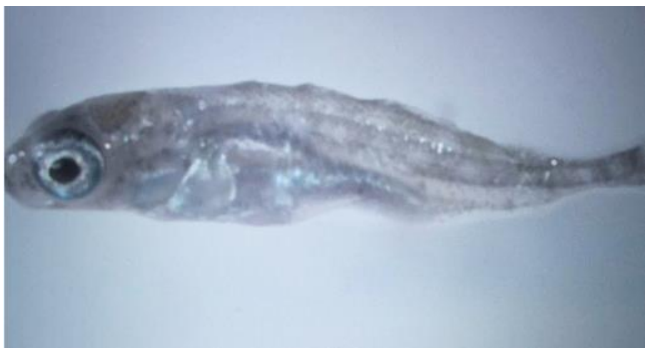
Generation of Stickleback intestinal organoid protocol

Arshad A. Padhiar[1], Xihao Huang[1], Daniel Bolnick[1]

1. Department of Ecology and Evolutionary Biology, University of Connecticut; 75 North Eagleville Rd, Storrs CT 06269 USA

Figure 1

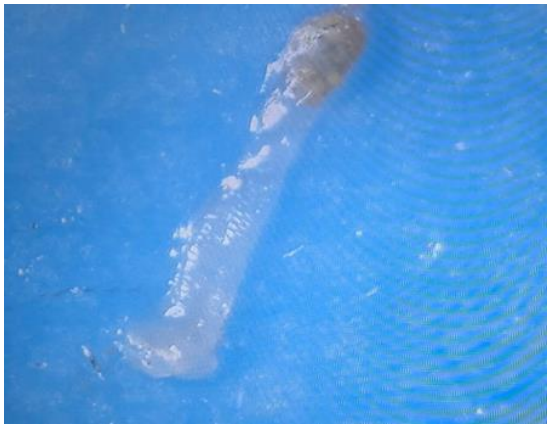


Euthanized juvenile stickleback positioned on a 2% agar plate under a dissection microscope. The translucent agar provides optimal contrast for visualizing the intestinal tract

Figure 2

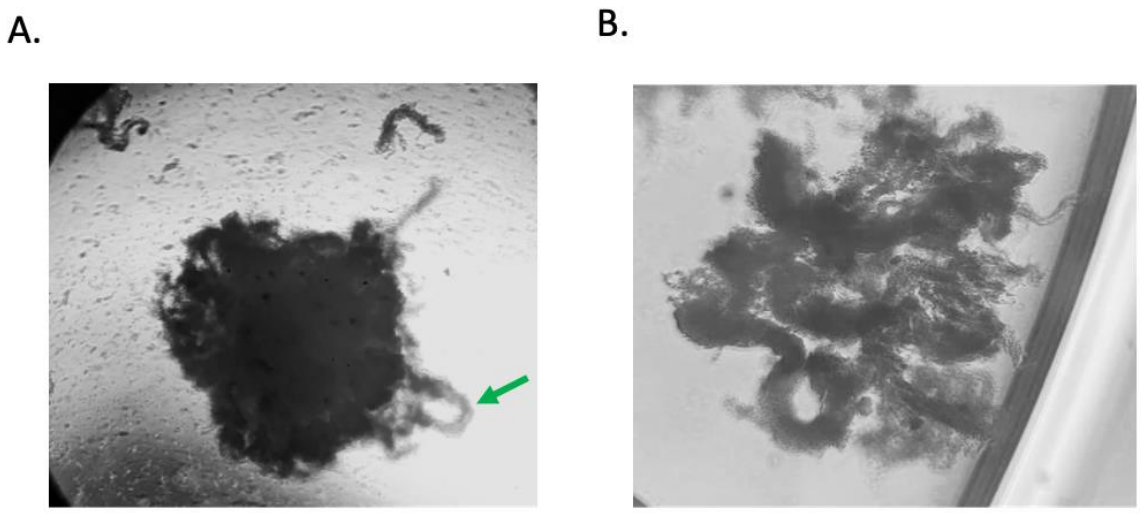
The panel shows the intact intestinal tract being carefully extracted from a 35 dpf stickleback larva under a dissection microscope. The gut has been pulled proximally towards the head after midline incision.

Figure 3



The isolated intestinal tract of a juvenile stickleback following removal of fecal contents by squeezing with closed tweezers

Figure 4



A) Primary intestinal organoids derived from juvenile stickleback, cultured for 5 days. The structures display characteristic 3D cystic morphology with evident crypt-like protrusions (arrowheads), indicative of preserved epithelial polarity and budding activity. **(B)** Crypt domains have detached from the organoid and initiated autonomous growth outside the Matrigel matrix. The central lumen is surrounded by a continuous epithelial monolayer, from which budding crypt-like domains are emerging, indicating preserved epithelial polarity and proliferative capacity