Rheology of skin mucus from yolk-sac salmon fry

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ABSTRACT

As part of our project "Microbial contributions to the Atlantic salmon (*Salmo salar*) skin mucosal barrier" we have explored techniques to investigate and characterise the rheological properties of the skin mucus of yolk-sac salmon fry. Here we describe the use of axial oscillation (or oscillatory squeeze flow[1]) to investigate rheology of small volumes of mucus.

INTRODUCTION

Skin mucus forms a primary barrier between fish and the aquatic environment, and as with all mucus secretions, mucus rheology is central to function. The yolk-sac salmon fry is small, and the intact skin mucus is thus ideally suited to *in situ* particle tracking microrheology studies, but we have an additional interest in characterising the macrorheology under applied deformation to gain a more complete understanding of the mucus rheology. Here the small skin surface area, and hence small volume of mucus available for testing provides a challenge. Oscillatory shear rheology measurements suffered from poor signal to noise ratio and it was challenging to obtain reliable and reproducible data. By utilizing the rheometer (Malvern Kinexus Ultra+) in axial oscillation mode we were able to obtain satisfactory deformation and response curves that indicated that the skin mucus of the yolk-sac salmon fry shows a range of typical mucus like behaviours.

METHODS

A Malvern Kinexus Ultra+ rheometer fitted with 20 mm diameter parallel plates was cooled to 8°C. A sequence was designed to sequentially run scripts to oscillate the normal force motor at a designated amplitude and frequency under constant temperature control while collecting raw data (gap and normal force). A micropipette was used to gently remove skin mucus (70 µl per fish) from the body of freshly euthanised fish moving slowly towards the tail taking care not to contact the delicate epithelium with the pipette tip. Mucus was placed directly onto the lower rheometer plate and the upper geometry lowered to a gap of 0.2 mm (theoretical volume of 63 µl) before running the sequence described above. Gap and normal force data were collected at a data rate of 200 and converted to strain and stress for further analysis. Experiments were carried out in a laboratory with temperature and humidity control and sample drying was found not to be a problem at the experimental temperature and timescale.

RESULTS

Example results obtained at a frequency of 1 Hz and strains of 5 and 10 % are shown here. In axial oscillation mode the driven sinusoidal strain wave resulted in a corresponding approximately sinusoidal waveform in the normal force signal with acceptable signal to noise ratio (Figure 1).

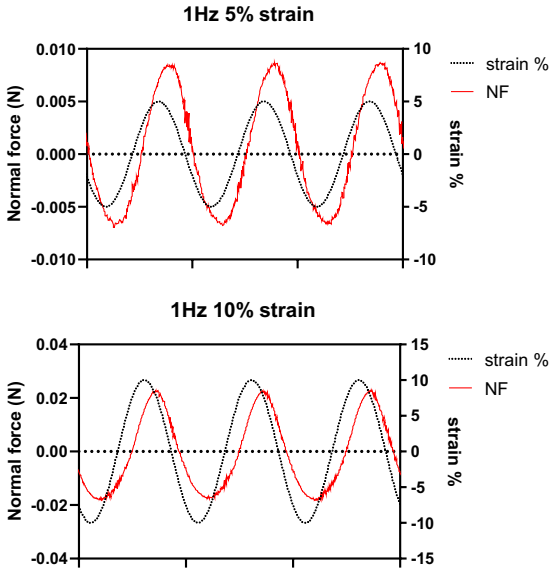

Figure 1. The strain and normal force signals over 3 oscillatory cycles at a frequency of 1 Hz and strain amplitudes of 5 and 10 %.

Over 30 seconds (30 cycles) the amplitude of the normal force curve shows some variation, tending towards greater amplitudes at 5 % strain and smaller amplitudes at 10 % strain (Figure 2).

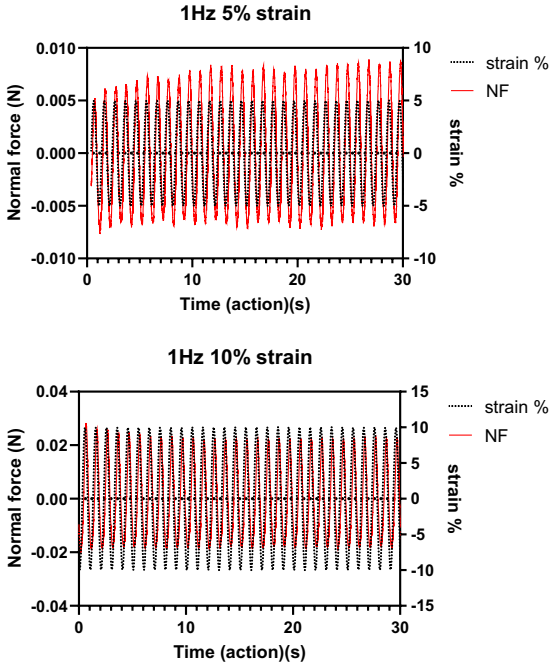

Figure 2. Stability of the normal force amplitude over 30 seconds.

This may reflect a general dynamic variability in the sample, inherent hardening or softening behaviours that are common to mucus samples[2-4], or potentially in the case of 10 % strain there may be a squeeze flow effect that leads to an effective reduction in the volume of mucus between the plates over consecutive strain cycles, thus lowering the total response signal from the material.

Non-linear behaviour in mucus, including hardening and softening behaviour often becomes obvious when we examine the Lissajous curve of stress against strain during the oscillatory cycle. Whilst in oscillatory shear we expect such curves to have rotational symmetry as the forward and back direction are essentially equivalent, the same is not true in the case of axial oscillation where compression (or loading) is fundamentally different to extension (or unloading)[5,6]. In this case the mucus is inherently sticky and forms a good contact with both the upper and lower geometry during the whole oscillatory cycle, so we use the term extension rather than unloading. If we plot the Lissajous curve for the final 3 cycles of the 5 % strain test we can see that the curve is relatively symmetrical although the maximum force in compression (top right quadrant) is larger than the maximum (negative) force in extension (lower left quadrant) (Figure 3). To assess potential additional non-linearities we can use the method of Ewoldt *et al*[7] to investigate the minimum-strain modulus or tangent modulus at γ=0 and the large-strain modulus or secant modulus evaluated at the maximum imposed strain. As we are working in axial rather than shear mode, we term these E'_M (minimum strain modulus) and E'_L (large strain modulus) and we differentiate between the compression ($E'_{M\,cp}$ and $E'_{L\,cp}$) and extension ($E'_{M\,ex}$ and $E'_{L\,ex}$) portion of the curve. In this case we see only slight variations between the 4 moduli plotted as gradients in Figure 3, indicating that 5 % strain at 1 Hz is around

the limit of the linear viscoelastic region for this sample.

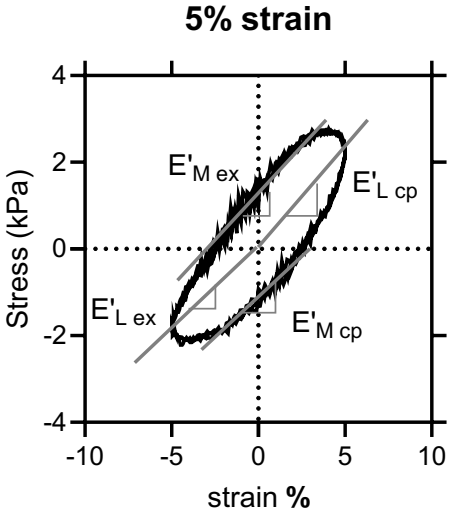

Figure 3. Lissajous plot for the final 3 oscillation cycles at maximum 5 % strain showing the gradients for the calculation of the moduli $E'_{M\ cp}$, $E'_{L\ cp}$, $E'_{M\ ex}$ and $E'_{L\ ex}$.

When the maximum strain amplitude is increased to 10 % the non-linearity of the behavior becomes pronounced, particularly in compression, with the gradient $E'_{L\ cp}$ being substantially larger than $E'_{M\ cp}$ indicating intracycle strain hardening in compression (Figure 4).

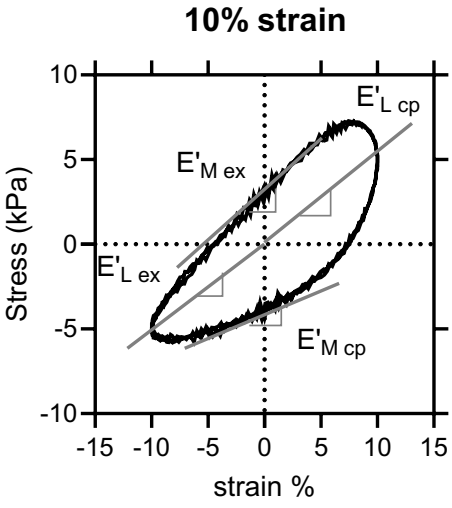

Figure 4. Lissajous plot for the final 3 oscillation cycles at maximum 10 % strain.

showing the gradients for the calculation of the moduli $E'_{M\ cp}$, $E'_{L\ cp}$, $E'_{M\ ex}$ and $E'_{L\ ex}$.

Given that the behaviour at 10 % strain is clearly out of the linear range we can consider that experiments under these conditions should be appropriately termed large amplitude oscillatory compression of LAOC measurements. Given the intracycle strain hardening it is tempting to assume that the reduction in total normal force response with repeated cycles may be due to the previously mentioned squeeze flow effect leading to an effective reduction in the volume of mucus between the plates over consecutive strain cycles. However, such intracycle hardening has previously been shown to occur concomitantly with bulk softening in mucus, so these data should be interpreted cautiously.

CONCLUSION

The use of axial oscillation rheology may provide useful rheological data for small sample volumes.

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REFERENCES

1.Bell, D., D. M. Binding, and K. Walters. "The Oscillatory Squeeze Flow Rheometer: Comprehensive Theory and a New Experimental Facility." *Rheologica Acta* 46, no. 1 (Oct 2006): 111-21.

2.Ewoldt, R. H., C. Clasen, A. E. Hosoi, and G. H. McKinley. "Rheological Fingerprinting of Gastropod Pedal Mucus and Synthetic Complex Fluids for Biomimicking Adhesive Locomotion." *Soft Matter* 3, no. 5 (2007): 634-43.

3.Nordgard, C. T., and K. I. Draget. "Dynamic Responses in Small Intestinal Mucus: Relevance for the

Maintenance of an Intact Barrier." *European Journal of Pharmaceutics and Biopharmaceutics* 95 (Sep 2015): 144-50.

4. Taylor, C., K. I. Draget, J. P. Pearson, and O. Smidsrod. "Mucous Systems Show a Novel Mechanical Response to Applied Deformation." *Biomacromolecules* 6, no. 3 (May-Jun 2005): 1524-30.
5. Kim, J. H., K. H. Ahn, and S. J. Lee. "Model Prediction of Non-Symmetric Normal Stresses under Oscillatory Squeeze Flow." *Korean Journal of Chemical Engineering* 29, no. 8 (Aug 2012): 1010-18.
6. Xu, Y. G., R. Tao, and G. Lubineau. "Nonlinear Viscoelasticity of Pre-Compressed Layered Polymeric Composite under Oscillatory Compression." *Composites Science and Technology* 162 (Jul 2018): 188-97.
7. Ewoldt, R. H., A. E. Hosoi, and G. H. McKinley. "New Measures for Characterizing Nonlinear Viscoelasticity in Large Amplitude Oscillatory Shear." *Journal of Rheology* 52, no. 6 (Nov-Dec 2008): 1427-58.