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Title: Macro-Rheology Characterization of Gill Raker Mucus in the Silver Carp, *Hypophthalmichthys molitrix*

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **17**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Kartik Bulusu**: The significance of this protocol is that it allows an accurate rheological characterization of non-Newtonian, biological fluids such as mucus, especially when only very small sample volumes are available **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Michael Plesniak**: This technique facilitates the characterization of the apparent yield stress and viscoelasticity of complex structured biological fluids such as gill raker mucus **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Kartik Bulusu**: This method enables the yield stress estimation of gill raker mucus and facilitates the rheological characterization and protocol development of similar biological fluids, such as human, animal, and plant secretions **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.4. **Michael Plesniak**: Demonstrating the procedure with Kartik Bulusu will be Samantha Racan, a graduate student from our laboratory **[1][2]**.

1.4.1. INTERVIEW: Author saying the above
1.4.2. The named demonstrator(s) looks up from rheometer workbench and acknowledges the camera

Protocol

2. Mucus Solution Preparation

2.1. To prepare approximately 2 milliliters of 100 milligrams per milliliter concentrated fish mucus solution, perform serial dilution of 2 milliliters of 400 milligrams per milliliter concentration by adding deionized water in 1-to-2 ratio, twice **[1-TXT].**

2.1.1. WIDE: Talent adding water to mucus Videographer: Important step **TEXT: Mucus extraction was performed by L. Patricia Hernandez, Biological Sciences Dept, GWU before the protocol step.**

2.2. Place the vial of mucus solution onto a shaker **[1]** until the solution is adequately homogenized **[2]** and any mucus particulate agglomeration has been mitigated **[3]**.

2.2.1. Talent turning on shaker/vial shaking

2.2.2. Shot of homogenized solution 100 milligrams per milliliter solution.

2.2.3. Shot of the table with the excised gill raker and mucus solutions of various concentrations with concentration labels visible in frame (Our videographer, Kevin McRoberts took a few extra shots that captured the excised gill raker and the mucus vials). **NOTE: Use as 2.1.2.**

3. Rheometer Calibration and Preparation

3.1. To calibrate the rheometer, launch the rheometer instrument control software **[1]** and select the **Calibration** and **Instrument** tabs **[2-TXT]**.

3.1.1. WIDE: Talent launching software, with monitor visible in frame

3.1.2. SCREEN: 3.1.2: 00:23-00:34 **TEXT: Repeat calibration x3**

3.2. Click **Calibration** and **Calibrate** to ensure that the calibration values are within 10% of each other and click **Accept [1-TXT]**.

3.2.1. SCREEN: 3.2.2: 00:00-00:51 *Video Editor: please speed up and please emphasize Inertia: 21.346 microN m.s.2 and green check row when it appears*

at about 00:37 **TEXT: Record instrument inertia calibration values in micronewtons/meters/seconds squared**

3.3. Turn the shaft on top of the rheometer to install the cone angle geometry **[1]**. The software will detect the 40-millimeter diameter, 1-degree, 0-minute, 11- second cone angle geometry **[2]**.

3.3.1. Talent turning shaft and installing the cone angle geometry *Videographer: Important step*

3.3.2. SCREEN: 3.3.2: 00:25-00:36

3.4. To perform the rheometer geometry calibration, click **Calibration** under **Inertia** and click **Calibrate [1]**. Click **Accept** to accept the calibration **[2]**. Click **Calibration** under **Friction** and click **Calibrate [3]**. Click **Accept** to accept the calibration. The geometry inertia and friction calibration values should be recorded in the appropriate units **[4-TXT]**.

3.4.1. SCREEN: 3.4.1: 00:04-00:29 *Video Editor: please speed up*

3.4.2. SCREEN: 3.4.1: 00:29-00:37 *Video Editor: can speed up*

3.4.3. SCREEN: 3.4.1: 00:38-01:14 *Video Editor: please speed up*

3.4.4. SCREEN: 3.4.1: 01:14-01:22 **TEXT: Repeat calibration x3**

3.5. To perform the zero-gap initialization, click **Gap** and **Options**. In the popup window, set the **Axial Force** to 1 Newton and click **OK**. Then click the **Zero Gap** icon **[1]**. The initialization is complete when the axial force experienced by the geometry becomes greater than or equal to 1 Newton as it touches the Peltier plate **[2]**.

3.5.1. SCREEN: 3.5.1: 00:08-00:40 *Video Editor: please speed up*

3.5.2. SCREEN: 3.5.2_Modified *Video Editor: please emphasize value becoming about 1N at 00:03 by placing a box around the axial force value located under "control panel" to the right of the screen.*

3.6. To ensure that the rheometer gap is zeroed so that its reference position is accurate, use the up and down arrows to raise the geometry to any arbitrary height **[1]**.

3.6.1. Talent using Up and Down arrows on the instrument to raise and lower the geometry

3.7. The control screen on the rheometer instrument **[1]** and the control panel of the rheometer instrument control software will both display the gap height **[2]**.

3.7.1. Shot of control screen showing gap height

3.7.2. SCREEN: 3.7.2: 00:06-00:10 *Video Editor: please emphasize/zoom to control panel area indicated by mouse*

4. Linear Viscoelastic Range (LVR) Rheological Analysis

4.1. To perform the rheological experiment in the linear viscoelastic range of a mucus solution of interest, load approximately 300 microliters of the fish mucus solution into a 1-milliliter pipette tip **[1-TXT]** and load the solution onto the Peltier plate **[2]**.

4.1.1. WIDE: Talent loading pipette *Videographer: Important/difficult step* **TEXT: e.g., here 100 mg/mL mucus solution used**

4.1.2. Talent adding mucus to Peltier plate *Videographer: Important/difficult step*

4.2. Press the **Go to Trim Gap** icon to lower the geometry onto the Peltier plate **[1-TXT]**, use the pipette to remove any excess mucus solution while applying a small angular velocity to the motor **[2]** and, click the **Stop** and **Apply** icons until the torque value reaches minimum **[3]**.

4.2.1. Talent pressing trim gap button **TEXT: 40 mm-diameter, 1º 0′ 11″ cone angle geometry trim gap = 28 micrometers. NOTE: Screen capture.**

4.2.2. Mucus being removed *Videographer: Important step*

4.2.3. Talent clicking "stop" and "apply" icons until torque value reaches min **NOTE: Screen capture.**

4.3. To perform oscillation amplitude experiments, in the **Procedures** menu of the **Experiments** tab, set the **Oscillation and Amplitude** and set the **Temperature** to 22 degrees Celsius. Set the **Soak Time** to 120 seconds and check **Wait for Temperature [1]**.

4.3.1. SCREEN: 4.3.1: 00:31-00:49 *Video Editor: please speed up* **TEXT: e.g., 22 °C, recorded temp at fishing site**

4.4. Set the **Frequency** to 1 hertz, the **Torque** to 10-10,000 micronewton meters, and the **Points per decade** to 10 **[1]**.

4.4.1. SCREEN: 4.3.1: 00:50-01:10 **TEXT: e.g., 1 Hz, estimated silver carp mastication**

4.5. Next, click the **Insert Duplicate Step** icon. Change **Amplitude** to **Frequency [1]**. Set the **Temperature** to 22 degrees Celsius and set the **Soak Time** to 0 seconds as necessary **[2]**.

4.5.1. SCREEN: 4.4.1: 00:00-00:10

4.5.2. SCREEN: 4.5.1: 00:03-00:10

4.6. Set the **Strain percent** to 1. In the **Logarithmic Sweep** menu, set the **Frequency** to 20 to 1 hertz and the **Points per decade** to 10 **[1]**. To set up the **Flow Sweep** procedure, click the **Insert Duplicate Step** icon and then change **Oscillation** to **Flow** and select **Sweep** to set the **Temperature** to 22 degrees Celsius and set the **Soak Time** to 0 seconds as necessary **[2]**.

4.6.1. SCREEN 4.6.1: 00:00-00:32 *Video Editor: please speed up* **TEXT: Strain % determined to fall within gill raker mucus linear viscoelastic regime**

4.6.2. SCREEN: 4.7.1: 00:08-00:27 *Video Editor: please speed up*

4.7. Set the **Shear rate** to 1-10,000 per second and the **Points per decade** to 10 and check the **Steady state sensing** box **[1]**. Press the **Go to Geometry Gap** icon **[2]** to lower the geometry to the preset suitable gap per specific geometry **[3-TXT]** and click **Start** in the instrument software **[4], and observe the motion of the cone geometry [5]**.

4.7.1. SCREEN: 4.8.1: 00:02-00:14

4.7.2. Talent pointing to geometry settings tab with Trim and Truncation Gap and pressing the button

4.7.3. Geometry being lowered **TEXT: Geometry gap for 40 mm diameter, 1° 0′ 11″ cone angle geometry fixed at 24 micrometers NOTE: Shot together with 4.7.2.**

4.7.4. Talent pressing Start, with monitor visible in frame

4.7.5. Added shot: Shot of the oscillating cone geometry on the Peltier plate of the rheometer (Our videographer Kevin McRoberts took this additional shot during our filming session)

4.8. A real time plot that reports the loss and storage moduli will be generated by the rheometer in the main window **[1]**. Right-click to select the **Graph Variables** tab and set the x-axis of the plot to **Oscillation strain percentage [2]** to view the data of interest **[3]**.

4.8.1. SCREEN: 4.10.1: 00:10-00:15

4.8.2. SCREEN: 4.11.1: 00:08-00:20 *Video Editor: please speed up*

4.8.3. SCREEN: 4.11.1: 00:21-01:28 *Video Editor: please speed up*

4.9. To view the angular frequency data, right-click **Frequency sweep** and select **Send to New Graph [1]**.

4.9.1. SCREEN: 61379_4.11.2_Plot2.mp4: 00:02-00:10

4.10. To view the viscosity and shear stress data in real time, right-click **Flow sweep** and select **Send to New Graph [1]**.

4.10.1. SCREEN: 4.11.3_Plot3_Modified: 00:03-00:10

4.11. Then save the file that contains both the experimental procedure and results in the native file format of the rheometer instrument control software **[1]**.

4.11.1. SCREEN: 4.12.1: 00:03-00:20 *Video Editor: please speed up*

4.12. For graphical analysis, export the mucus concentration data into a spreadsheet **[1]** and run supplemental codes to generate plots of the apparent viscosity for varying shear strain rates of the loss modulus, storage modulus, and phase angle for varying oscillation stresses **[2]** and to generate graphs of the results **[3]**.

4.12.1. SCREEN: 4.13.1: 00:03-00:17 *Video Editor: please speed up*

4.12.2. SCREEN: 4.13.2. *Video Editor: please speed up*

4.12.3. SCREEN: 4.13.3: 00:02-00:32 *Video Editor: please speed up*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.

2.1., 3.3., 4.1., 4.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
4.1. To ensure the success of finding the linear viscoelastic region, careful analysis of the generated plot is used to determine the region. Region selected must be a linear portion of the log-log plot generated.

Results

5. Results: Representative Gill Raker Mucus Marco-Rheology Characterization

5.1. As observed in this representative analysis **[1]**, the storage modulus of the 200 milligram per milliliter concentration decreased **[2]** and crossed over the loss modulus **[3]** within the stress range **[4]**.

5.1.1. LAB MEDIA: Figure 7B
5.1.2. LAB MEDIA: Figure 7B *Video Editor: please emphasize magenta data points*
5.1.3. LAB MEDIA: Figure 7B *Video Editor: please emphasize blue data points*
5.1.4. LAB MEDIA: Figure 7B *Video Editor: please add/emphasize dashed lines with arrows and text*

5.2. The phase angle data for this concentration **[1]** demonstrated a steady transition to viscoelastic liquid with a transitional region of yield stresses **[2]**.

5.2.1. LAB MEDIA: Figure 7B *Video Editor: please maintain emphasis as for 5.1.4.*
5.2.2. LAB MEDIA: Figure 7B and 7E *Video Editor: please add/emphasize vertical dashed lines and horizontal dashed lines with arrows and text in Figure 7E*

5.3. Modulus data for the 400 milligrams per milliliter concentration **[1]** demonstrated a yielding phenomenon **[2]** with a crossover point **[3]** between the storage and loss moduli that occurred after a sharp decline in the loss modulus **[4]**.

5.3.1. LAB MEDIA: Figure 7C
5.3.2. LAB MEDIA: Figure 7C *Video Editor: please emphasize magenta and blue data points from left side of graph to vertical dashed line*
5.3.3. LAB MEDIA: Figure 7C *Video Editor: please emphasize dashed line*
5.3.4. LAB MEDIA: Figure 7C *Video Editor: please emphasize magenta and blue data points from dashed line to end of graph.*

5.4. The phase angle data for the 400 milligrams per milliliter concentration **[1]** demonstrated a sharp transition to viscoelastic liquid with a crossover point at approximately 0.27 Pascals **[2]**.

5.4.1. LAB MEDIA: Figure 7C *Video Editor: please maintain emphasize as for 5.3.4.*
5.4.2. LAB MEDIA: Figures 7C and 7F *Video Editor: please emphasize vertical dashed line and dark orange arrows and text in Figure 7F*

5.5. The 100 milligrams per milliliter concentration **[1]** exhibited a constant viscosity **[2]** and a negligible flat region **[3]** and behaved as a Newtonian fluid **[4]**.

5.5.1. LAB MEDIA: Figure 8
5.5.2. LAB MEDIA: Figure 8 *Video Editor: Please emphasize red data line and grey and tan shaded regions in Figure 8A*
5.5.3. LAB MEDIA: Figure 8 *Video Editor: please emphasize the red data line in Figure 8B*
5.5.4. LAB MEDIA: Figure 8

5.6. The 200 milligrams per milliliter concentration demonstrated non-Newtonian behavior and a propensity to yield **[1]** with a pronounced flat region **[2]**.

5.6.1. LAB MEDIA: Figure 8 *Video Editor: Please emphasize blue data line in Figure 8A*
5.6.2. LAB MEDIA: Figure 8 *Video Editor: please emphasize blue data line in Figure 8B*

5.7. The 400 milligram per milliliter concentration **[1]** is the closest to the gill raker mucus composition, exhibiting a clear change in the state of the mucus from gel-like to a shear thinning fluid after yielding **[2]**.

5.7.1. LAB MEDIA: Figure 8 *Video Editor: please emphasize black data line in Figure 8A*
5.7.2. LAB MEDIA: Figure 8 *Video Editor: please emphasize black data line in Figure 8B*

Conclusion

6. Conclusion Interview Statements

6.1. **Kartik Bulusu**: As outlined in the protocol manuscript, it is important to determine the oscillation strain percentage value within the linear viscoelastic regime of the gill raker mucus before running dynamics sweeps **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (Step 4.1., 4.5.)

6.2. **Samantha Racan**: Our protocol can be used to ascertain the apparent yield stress of sticky and gel-like biological fluids and can be extended to “tack and peel” tests for a full characterization of the adhesivity of mucus-like materials **[1]**.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

6.3. **Michael Plesniak**: This protocol paves the way for the hydrodynamic investigation of filter feeding with mucus-like materials, the creation of analytical models, and the advancement of crossflow and membrane filtration technologies **[1]**.

6.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera