

Fishes that suck: comparison of the adhesive discs of three fishes of the Pacific Northwest

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Abstract:

There are more than ten different species of fishes in the Pacific Northwest with ventral adhesive organs that facilitate adhesion to marine substrates. The performance of these adhesive discs have been measured in three species. These include the Northern clingfish (*Gobiesox maeandricus*), Pacific spiny lump sucker (*Eumicrotremus orbis*), and tide pool snailfish (*Liparis florae*). While the clingfish that lives in the intertidal zone where waves continually crash down, it is the other two who have been shown to have stronger discs. Scanning electron microscopy and photography of cleared and stained individuals was used to look for differences in morphology that might explain these performance differences. High speed videography was used to determine the mechanism of detachment for *E. orbis* and *L. florae* because this is unknown. All three species have different disc morphology (Fig. 1); *E. orbis* and *L. florae* have much larger papillae than *G. maeandricus* and a more rigid support to their disc which may help to explain the difference in disc performance. Papillae may increase friction which prevents the disc from slipping and rigid pelvic spines resist bending of the disc. Both *E. orbis* and *L. florae* seem to use abduction of their pelvic fins and operculum to brace themselves as they pull back the anterior edge of their disc, causing failure and allowing the disc to be peeled back in the anterior direction. Modeling discs based off these morphologies could be done to create a strong, bio-inspired suction cup for human use.

Introduction:

In the Pacific Northwest, there are many different species of fish that have ventral adhesive organs that allow them to adhere to different surfaces in the marine environment

(Lamb & Edgell, 1986). There are many fish in the families Liparidae and Cyclopteridae that have these discs, as well as the family Gobiesocidae. The latter family includes the Northern clingfish, *Gobiesox maeandricus*. This fish has been previously been shown to be very good at sticking to rough surfaces, even better than it sticks to smooth surfaces such as glass (Wainwright *et al.*, 2013). It has also recently been shown that these same fish are also very good at sticking to fouled substrates; substrates covered in biofilms and invertebrates and other small organisms. On these surfaces, clingfish can still support nearly 150 times their own body weight (Ditsche *et al.*, 2014). Despite their ability to stick to this variety of rough substrates, previous studies have found that other species with adhesive discs actually perform better than the clingfish. The tide pool snailfish, *Liparis florum*, has been found to tolerate higher levels of stress than the clingfish on substrates of varying roughness (Wainwright & Summers, unpublished). It has also been shown that *Eumicrotremus orbis*, the Pacific spiny lumpsucker, has a much stronger adhesive disc compared to the clingfish on a smooth surface. *E. orbis* can support a maximum of nearly 850 g/cm² of disc area; this is nearly 400 g more than the clingfish which maxes out just under 454 g/cm² (Arita, 1967).

This difference in performance may seem strange initially, as it is the clingfish that live in the intertidal zone where the surf is constantly crashing and splashing upon the rocks where the clingfish lives. The lumpsucker and snailfish live near the shoreline, but in deeper water where they forces they must deal with are that of currents and the flow of tides instead of crashing waves. The first goal of this study was to look and see what was it morphologically about the discs of these three species could be used to relate back to this difference in adhesive disc performance. When a fish with an adhesive disc

sticks to a surface, it must have some mechanism to release itself and move around in its environment. Clingfish have an interesting mechanism of release in which the fourth pelvic lepidotrich is rotated about ninety degrees. When this fin abducts, a channel is opened between the anterior and posterior parts of the disc and water will flow in allowing the disc to fail (Summers, personal communication). However, the mechanism of release in lumpsuckers and snailfish has never been documented. The second goal of this study was to try and determine how these fish manage to detach themselves from the substrate.

Methods:

Imaging –

Fishes were collected while night-lighting off the dock at the Friday Harbor Laboratories on San Juan Island, Washington. Capture occurred between 10pm and 1am with a dip net when fish swam up to the light. Two *Eumicrotremus orbis* and *Liparis florum* were euthanized via submersion in a concentration of 0.5 g/L MS 222 for 15 minutes.

Specimens were then bled out to prevent any tissue damage via freezing. Standard length, weight, and the two axis of the disc were recorded; the formula for the area of an ellipse, $A = \pi * r_1 * r_2$, was used to describe the adhesive disc's area as their discs are slightly elongate. Specimens were then fixed in 10% formalin for an hour, let to sit in water for an hour, then put through a series of ethanol washes: 50%, 75%, 95%, 100% at an hour in each solution. The Samdri – 790 Critical Point Dryer (Rockland, Maryland) was used to dehydrate the specimens with carbon dioxide; these were twice sputter coated with gold in the SPI Sputter (West Chester, Pennsylvania). Two pre-prepared *Gobiesox maeandricus* specimens were used that had been previously prepared by

another student, Dylan Wainwright, in 2012. A tabletop NeoScope JCM 5000 tabletop scanning electron microscope, SEM, (Melville, New York) was used to image specimens. For the lumpsuckers and snailfish, large pads were counted on the disc and averaged over all specimens in the lab (*E. orbis* n = 6, *L. floriae* n = 7). For all species the average diameter of pads (n = 30) and their microstructure, papillae and microvilli (n = 30), and were determined using ImageJ (National Institutes of Health, <http://imagej.nih.gov/ij/>). Cleared and stained specimens were obtained from the museum in Lab 8 at the Friday Harbor Laboratories. A Zeiss Discovery V.20 stereo microscope with AxioCam HRc was used to image the bone structure of these fishes.

Videography –

One *E. orbis* and one *L. floriae* were filmed using high speed videography to attempt to determine how they detach from the substrate. A dark green food dye was injected into the water surrounding the fish to visualize where the disc failed during detachment. Fishes were left to settle in a high rimmed glass petri dish with seawater being exchanged every five minutes until the fish settled and a detachment event was recorded. Fish were transferred back to a sea table after filming. *E. orbis* was filmed at fifty frames per second and *L. floriae* at sixty frames per second with a Trouble Shooter model LE500MS Fastec high speed video camera (San Diego, California). Videos were processed using QuickTime Player and edited using Photoshop CC 2014.

Results:

Imaging –

The SEM imaging reveal that the microstructures of the discs of these three species are quite different. Table 1 summarizes some of the differences between these species seen

in Figure 2. In *Eumicrotremus orbis*, the rim of the pad is surrounded by two layers of fimbriae. The inner disc rim has 13-16 (n=6) large pads with smaller pads between the rim of fimbriae and larger pads. *Gobiesox maeandricus* only has fimbriae around its posterior edge. The rest of the rim of the disc is surrounded by pads of papillae that break up into microvilli; these pads increase slightly in diameter towards the center of the disc. *Liparis florae* has no fimbriae or small pads. Its large pads, which number from 13-15 (n=6) are covered only in papillae without microvilli that are slightly smaller in diameter than those geometric papillae of *E. orbis*. The center of the discs of these fishes are also quite different; both *G. maeandricus* and *L. florae* have a more porous surface epithelium while *E. orbis* has a much smoother epithelium with very obvious holes or pores in its surface (Fig. 3).

The osteology of these adhesive discs is not consistent between each of these species. While *E. orbis* and *L. florae*'s discs are supported by calcified pelvic fin rays from the pelvic girdle, the disc of *G. maeandricus* has cartilaginous supports that extend to the edge of the disc in addition to its pelvic lepidotrichia (Fig. 4).

High Speed Videography –

The high speed video taken of the detachments of *Eumicrotremus orbis* and *Liparis florae* seem to reveal a much different mechanism than what is used by the *Gobiesox maeandricus*. The flow of the dye shows that in both species, the disc tends to fail at its anterior end and the flow of dye is always towards the posterior end. Detachments are usually accompanied by an expansion of the opercula and pectoral fins which appear to be involved in helping fail the anterior edge of the disc which then allows for a peeling off of the disc in the posterior direction (Fig. 5). It can also be seen in the video of *L.*

florae that the water under the disc appears to be evacuated just before detachment which would make failing the disc easier (Fig. 6).

Discussion:

The SEM images of the discs of these three species show that there are in fact a different organization of the adhesive disc in these fish as well as differences in the microstructure that makes up these discs. Both *Liparis florae* and *Eumicrotremus orbis* discs are more similar to each other than either is to the *Gobiesox maeandricus* adhesive disc. This seems logical as the Liparidae and Cyclopteridae families are only one node from each other as opposed to the Gobiesocidae which are much farther away on the tree published by Near *et al* (2012). There is nothing obviously apparent about the arrangement of pads on the discs that would seem to make one disc stronger than the others. The size of the papillae that make up the disc could be used to explain the differences in adhesive performance between *L. florae*, *E. orbis*, and *G. maeandricus*. In *L. florae* and *E. orbis*, the papillae are much larger than the microvilli on the surface of the pads of *G. maeandricus*. This means that there is a lot more surface area in contact with the surface the disc has adhered to. When the fish is pulled away from the substrate, either by currents or curious scientists, the disc will fail when the edges slip towards the middle. One way to prevent the disc from failing is by increasing the frictional forces where the disc is in contact with the surface. Large sized papillae have more surface area which means they should have more friction when pulled over a surface than the microvilli of the clingfish. While the size of the papillae on the surface of the pads on the adhesive discs are larger in both the lumpsucker and snailfish, there is still area between the papillae where mucus might be located to help form a tight seal on the substrate. The

small size of the papillae in all three species will help with their discs to mold to the surface of attachment even if it is rough. Experiments with *E. orbis* need to be completed to see if it is actually better at sticking to rough surfaces as opposed to glass, the only substrate to date in which comparative studies have been completed.

There is also a large difference in the bone structure that makes up the disc of these fishes. Both *L. florum* and *E. orbis* have discs who are supported by heavily calcified pelvic rays. This support system at the roof of the disc could also be used to help explain why their adhesive ability has been shown to be stronger than that of the clingfish. When the fish is pulled away from the substrate, these stiff bones will resist bending and tend to keep the disc in its original, sucked-down position. The forces trying to remove the fish are not only fighting against the suction forces, but also the tensile force of these fin rays. In the clingfish, the lepidotrichia also have cartilaginous extensions that reach out to the rim of the disc. These more flexible extensions mean that when the fish is pulled upon, this support system should be able to bend more easily. When bending occurs, a larger volume will be created below the disc which will create a greater negative pressure and should help to keep the fish stuck to the substrate. Just based off the osteology of the disc support, it seems as though having a rigid support system might confer extra strength to the disc that clingfish cannot achieve due to their disc's morphology. The microstructure along with the osteology both offer possible reasons why the clingfish disc is weaker than that of the lumpsucker and snailfish, though there are undoubtedly other factors that this study did not look at, such as the mucus of the disc and the size and strength of the muscles involved in disc movement.

The videos taken of both the lumpsucker and snailfish show that their release mechanism is very different from that of the clingfish. It appears that these fish are using their pelvic fins and opercular expansion to brace themselves and lift up the very anterior edge of the disc. When this edge fails, the fishes could then use a wave of disc muscle contraction in the posterior direction to peel their disc off the substrate just as a person pulls off a suction cup by peeling from the edge. The video of the snailfish also seems to show that some of the volume under the disc is being evacuated just before detachment. This forcing out of some of the volume of the adhesive disc would make it easier to release and might be why they are able to fail the anterior edge. More video needs to be taken to confirm this, but these videos certainly seem to support the notion that detachment is a process regulated by muscular movement. Arita (1967) showed that lumpsuckers were unable to detach themselves when the nerves innervation the pectoral fins and disc were severed which also lends support to the idea that the abduction of the pelvic fins is also key to detachment along with the disc's musculature. This study looked to try and determine what caused the difference in performance seen between lumpsuckers and clingfish and snailfish and clingfish. It also was able to determine that these fish use a very different mechanism than the clingfish when they detach themselves. This study shows how though these fish are faced with the same problem of adhering to substrates in the marine environment, they have all evolved slightly different ways of doing this task, and doing it well. Now that the morphology of these discs has been well described, it is possible to start attempting to tease out what is most important for a strong adhesive disc. Models can be made that have all the components found in these fish, including fimbriae, papillae, and some kind of support

structure. This ‘ideal’ model can then be tweaked; things like the size of the fimbriae or amount of pads can be changed and their effect on disc performance can be evaluated.

This method of model testing could be used to determine what most important structures are for disc integrity. Eventually, this information could be used to build a biologically inspired suction disc that could be used for human purposes.

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Figure 1. Images of the adhesive disc of three different fishes of the Pacific Northwest. From left to right: the Pacific spiny lumpsucker (*Eumicrotremus orbis*), the Northern clingfish (*Gobiesox maeandricus*), and the tidepool snailfish (*Liparis florae*). These pictures are meant to show the differences in the structure of the adhesive disc that are visible to the naked eye.

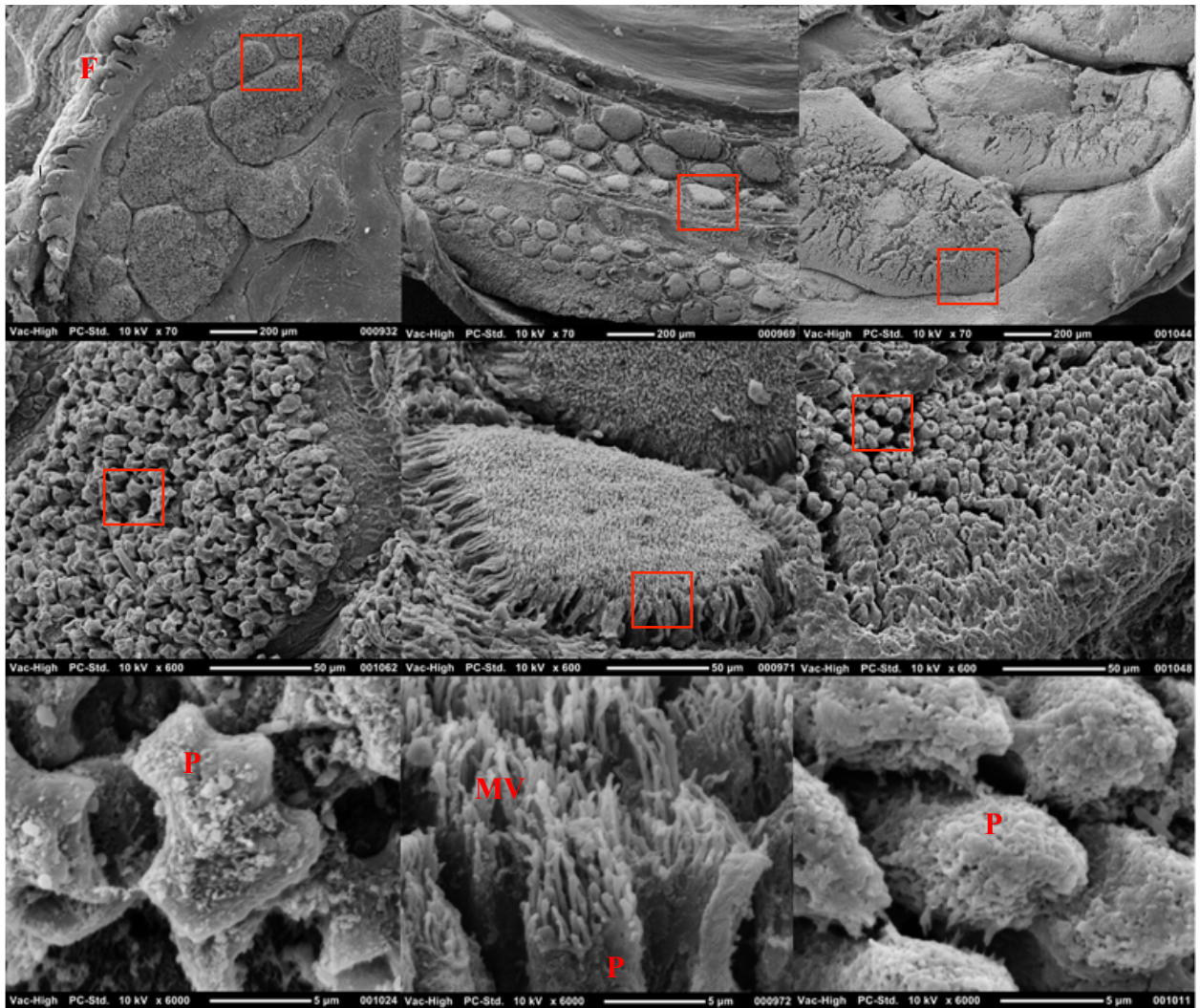


Figure 2. Scanning electron microscopy images of *Eumicrotremus orbis* (left column), *Gobiesox maeandricus* (middle), and *Liparis florae* (right). Each row is taken at the same magnification and pictures have their own scale bar. Each species has its own organization of its adhesive disc with variation in the microstructure of their pads. Red boxes show the area where the next picture was taken at a higher magnification. All images were taken from the anterior end of the specimen for ease of comparison. P; papillae, MV; microvilli, F; fimbriae.

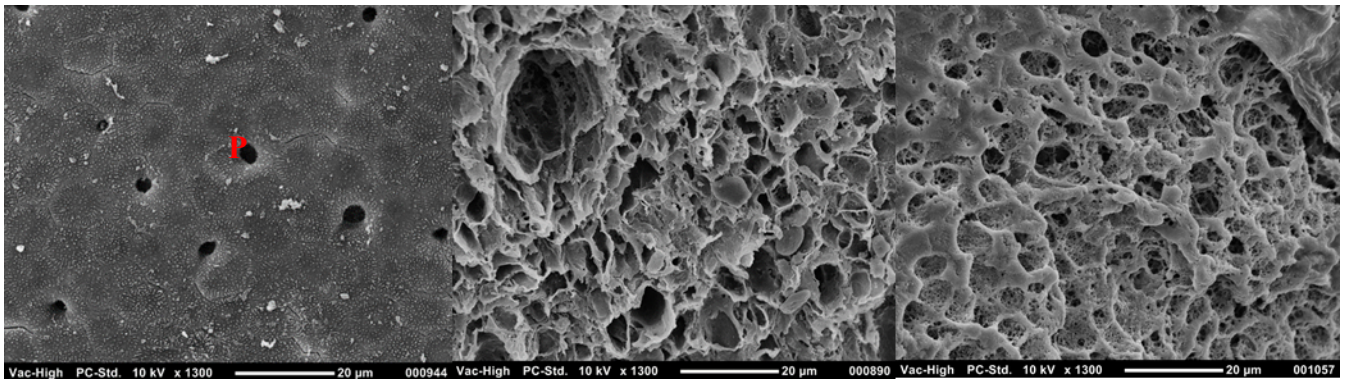


Figure 3. SEM images of the internal area of the discs of *Eumicrotremus orbis* (left), *Gobiesox maeandricus* (middle), and *Liparis florae* (right). The epithelial tissue of the internal roof of the disc of *E. orbis* is much smoother and have very obvious pores (P). It has not been confirmed if they are mucus pores or neuromast pores.

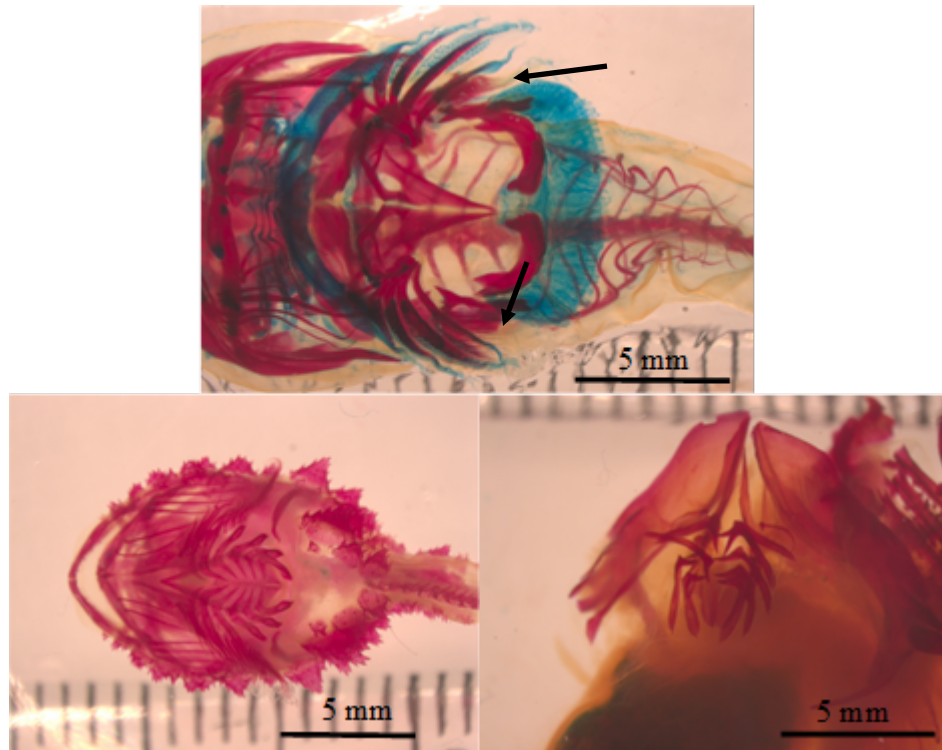


Figure 4. Pictures taken of cleared and stained *Eumicrotremus orbis* (left), *Gobiesox maeandricus* (top), and *Liparis florae* (right). Individuals were stained with Alcian Blue (cartilage) and Alizarin Red-S (calcified material). Arrows in the top picture point to the rotated, fourth pelvic lepidotrich that acts as the release mechanism of detachment in *G. maeandricus*. Note the heavy calcification of the pelvic rays of *E. orbis* and *L. florae* and the amount of cartilage associated with the lepidotrichia in *G. maeandricus*.

Table 1. Information about the two lumpsucker and snailfish that were used for the SEM imaging. Clingfish discs were pre-prepared so the size of the original fish was unknown. All measurements are recorded with \pm standard deviation. For the standard length, weight, and area of disc, $n = 2$; for pad diameter and papillae width $n = 30$.

	<i>Eumicrotremus orbis</i>		<i>Gobiesox maeandricus</i>	<i>Liparis florae</i>
Average Standard Length (cm \pm SD)	1.83 \pm 0.523		NA	5.92 \pm 1.68
Average Weight (g \pm SD)	0.65 \pm 0.49		NA	4.25 \pm 3.46
Average Area of Disc (cm ² \pm SD)	0.919 \pm 0.844		1.851 \pm 0.254	0.58 \pm 0.48
Pad Sizes	Small	Large	Small to Medium	Large
Average Pad Diameter (μ m \pm SD)	217.5 \pm 58.08	630.8 \pm 98.21	139.7 \pm 31.67	1064.7 \pm 110.77
Shape of Papillae	Globular or geometric		Microvilli topped	Conical-shaped
Width of Papillae (μ m \pm SD)	9.309 \pm 1.547		0.761 \pm 0.262	6.956 \pm 1.211
Fimbriae	Present		Present	None

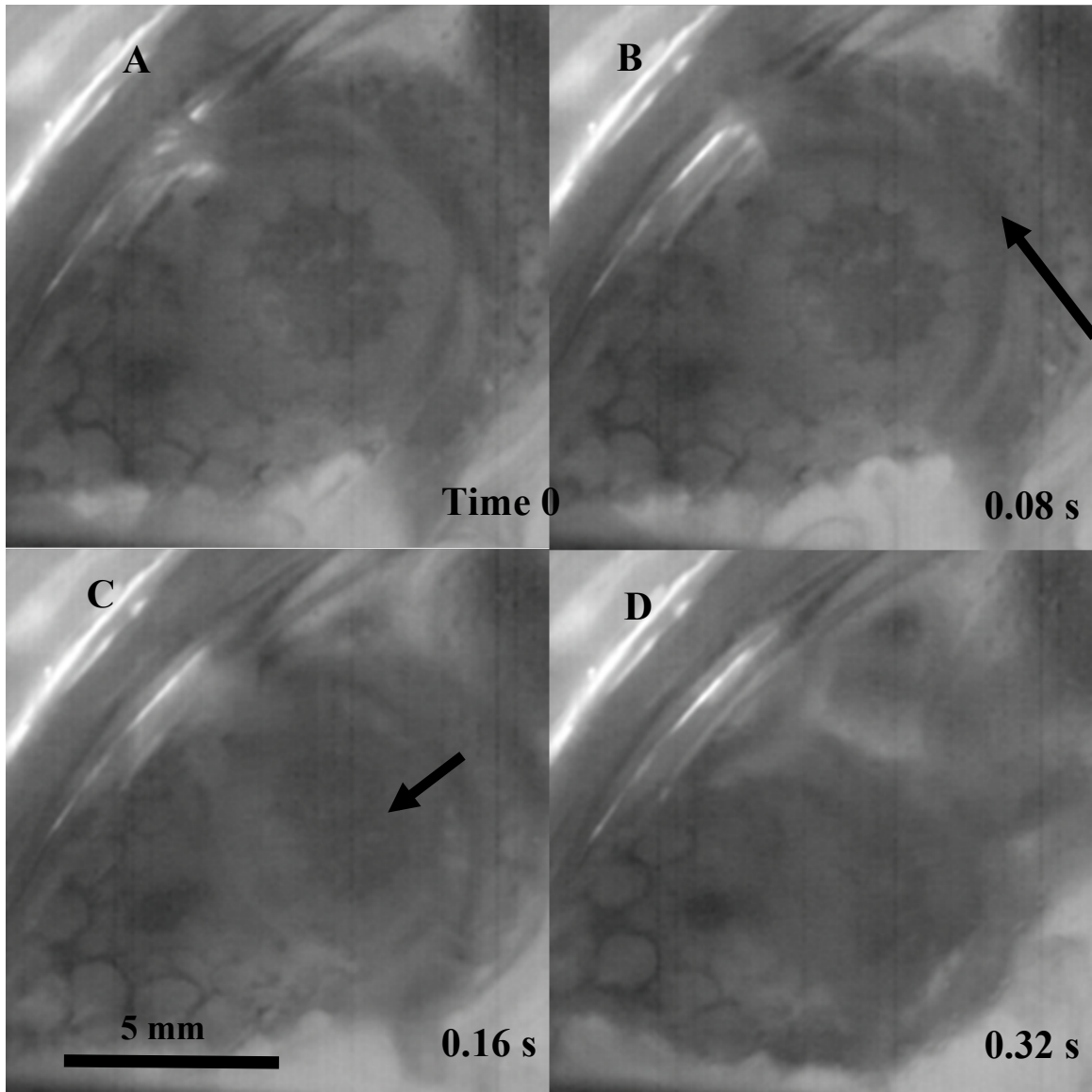


Figure 5. Images taken from high speed video of detachment in *Eumicrotremus orbis*. The video was recorded at fifty frames per second. **A**; *E. orbis* is fully attached to the substrate as green dye is injected around it. This is also time zero. **B**; pectoral fins are abducted and dye slips under the very anterior edge of the disc (arrow). The disc is still nearly fully attached. **C**; all but the posterior edge of the disc is detached and dye has flowed into the disc from the anterior end. Arrow represents the movement of flow. **D**; *E. orbis* has become fully separated from the substrate and swims off. Scale bar is the same for all images.

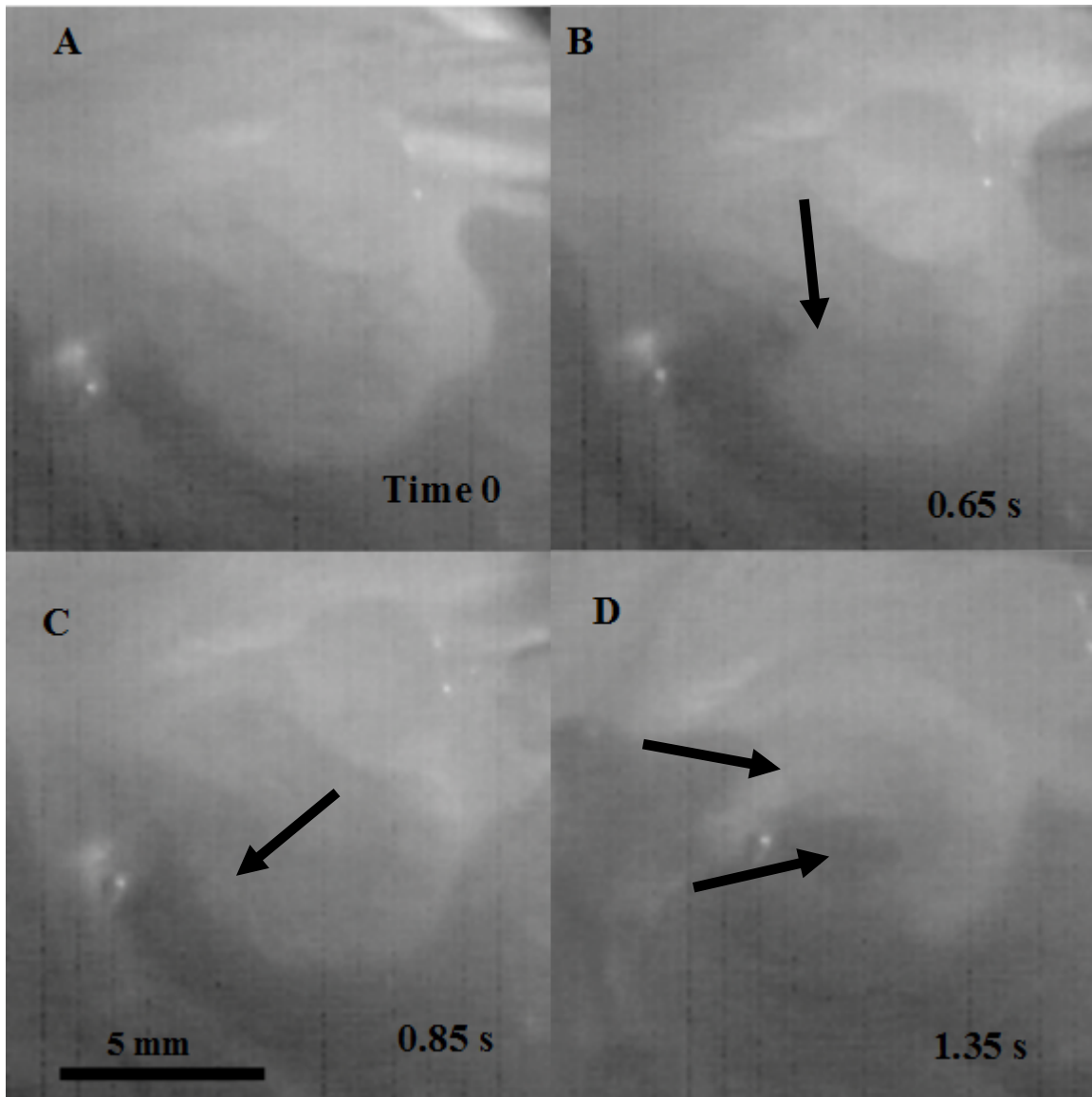


Figure 6. Images taken from high speed video of detachment in *Liparis florae*. This video was recorded at sixty frames per second. **A**; *L. florae* is fully attached and the dye is surrounding the disc. **B**; *L. florae* is still attached but some dye has leached under the disc near the anterior end (arrow). **C**; The dye has now been evacuated from under the disc where the arrow is pointing. **D**; The disc now fails and dye flows in from the anterior direction (arrows).

