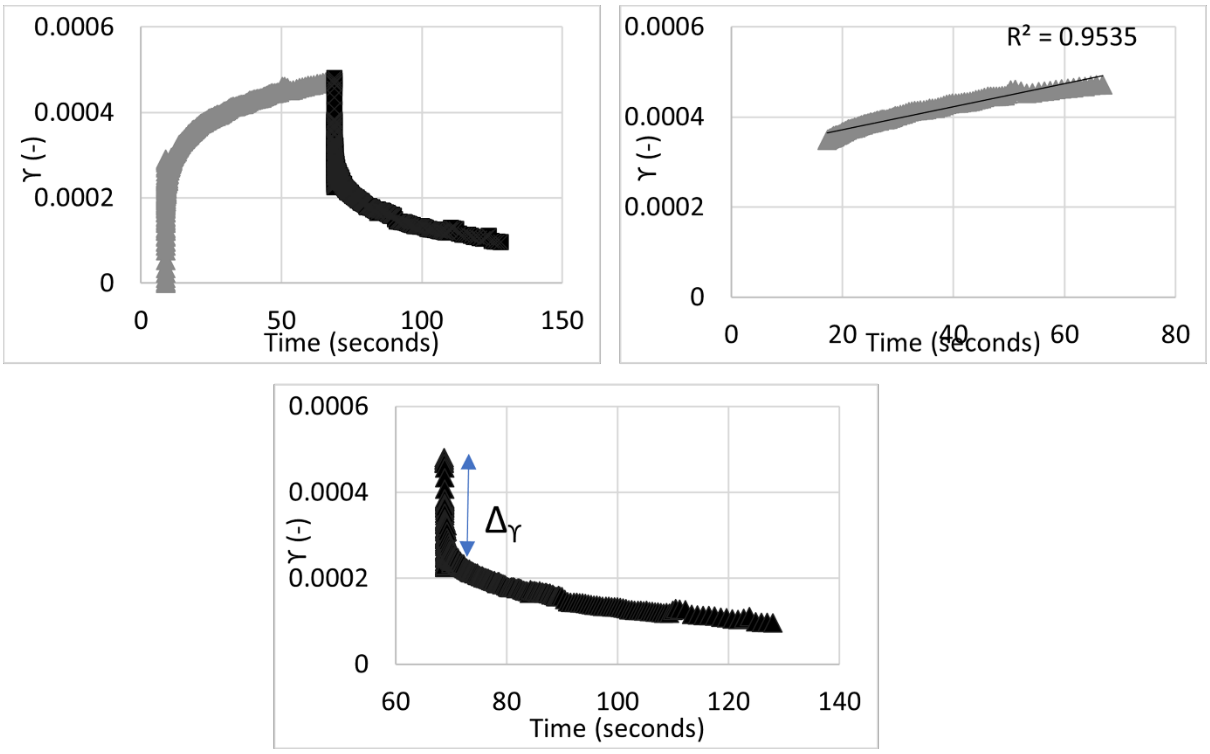

Figure S2. (a) Representative creep-recovery data for a PVC replicate, where grey is the creep portion of the experiment and black is the recovery, (b) η was calculated using the linear viscous region during creep. The linear viscous region was taken as the part of the slope where $R^2 > 0.95$. (c) G was calculated using the length of the elastic recovery response ($\Delta\gamma$). The elastic response is the measured using the immediate vertical drop in strain that occurs when stress is removed.

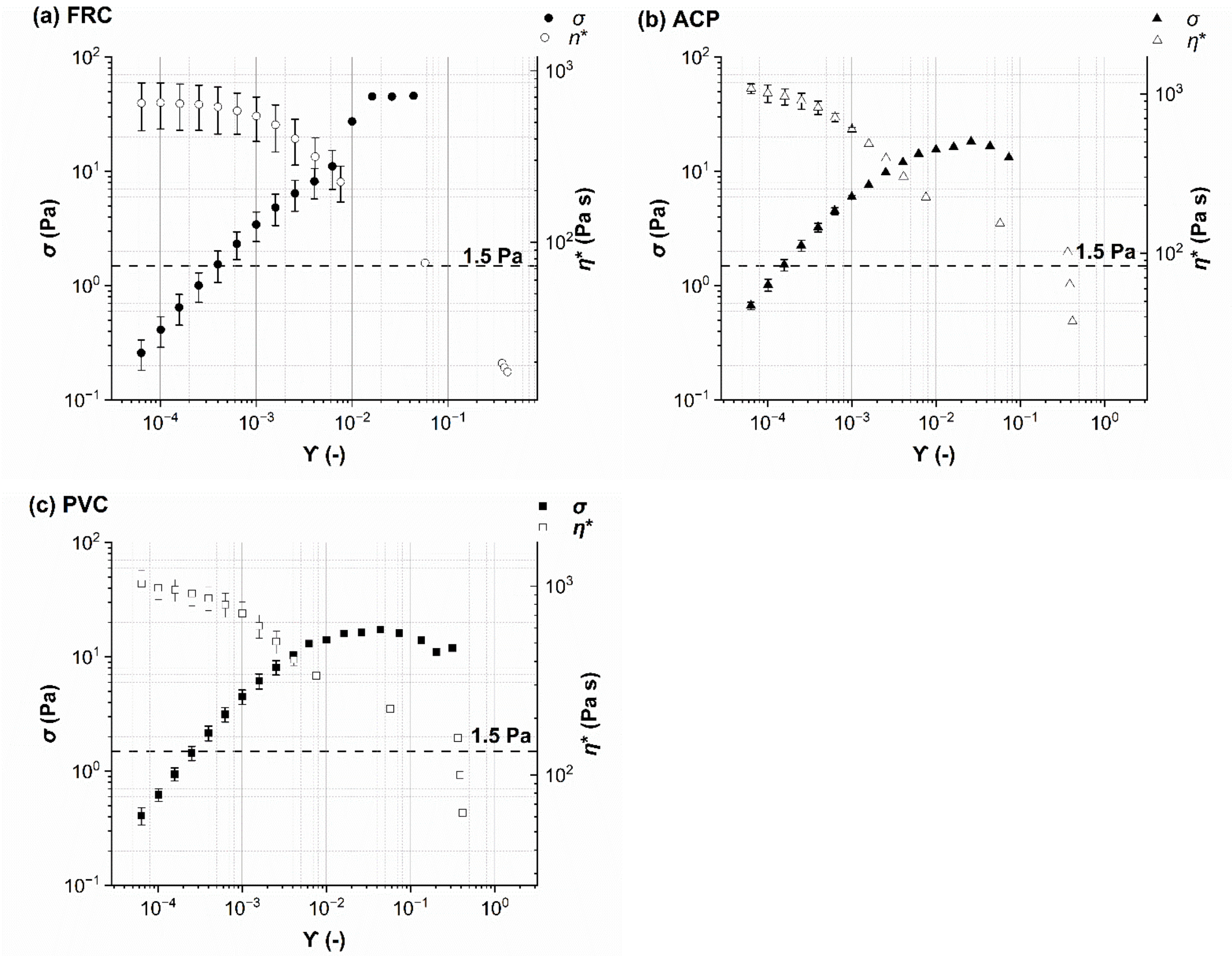

Figure S3. Stress, σ (Pa) and complex viscosity, η^* (Pa s) *vs.* against strain, γ (-) for: (a) FRC, (b) ACP and (c) PVC. Data is presented as mean ± SD. A horizontal line has been added at 1.5 Pa to show that this was within the LVR for all coupons and was therefore used for further analysis.

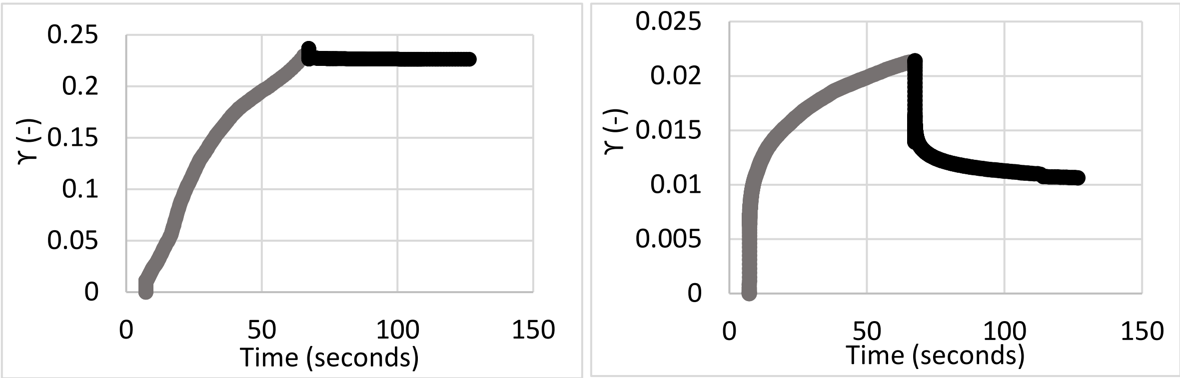

Figure S4. Creep recovery data for: (a) FRC (b) ACP biofilms tested at a shear stress of 20 Pa. This shear stress was out of the LVR for both biofilms which react very differently. The FRC biofilm shows very little elastic response and no recovery once the stress was removed which is characteristic of a liquid. The ACP biofilm, however, shows evidence of some permanent deformation caused by a viscous-dominated response to shear, but there is still evidence of some elastic recovery.

<u>**References for Supplemental**</u>

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2. Blauert F. Investigating biofilm deformation using optical coherence tomography and fluid-structure interaction simulation [Internet]. 2017 [cited 2020 Apr 9]. Available from: http://wasserchemie.ebi.kit.edu/

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