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SUCTION PRESSURE MEASUREMENT AND BEHAVIORAL OBSERVATIONS OF SPAWNING-RUN SEA LAMPREYS (*PETROMYZON MARINUS)*

by

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Thesis

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in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

in

General Biology

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ABSTRACT

Sea lampreys utilize attachment by suction throughout their life cycle. Despite the importance of this behavior, the role of surface texture in the attachment process has not been fully understood. The goal of this study was to develop a technique to quantitatively examine attachment abilities on surfaces of different texture and then to test whether the lampreys would seek out the surface that best promoted attachment. Results of the first experiment showed that the ability to attach depended on the interaction between a surface's characteristics and the sea lamprey's oral disk anatomy. For management applications where lamprey attachment is a concern, this pressure-measurement method would be ideal for surface creation and testing. In the second experiment, sea lampreys displayed little preference among surfaces with varying levels of texture. Repeating this experiment in a high sheer environment may yield more insight into the management implications of surface preference and attachment.

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INTRODUCTION

Sea lampreys (Petromyzon marinus) rely on sucker attachment in two of the three stages of their anadromous life cycle. In the parasitic adult stage, sea lampreys use suction to feed on host fishes. During the reproductive phase, attachment is used in the spawning process, and attachment to substrate aids migratory movement through swift currents (Hardisty and Potter 1971). Despite the implications for survival, little research has gone into the study of the sea lamprey attachment process. Currently, information exists on sea lamprey oral anatomy (Lennon 1954; Lethbridge and Potter 1979; Youson 1981) and suction-pressure creation (Reynolds 1931; Lanzig 1958; Gradwell 1972); however, a quantitative relationship between surface characteristics and attachment ability has not yet been established.

In contrast to the mechanics of suction attachment, many studies have addressed the management of anadromous lamprey populations. In the Great Lakes, parasitism by invasive sea lampreys is deemed responsible for the decline of Lake Trout (Salvelinus namaycush) and other important commercial fishes (Smith and Tibbles 1980). In other regions of the world, such as the West Coast of the United States (Ocker and others 2001) and Central and Eastern Europe (Renaud 1997), anadromous lampreys are in decline as a result of habitat destruction and poor water quality. Although their management goals differ, resource managers from all regions may benefit from research on lamprey attachment. The manipulation of attachment behaviors may enhance current management strategies, such as trapping or low head barriers in the Great Lakes (Hunn and Youngs 1980; Lavis and others 2003) and dam passage in Europe and the West Coast of the United States (Ocker and others 2001).

Before the experiments for this thesis study can be discussed in detail, it is important to provide a review of current knowledge of sucker attachment. All that has been published about sea lamprey suction formation and attachment behavior is compiled in the sections that follow.

Literature Review

Oral Anatomy

The mouth of a lamprey is the region between the outer edges of the oral disk and the esophagus (Figure 1). In that span, there are two main compartments important to the creation of suction: the buccal funnel and the pharyngeal cavity (Youson 1981). The first compartment, the buccal funnel, is composed of the oral disc, the external opening, and the buccal cavity. The oral disc is described as the rim of tissue surrounding the external opening and is made up of an epidermis, muscle, and connective tissue (Youson 1981). Epidermal teeth are arranged on the outside surface of the oral disc (Youson 1981) and most likely provide the lamprey with an enhanced grip on a surface before and during suction. The integument of a lamprey's oral disc consists of a series of finger-like projections called fimbriae (Lethbridge and Potter 1979). The fimbriae are lined with mucus-secreting cells and are very flexible, a combination allowing the lamprey to form a complete seal on a variety of surfaces. Papillae are shorter extensions of the skin located on the periphery of the fimbriae (Khidir and Renaud 2003). Lethbridge and Potter (1981) suggest that the location of these tissues may indicate that they function as sensory receptors that provide the lamprey with surficial texture information. The buccal cavity is located on the inside of the lamprey's mouth between the external opening and the oral passage found posterior to the tongue.

Bilobed laminae, found at the anterior end of the tongue, provide the main cutting edge in a parasitic attachment event (Lennon 1954). The protractor and retractor muscles help provide piston-like control to the tongue. The annularis muscle, situated around the edges of the disc, provides control over the size of the buccal cavity and, thus, the shape of the buccal funnel (Lanzig 1958).

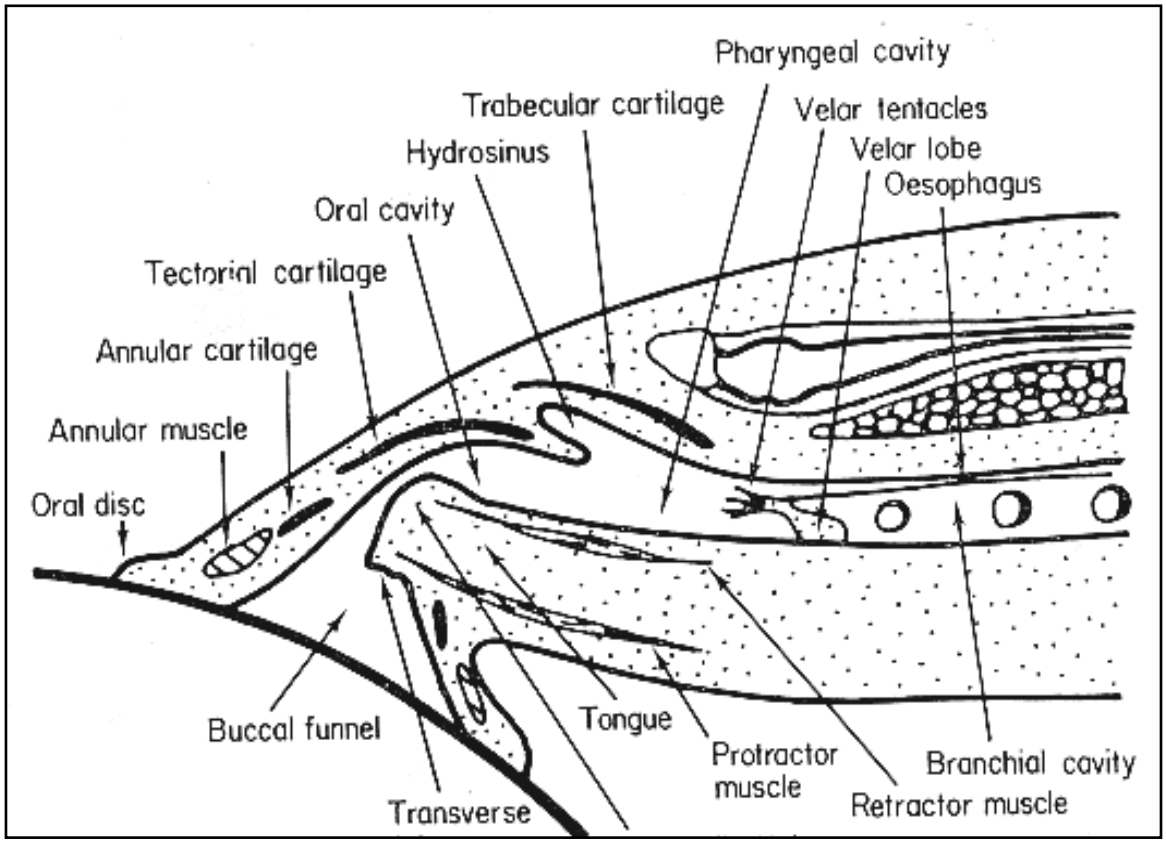

Figure 1 Schematic of sea lamprey mouth

Lateral cross-section of the lamprey head reveals the important structures required for attachment. Reproduced from Hardisty and Potter (1971)

The second compartment, the pharyngeal cavity, lies between the oral passage to the beginnings of the esophagus and the branchial cavity. Located dorsally in the pharyngeal cavity is the hydro-sinus. Reynolds (1931) stated that compressions of the hydro-sinus are responsible for the expulsion of water from the pharyngeal cavity through the branchial cavity. These expulsions may be necessary to clean the cavity of dirt or other unwanted particulates (Reynolds 1931). Guarding the entrances of both the esophagus and branchial cavity from the pharyngeal cavity is a one-way valve termed the velum. The dorsal portion of the velum serves as a filter on particles entering the esophagus. The ventral lobes of the velum face backwards toward the branchial cavity and do not allow expulsions of air and water back into the pharyngeal cavity. This device plays an integral role in the development of suction (Lanzig 1958).

SUCTION FORMATION

The following is a step-by-step description of suction formation. First, the lamprey approaches the desired surface, contracts its annularis muscle, and the buccal funnel spreads over the surface (Lanzig 1958). The oral disk then conforms to the target surface and completes a seal through a combination of fimbriae flexibility and mucus secretion (Lethbridge and Potter 1979). Next, the tongue retracts into the oral passage and seals off the buccal funnel from the pharyngeal cavity. The buccal cavity expands, which creates a vacuum inside this cavity and a corresponding suction force on the surface. The sea lamprey can now maintain a suction attachment until its tongue is shifted. In the next step, water is pushed out of the pharyngeal cavity through the compression of the hydro-sinus. This creates an area of low pressure within the pharyngeal cavity. The velar apparatus seals the

pharyngeal cavity from water directed from the higher pressure branchial cavity (Hardisty 1979). Finally, the tongue protracts, and water flows into the buccal funnel from the pharyngeal cavity until the pressures are equal. The lamprey can now use its piston-like tongue to rasp at its prey and digest its fluids while it maintains its suction grip (Lanzig 1958). For a simplified schematic of this suction creation process, see Figure 2.

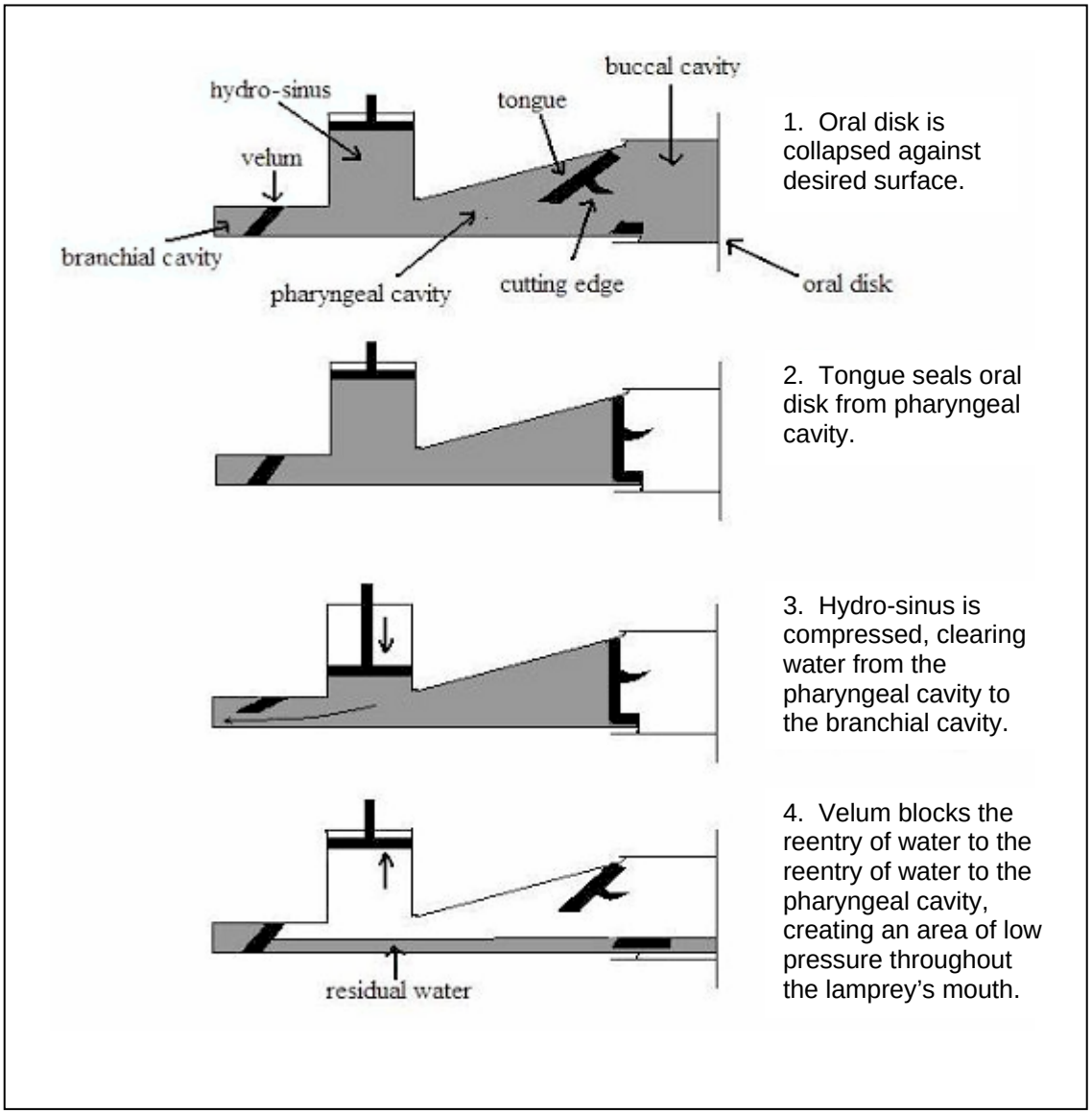

Figure 2 Schematic of pressure creation within the sea lamprey mouth

A simplified schematic of the suction development process. Adapted from Reynolds (1931).

SUCTION MEASUREMENT

Norman Gradwell (1972) was the first to measure suction pressures created by sea lampreys. First, he inserted cannulae (flexible surgical tubing) in the gill pouches and naris of four prespawn adult lampreys. He then connected pressure transducers to these cannulae, which allowed for the measurement of each cavity's pressure. To obtain the total pressure exerted by the lampreys on an acrylic surface, he connected a pressure transducer to a hole in the designated attachment area. He monitored all pressures, using a polygraph. He monitored lamprey movements, using a balanced lever resting against the fish. Results of his study showed that the total suction-pressure waveform created during a suction event was composed of a variety of factors, the greatest of these being branchial pressure and tongue movement. In the absence of tongue movements, the measured sucker pressures varied from -0.049 to -00.59 kPa and had frequencies and amplitudes that were highly correlated with branchial pressures. Because of the lampey's ability to seal the oral cavity, tongue movements caused much larger fluctuations in suction pressures. Gradwell (1972) found that with all initial suction engagements, one or two cycles of tongue movements were recorded. When his animals were left attached to the surface and undisturbed for over an hour, spontaneous tongue movements and ensuing large pressure fluctuations occurred at regular intervals. Tongue movements were also elicited when a fish was lightly touched, allowing for a reinforcement of suction pressure on the surface. When the author attempted to dislodge the oral disk from the control surface, the resistant pressures exceeded -11.76 kPa. No other surfaces besides the smooth acrylic were tested in the study.

ATTACHMENT BEHAVIORS

Feeding Through visual (Lennon 1954) and olfactory (Hardisty and Potter 1971) cues, a sea lamprey selects its host. As the lamprey closes in on its prey, its suctorial mouth widens over the host's body. Once in contact with the flesh, the lamprey maintains its hold by use of suction (Lennon 1954). Through the rocking action of its tongue, the lamprey is able to rasp a hole into its victim, creating a pathway for feeding on the host's bodily fluids (Lennon 1954). A secretion released to the wound from the buccal glands enhances the breakdown of the prey's tissues to fluids. The secretion, termed Lamphedrin, exhibits anticoagulatory, cytolytic, and hemolytic properties (Lennon 1954). Although the lamprey may slide along the host's body to gain a more favorable position for feeding, suction is rarely broken until the lamprey is finished with the host (Hardisty and Potter 1971).

In its selection of a host, the sea lamprey does not appear to have a preference as to the host's flesh type. Hardisty and Potter (1971) referred to a study done by Surface in 1898 in which 74 fish species were known victims of sea lamprey in Lake Cayuga, New York. Among these fishes were *Acipenser fulvescens* and *Ictalurus nebulosus*, which have relatively smooth flesh types, and *Lepisosteus ossues,* with much rougher scales. Observations from the Great Lakes indicate that the smooth-skinned *Lota lota maculosa* is just as likely to be attacked as a rough-scaled *Perca flavescens* (Hardisty and Potter 1971).

Motility As a result of the absence of (a) paired fins and a hydrostatic organ, (b) poorly developed ventral and caudal fins (Beamish 1974), and (c) anguilliform swimming (McAuley 1996) lampreys are poor swimmers compared to other fish. To compensate for this limitation, sea lampreys have developed an alternative behavior for passage through areas

with high water velocities. Sea lampreys have the ability to attach to a substrate, rest, and then burst forward. Repeated bouts of this behavior allow lamprey passage in currents that would otherwise have restricted upstream movement (Hardisty and Potter 1971; McAuley 1996).

Spawning During redd construction, mating, and redd defense, sea lampreys utilize attachment behaviors (Hardisty and Potter 1971). Nest building involves lifting of stones (the lamprey attaches to the stone and then releases it outside of the nest) and vibration (the male will attach to one stone and then vibrate in short bursts to stir up loose stone that is swept downstream). During the initiation of the spawning act, the female first attaches to a rock in the redd, and then, the male attaches to the head of the female for stability. In redd defense, primary males have been observed to be antagonistic towards other males intruding on the nest. These primary males will attach to the intruder male and, by a violent wriggling motion, remove the male downstream from the redd.

Behavioral Observations

In his thesis research, McAuley (1996) sought to develop and test a sea lamprey velocity barrier. Recognizing the ability of sea lampreys to use attachment when traversing higher stream velocities, he lined his barrier with a nonattachable surface. This surface was plastic meshing, chosen from six different surfaces tested for sea lamprey attachment (McAuley 1993). In this preliminary study, the ability of the lamprey to attach to a surface was not quantified; rather, the surfaces were shown to either facilitate or prohibit attachment. The surface that best prohibited attachment was used for the barrier. The reasoning behind

the sea lampreys' inability to attach to the surface was not fully examined; however, the most textured surfaces provided the most hindrance to attachment.

In studying passage efficiency of migrating Pacific lamprey around dams in the Columbia River, Ocker and others (2001) found that passage success was lowest in areas with little or no attachable surfaces. The flooring in these areas (collection channels and transition areas of the dams) consisted of metal grating and few spots of cement. In spots where cement was available around the gratings, lamprey success was 31% to 39%. In sections with long strips of the grating and little or no cement passage, success was 5% to 6% (Ocker and others 2001).

Research Objectives

The first goal of this research study was to quantify the relationship between surface characteristics and sea lamprey attachment ability. To accomplish this, fish were placed into a test apparatus that guided them to a target attachment surface. The construction of this apparatus allowed easy exchange of surfaces with quantifiable variations in texture. Pressures exerted on each surface were recorded with an absolute pressure sensor, and digital photographs were taken of the oral disk at the onset of each attachment event. In the pressure recordings, quantitative metrics were derived from characteristics denoting water intrusion to the oral disk. In the visual analysis, contortion of the oral disk was examined. The combination of these analyses provided the quantifiable evidence required to contrast surface characteristics with suction establishment and maintenance. This research expands upon the current knowledge of the pressure characteristics of suction attachment (Reynolds 1931;

Lanzig 1958; Gradwell 1972) and provides insight into surface characteristics that may be useful for the management of lampreys.

The second goal was to document the preferences of sea lampreys presented a choice of textured surfaces for attachment. The surfaces used in this second experiment had the same surface characteristics as those tested in the first experiment. If sea lampreys preferred those surfaces that best promoted suction maintenance, there is a possibility of exploiting this behavior for management purposes.

MATERIALS AND METHODS

Test Fish

The sea lampreys used in the Summer 2005 thesis experiments were trapped during their spawning run up the Ocqueoc River (Presque Isle County, Michigan) in mid-May 2005 by United States Geological Survey research technicians. The trapped lampreys were taken back to the Hammond Bay Biological Station (HBBS), located in Millersburg, Michigan, where the sexes were divided into separate holding tanks. The males were set aside for a male sterilization project (Twohey and others 2003) carried out at HBBS, whereas the females were reserved for other lamprey research projects in the region. On 27 May 2005, 120 female sea lampreys were transferred from HBBS to the Aquatic Ecology Laboratory at Eastern Michigan University (Ypsilanti, Michigan). They were kept in two 1133-L recirculating tanks at 8 °C. The total lengths of these female lampreys ranged from 384 mm to 562 mm, with a mean length of 467.2 mm.

Experiment 1: Suction Pressure Measurement

EQUIPMENT AND SETUP

From 31 May 2005 to 3 August 2005, an absolute pressure sensor was used to monitor negative pressures exerted by sea lampreys on test surfaces (Qubit Systems, Kingston, ON). Four 301-L recirculating tanks kept at 8 °C were used in these experiments. One tank contained the equipment necessary for pressure measurement, whereas the other three were used to maintain separation of experimental individuals between trials.

The base of the pressure measurement apparatus was a 63.5-cm-long acrylic tube with three-quarters of its length cut in half along the horizontal plane (Figure 3). A 92-mm-

wide by 121-mm-long acrylic flange was glued to the intact end of the tube to provide easy exchange of experimental surfaces. A gasket of foam sheeting prevented water from flowing between the flange and the experimental surface (Figure 4).

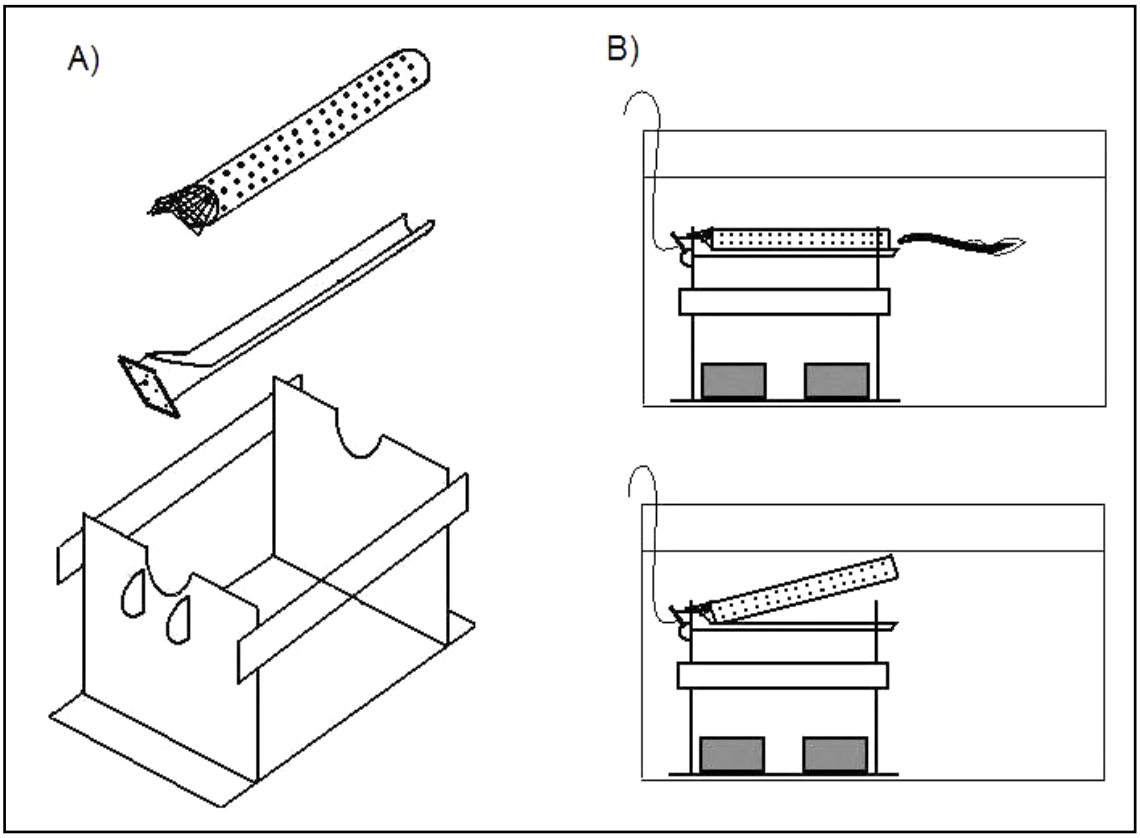

Figure 3 Schematic of the pressure-management apparatus

Schematic of the experimental apparatus used to measure suction pressures of sea lampreys (A) A stand held the holding tube and desired attachment surface in place. (B) A lamprey was inserted into the holding tube. Once the tube was lifted, the lamprey slid toward the attachment surface.

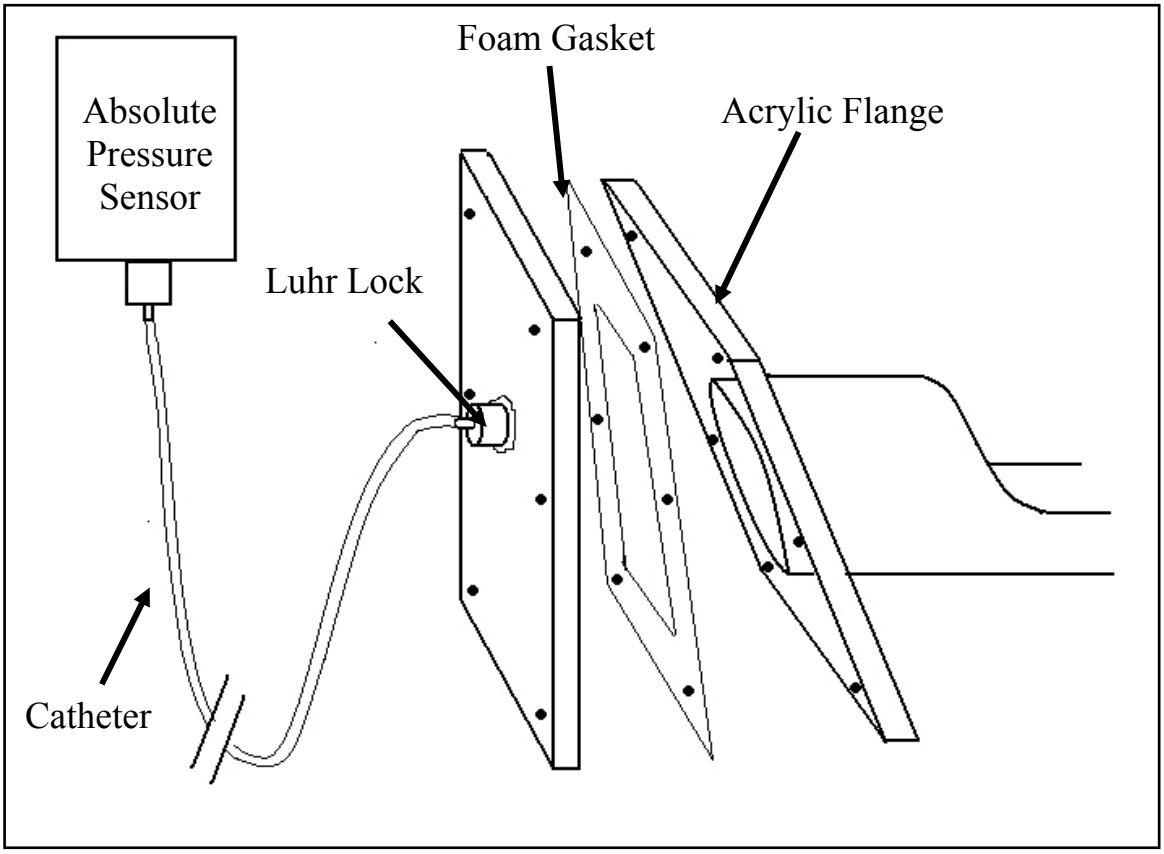

Figure 4 Schematic of flange/experimental surface components

Close-up schematic of the primary components of the pressure measurement system. The experimental device was held in place to a flange on the acrylic tube. A foam gasket prevented water from flowing between the two surfaces. A luhr lock was glued into a hole drilled into the experimental surface. A catheter connected to the luhr lock allowed pressures exerted over the hole to be monitored by the absolute pressure sensor.

Experimental surfaces were 90 mm wide by 114 mm long. In the middle of each surface, a 6-mm-diameter hole was cut 46 mm from the top and 46 mm from each side. A luhr lock glued into the hole connected the surface to the absolute pressure sensor via a 1.6-mm-diameter catheter (see Figure 4). Pressure data from the sensor were directed through a Vernier LabPro interface and recorded on Vernier Logger Pro 3 software (Vernier Software and Technology, Beaverton, OR). Experimental time modifications, equipment zeroing, and

data collection were all done with the software package. In all experiments, pressures were measured in kilopascals and recorded every 0.1 seconds.

Inside the experimental tank, an acrylic cradle held the pressure-measurement apparatus 18 cm underwater (see Figure 3). Prior to an experiment, a 61-cm-long Polyvinyl Chloride (PVC) tube with an inside diameter of 5.1 cm was freely set onto the half-cut section of the acrylic tube. Holes were drilled throughout the PVC tube to ensure that a lamprey could not attach inside the chamber. A 9-cm-wide by 9-cm-tall sheet of plastic window screen extended over the gap between the PVC tube and the acrylic tube. This screening concealed experimenter movements from the test lampreys and hindered escape from the apparatus (see Figure 3).

All pressure-measurement experiments were done with a randomized-block design with each fish as the blocking factor and the successive test surfaces as the treatment factors. An experiment was initiated once a lamprey was coerced inside the back end of the PVC tube. If the lamprey did not move forward, the back end of the tube was gently raised, and the lamprey slid toward the experimental surface. When a lamprey detached during an experiment, the back end of the PVC tube was lifted to reintroduce the lamprey onto the experimental surface. In addition to the pressure measurements, digital pictures of the oral disk at the initiation of most attachments to grooved surfaces were taken through the transparent flange. No lampreys were used in more than one pressure-measurement experiment.

EXPERIMENT 1A: TEST OF GROOVE DEPTH

From 31 May 31 2005 to 3 June 2005, 4 surfaces were introduced to 30 randomly selected sea lampreys in a randomized-block design. The first surface was made of smooth acrylic with no alterations. The remaining acrylic surfaces each had two grooves cut vertically along the length of the surface. On each surface, the two grooves were symmetrical to the catheter hole and 8 mm apart from their respective inside edges (Figure 5a). Grooves on each surface were 3 mm wide, but the grooves differed in depth (Figure 5b). Depths were 1 mm (shallow-grooved surface), 2 mm (medium-grooved surface), or 3 mm (deep-grooved surface). All fish were tested on the four surfaces in a randomized order. Pressure recordings lasted 12 minutes, and fish were given 24 hours before being tested on a different surface.

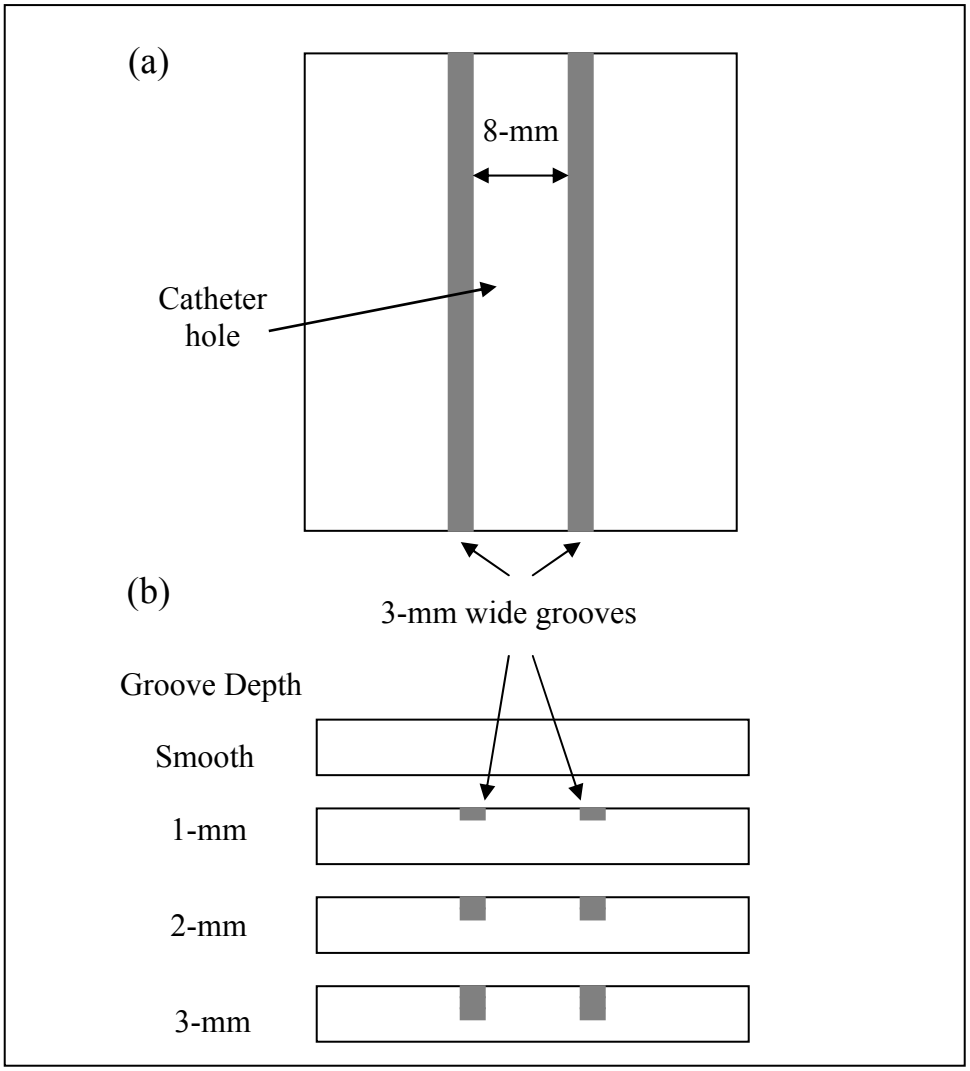

Figure 5 Schematic of the groove cuts for the shape experiment

(a) Drawing of a 3-mm-wide grooved surface with grooves cut symmetrical to the catheter hole. (b) Drawing of the four surfaces used in Experiment 1A. All grooves were 3 mm in width and varied in depth.

EXPERIMENT 1B: TEST OF SURFACES WITH RELEVANCE TO FIELD APPLICATION

From 14 June 2005 to 20 July 2005, a series of four pressure-measurement experiments was performed on laboratory sea lampreys to examine how specific arrangements of discontinuities would influence suction. The emphasis was on those surface textures that would be useful in field applications to either enhance or hinder suction attachment. In the first experiment, four surfaces with different groove dimensions were introduced to 30 sea lampreys. Two of the surfaces were repeated from Experiment 1A, that is, 3 mm wide by 1 mm deep (shallow-grooved) and 3 mm wide by 2 mm deep (medium-grooved), a third surface had grooves with the dimensions 1 mm wide by 3 mm deep, and the final surface had grooves measuring 1 mm wide by 6 mm deep. As with Experiment 1A, all grooves were rectangular in shape.

In a second experiment, two surfaces that differed in "biofilm" texture were presented to 15 sea lampreys. Because of time constraints and unwillingness to introduce an excess of algae to the tanks, natural biofilm was not used in this experiment. Instead, a thin layer of vegetable lard was used as a replacement for natural biofilm because of its ability to maintain a slimy texture while submerged in water over extended periods of time. During experimentation, one surface was covered with a film of vegetable lard, whereas the other remained unaltered. After each trial run, the vegetable lard was reapplied to the respective surface.

In a third experiment, two surfaces that differed in groove orientation were presented to 15 randomly selected sea lampreys. Each acrylic surface had two grooves that were 8 mm apart from their respective inside edges and were 3 mm wide by 2 mm deep (medium-

grooved surfaces). On one surface, grooves were oriented horizontally; in the other, grooves were oriented vertically.

In the final experiment, three surfaces that differed in shape of groove cut were introduced to 15 sea lampreys (Figure 6). All three surfaces had grooves with cross-sections of roughly 6 mm^2. One surface had two rectangular grooves 3 mm wide by 2 mm deep. This cut was made in a router with a drill bit fitting the desired measurements. The second surface had semicircular-cut grooves with a radius of 2 mm. A router blade fitting these dimensions was used in the creation of this cut. A third surface had triangular-cut grooves with the dimensions 6 mm wide by 2 mm deep. This cut was made with a router set at a defined angle at the desired incision length. The four experiments were all performed with the same technique as used in Experiment 1A. Pressures exerted on each surface were recorded in 12-minute trials.

Figure 6 Schematic of the three different groove cuts for the shape experiment

Drawing of the three types of cuts used to determine the effects of groove shape on attachment ability.

PRESSURE SENSOR CALIBRATION

The recorded pressures exceeded those measured by Gradwell (1972) and surpassed the plausible pressures dictated by the physical environment. The lampreys were shown to create pressures that exceeded ambient pressures, requiring a force of roughly 10 times the ambient pressure to induce detachment. To examine the problem with the sensor readings,

the pressure sensor was set to record pressures from a vacuum pump (Gast Manufacturing Inc. Model number DOA-P704-AA. Benton Harbor, MI).

Vacuum pump settings were measured from 100-550 mm Hg at increments of 50 mm Hg. Recordings lasted at least 30 seconds. The measured pressures were plotted against the nominal pump settings, with zero as the established origin. The resulting relationship was linear ($y = 10.38x$: $n = 10$, $r^2 = 0.993$, $p < 0.001$). The slope (10.38x) was used as the correction factor for all pressures recorded during experimentation with lampreys. Because all experimental pressures, Experiments 1A through 1C, were similar in magnitude, it was assumed that this pressure difference was constant throughout the pressure-measurement experimentation.

Data Handling for Experiments 1A and 1B

In the pressure analyses, an attachment event was defined as a period of suction attachment during which pressure was maintained below the ambient water pressure. In each pressure recording, the first attachment event that lasted at least 180 seconds was selected. The pressures recorded in the first 180 seconds of this attachment event were then extracted from the pressure dataset and analyzed.

From each extracted dataset, two metrics were chosen for quantitative comparison: (1) pumps per minute and (2) rate of pressure loss within a pump segment. *Pumps* were defined as a decrease in pressure greater than 0.51 kpa per 0.1 second (Figure 7). As described by Gradwell (1972), these large fluctuations were regular and voluntary, corresponding with the movement of the tongue. From current understanding of the tongue's role in suction creation and maintenance (Reynolds 1931; Lanzig 1958; Hardisty 1979), it

appears that the tongue pumps represented the expulsion of water that intruded to the oral disk as a result of a leak in the suction seal. By measuring the frequency of the tongue pumps during attachment to various surface textures, the lampreys' ability to attach to each surface was quantified. Differences in pumps per minute per surface were tested with a Randomized Block ANOVA at a significance value of $p_{(\alpha)} = 0.05$. Each fish in the experiment served as a blocking factor, whereas each surface tested was a treatment factor.

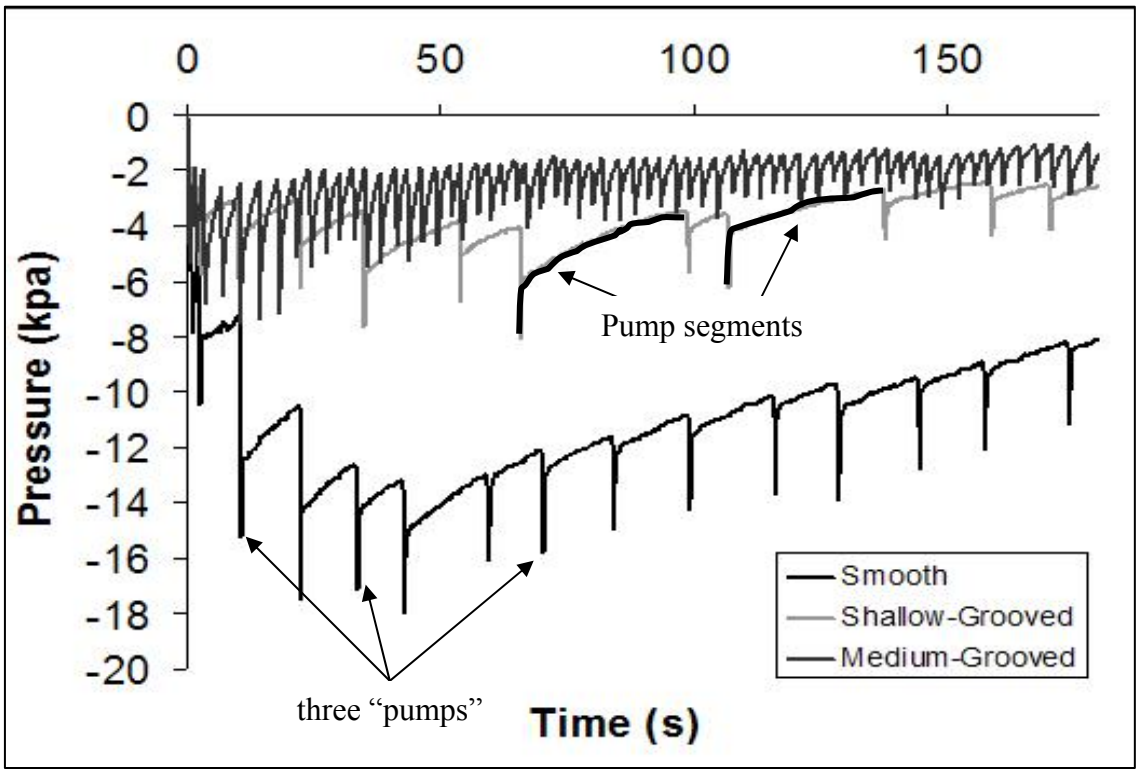

Figure 7 Graphical example of a pressure recording

Graphical example of 180-second extractions taken from the attachment records of an individual lamprey. This lamprey attached to all three surfaces. The magnitudes of pressures found in this graph are not indicative of the surface attachments of other lampreys.

Pressures recorded between the end of one pump and the initiation of the next were considered part of a *pump segment* (see Figure 7). During this segment, water seeps into the oral cavity, and the pressure inside the mouth gradually increases towards the ambient pressure. The pump segment ends when the lamprey pumps its tongue to flush the water out of its mouth to maintain its suction attachment. By measuring the rate of pressure lost within the pump segment, the rate of water intrusion may be estimated, and the lamprey's attachment ability to the surface may be quantified.

For an adequate representation of mean pressure-loss rate during an attachment event, 10 pump segments from each surface attachment were desired. For attachment events with greater than 10 pumps, this was done through the selection of 10 pump segments at random. However, in some instances there were 10 or fewer pump segments within the extracted attachment events. In those cases, all pump segments were chosen for analysis. Additionally, any pump segment lasting less than 0.6 seconds was excluded from pressure-loss-rate analysis. These short segments were termed *adjustment pumps* and generally occurred as a lamprey situated itself onto the surface (Gradwell 1972).

The loss rate of each pump segment was computed as the mean b-value in an exponential decay function fitted to the pump-segment data points by Microsoft Excel software (Equation 1). In the segments chosen, b-values were estimated and then averaged. Differences in mean b-values per surface were tested with a Randomized Block ANOVA at a significance level of $p_{(\alpha)} = 0.05$.

$$Y = c*e^{bx}$$

Equation 1 Exponential decay function where x is time, c is the y-intercept, e is the natural log, and b is defined as a constant rate

In this experiment, contortion was defined as the repositioning of the oral disk away from the typical circular shape. This concept is similar to the D_L ratio used to examine shoreline development of lakes in limnology (Wetzel 2000). The ratio refers to the length of the lake's shoreline divided by the circumference of a circle that has the same surface area as the lake (Equation 2). The closer the ratio is to 1.00, the more circular the shape of the lake.

$$D_L = SL/(2((\pi * A_0)^{1/2}))$$

Equation 2 Shore Line Development ratio where SL is the measured perimeter and A_0 is the calculated surface area

To compare the amount of oral disk contortion required to attach to the shallow-grooved and medium-grooved surfaces, digital pictures taken of these events were utilized. In all, there were 26 pictures of shallow-grooved attachments and 29 pictures of medium-grooved attachments. In the first step, the pictures were imported into Adobe Photoshop 6.0 and cleaned with the Auto Levels and Auto Contrast features. Next, the picture numbers were randomized and then opened individually in a graphics application called ImageJ version 1.36b (National Institutes of Health, Bethesda, MD). Within this application, an oral disk perimeter could be traced, and then the program calculated the total length of the trace as well as the surface area within the figure's perimeter. This tracing procedure was performed five times for each picture, and the average of the five was that picture's perimeter and area measurements. These measurements were inserted into the D_L ratio equation. D_L averages were then calculated for each surface type. A Two-Sample t-test was used to statistically compare the averages.

In Experiments 1A and 1B, the data were transformed to meet the equality of variance assumptions of the Randomized Block Analysis of Variance. This was performed

through residual analysis. The transformation that best fit each experimental dataset is included in the results.

Experiment 1C: Maximum Pressure

From 29 July 2005 to 3 August 2005, the maximum negative pressures exerted on a smooth acrylic surface were compared to oral disk areas in 12 randomly selected sea lampreys that had not been used before in the pressure-measurement apparatus. Each lamprey was placed into the pressure-measurement device and, once attached to the test surface, was pulled by hand until detached. This process continued until the lamprey was no longer able to attach to the surface or after a maximum of 10 recorded minutes. Because of the intense physical demands of this experiment, individuals were tested only once. The most negative pressure attained during each trial was considered the maximum.

After each individual test was completed, the lamprey was taken out of the tank and forced to attach to one side of a clear acrylic sheet. The oral disk height and width were then measured on the opposite side of the sheet to the nearest millimeter. The equation for the area of an ellipse was used to calculate individual oral disk areas. The areas were then plotted against the corresponding maximum pressures. The resulting relationship was statistically examined through linear regression.

Experiment 2: Preference of Attachment Site

Equipment and Setup

Surface preferences of sea lampreys were examined from 16 June 2005 to 17 July 2005. The study tank was an 1133-L recirculating tank maintained at 8° C by an in-line

chiller. Water entered the tank at the northern end and flowed toward the outflow in the southeast corner (Figure 8). Inside the tank was a 1.7-meter-long by 1.1-meter-wide arena that enclosed six boxes that each measured 0.55 m wide by 0.55 m long by 0.55 m tall and were open at the top (see Figure 8). The arena-box complex was made from egg-crate diffusers made for fluorescent lighting. This material was plastic, with a checkered pattern of square holes that prevented sea lamprey attachment.

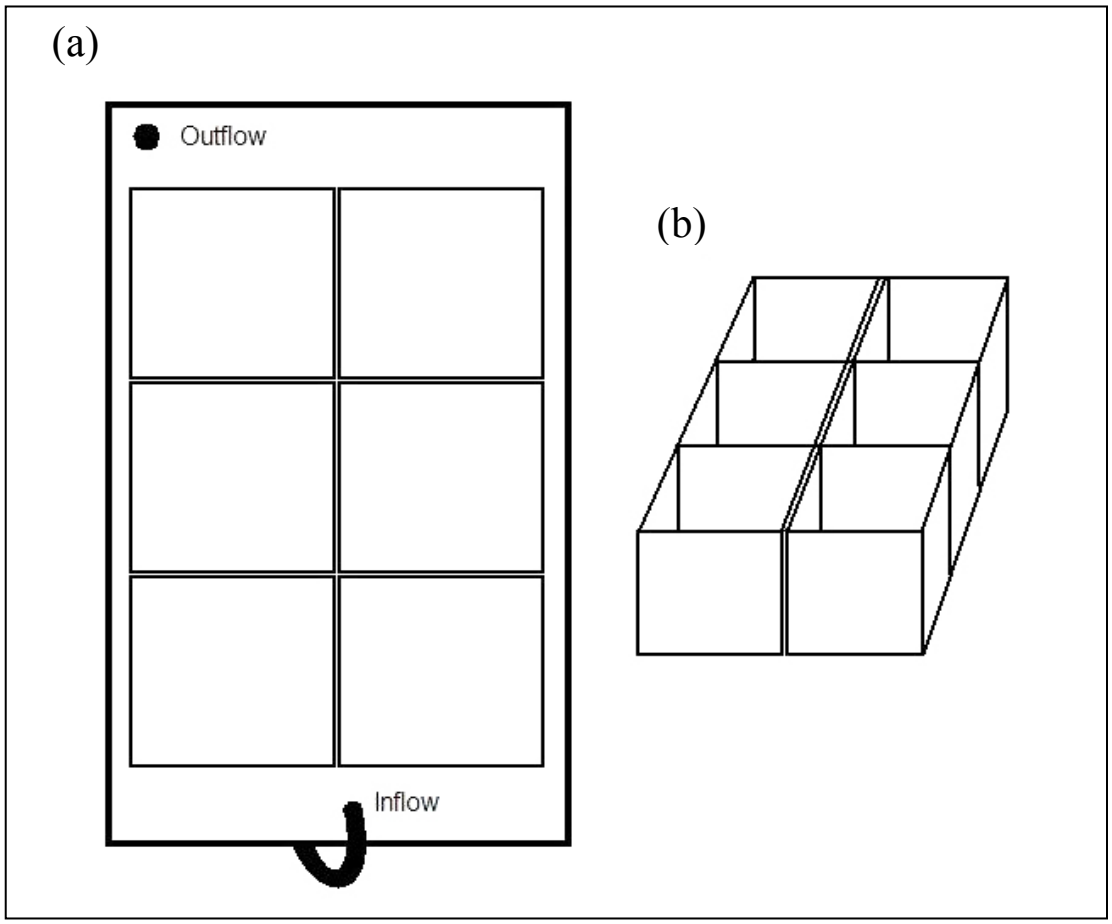

Figure 8 Schematic of the preference area used for Experiments 2A and 2B

Schematics of the arena used for surface preference experiments. (a) A top view of the experimental tank, the flow system, and the six individual test boxes. (b) Diagonal view of the six test boxes.

Digital video surveillance equipment was used to monitor attachment behaviors. Four 34-mm-long, low-light cameras were fixed roughly 1.3 m over the study tank. The lens of each camera measured 3.6 mm in diameter, providing a viewing angle of 92°. Two 100-watt floodlights with red filters provided the lighting over the tank. The red filters provided a continual impression of darkness to the test lamprey (Gordon McDonald, University of Guelph, personal communication, June 2004) yet supplied enough light for the cameras to function properly.

Recording was initiated once the first randomly selected sea lamprey was placed into its individual test box. Each lamprey was recorded for 24 hours by all four cameras. The resulting video was digitized through a PC Witness A/V-Digital converter, transferred to a laptop computer via a USB link, and then stored on the hard drive via PC Witness software (CCTV Wholesalers, New Orleans, LA). Surveillance scheduling, frame rates, and recording frequencies were all managed with the software package. In all behavioral experiments, the frame rate of each camera was set to 5 frames per second. These settings allowed for time-lapse recording such that 3 minutes and 53 seconds of recorded time corresponded to 30 minutes of real time.

Experiment 2A: Test of Groove Depth

Thirty sea lampreys were observed for preferences in attachment sites over 24-hour time periods between the dates 16 June 2005 and 30 June 2005. The multibox setup of the arena allowed for six individual preference tests per day. When the lampreys were placed inside the box, the only available attachable surfaces were 5-mm-thick acrylic tiles fixed to the walls. Each tile measured 30 cm tall by 30 cm wide and was either unaltered (smooth),

had 3-mm-wide by 1-mm-deep grooves (shallow-grooved), or had 3-mm-wide by 2-mm-deep grooves (medium-grooved). Grooves were cut 8 mm apart from their respective inside edges. At the beginning of each new test day, tiles were placed at random on the west, south, or east wall of every test box. When the tiles were fixed to the box walls, the grooves were oriented vertically from the floor of the tank.

Experiment 2B: Test of Surfaces With Relevance to Field Application

From 14 July 2005 to 16 July 2005, two preference experiments examined surface characteristics that may be important for field applications. In the first experiment, lampreys were presented with two acrylic tiles that differed in biofilm texture. Within each box, one tile was unaltered (smooth) and the other was covered with vegetable lard to simulate the texture of biofilm. At the beginning of every new test day, the vegetable lard was reapplied. In the second experiment, lampreys were introduced to tiles that differed in the orientation of grooving. Each surface had 3-mm-wide by 2-mm-deep grooves that were 8 mm apart. Tiles were situated such that the grooves of one tile were oriented vertically from the tank floor and the grooves from the other tile were oriented parallel with the tank floor.

The presence of biofilm and the orientation of grooves were tested with the same technique used in Experiment 2A. For consistency, tiles were placed on the east and west walls of each box. Twelve lampreys were randomly selected for each experiment.

Data Handling for Experiments 2A and 2B

Analysis of each 24-hour video involved the documentation of the location and total time of every attachment event. Because this was a preference study, only those lampreys that attached to each wall at least once were included in the analysis. Two metrics were used in the analysis of this experiment: (1) frequency of attachment attempts by each fish to each surface and (2) total time each fish attached to each surface. Both metrics were statistically compared with a Friedman's Test, in which the blocking factor was the individual fish number and the treatment factor was the surface type. Due to the nonnormality of the dataset, the data were best suited to a nonparametric test for statistical comparison purposes. Because the fish could either attach to a surface or choose to rest on the bottom of the test box, the assumption of independence was met.

RESULTS

Behavior within the Pressure Measurement Apparatus

Most of the lampreys that were forced into the experimental chamber quickly moved away from the handler toward the end of the tube with the attachment surface. If the fish did not have this response, the end of the tube was lifted to slide the fish to the attachment surface at the other end (see Figure 3). Once there, much of the lamprey's effort was directed toward escaping from the tube. This struggle continued until the fish located an area for attachment. As suction attachment was established, the escape behavior ceased, and the fish generally maintained the attachment until suction was lost because of pressure loss or experimenter manipulation. When grooved surfaces with large areas for water intrusion were tested, several attempts at attachment were typical and oral disk contortion was common. In instances where the fish could not form a suction seal on the attachment surface, the fish would continue efforts to escape and eventually tire to the point of resting inside the tube without an oral disk attachment. In all cases, experiments lasted 12 minutes.

Experiment 1: Suction Pressure Measurement

EXPERIMENT 1A

Only 6 of the 30 lampreys successfully attached to the 3-mm-wide by 3-mm-deep grooved surface over the 180-second time span; however, 25 of the 30 fish attached to the other three surfaces for the desired time period. These 25 fish were used in the comparison of surfaces. Figure 7 shows a representation of an attachment excerpt from one of the 25 lampreys tested, along with examples of the two quantitative metrics used for surface

comparison. The deepest grooved surface was not included in the analyses because of the small number of successful pressure recordings.

On average, fish attached to the smooth surface pumped at a rate of 2.09 pumps per minute, fish attached to the shallow-grooved surface pumped at a rate of 2.85 pumps per minute, and fish attached to the medium-grooved surface pumped at a rate of 21.5 pumps per minute (Figure 9a). The means were significantly different (ANOVA on log-transformed data: $F_{0.05, 2, 48} = 72.78$, $p < 0.001$), with significantly higher pump rates for the medium-grooved surface compared to the other two surfaces [Tukey HSD (MSE 0.08, df = 48): medium vs. smooth: $p_{(0.05)} < 0.001$; medium vs. shallow: $p_{(0.05)} < 0.001$; smooth vs. shallow: $p_{(0.05)} = 0.293$].

The average lamprey suction pressures dissipated most slowly when fish attached to the shallow-grooved surface (b-value = -0.14, $s_{\xi} = 0.03$), less slowly when fish attached to the smooth surface (b-value = -0.21, $s_{\xi} = 0.04$), and the fastest when they attached to the medium-grooved surface (b-value = -0.60, $s_{\xi} = 0.09$) (Figure 9b). The means were significantly different (ANOVA on square-root of data: $F_{0.05, 2, 48} = 19.63$, $p < 0.001$), and there were significantly higher pressure loss rates for the medium-grooved surface compared to the other two surfaces [Tukey HSD (MSE 0.056, df = 48): medium vs. smooth: $p_{(0.05)} < 0.001$; medium vs. shallow: $p_{(0.05)} < 0.001$; smooth vs. shallow: $p_{(0.05)} = 0.679$].

The visual impression of the attachments was that the lampreys distorted their oral disks the most when attached to the deeper (2-mm-deep and 3-mm-deep) grooved surfaces. This impression was confirmed statistically through the contortion analysis. The average Shore Line Development ratio was 1.04 ($s_{\xi} = 0.002$) for the shallow-grooved surface and 1.06 ($s_{\xi} = 0.003$) for the medium-grooved surface (Figure 10). These data were significantly

different ($t_{0.05\,(2),\,53} = -6.569$, $p_{(0.05)} < 0.001$), and more contortion was required for attachments to the medium-grooved surface.

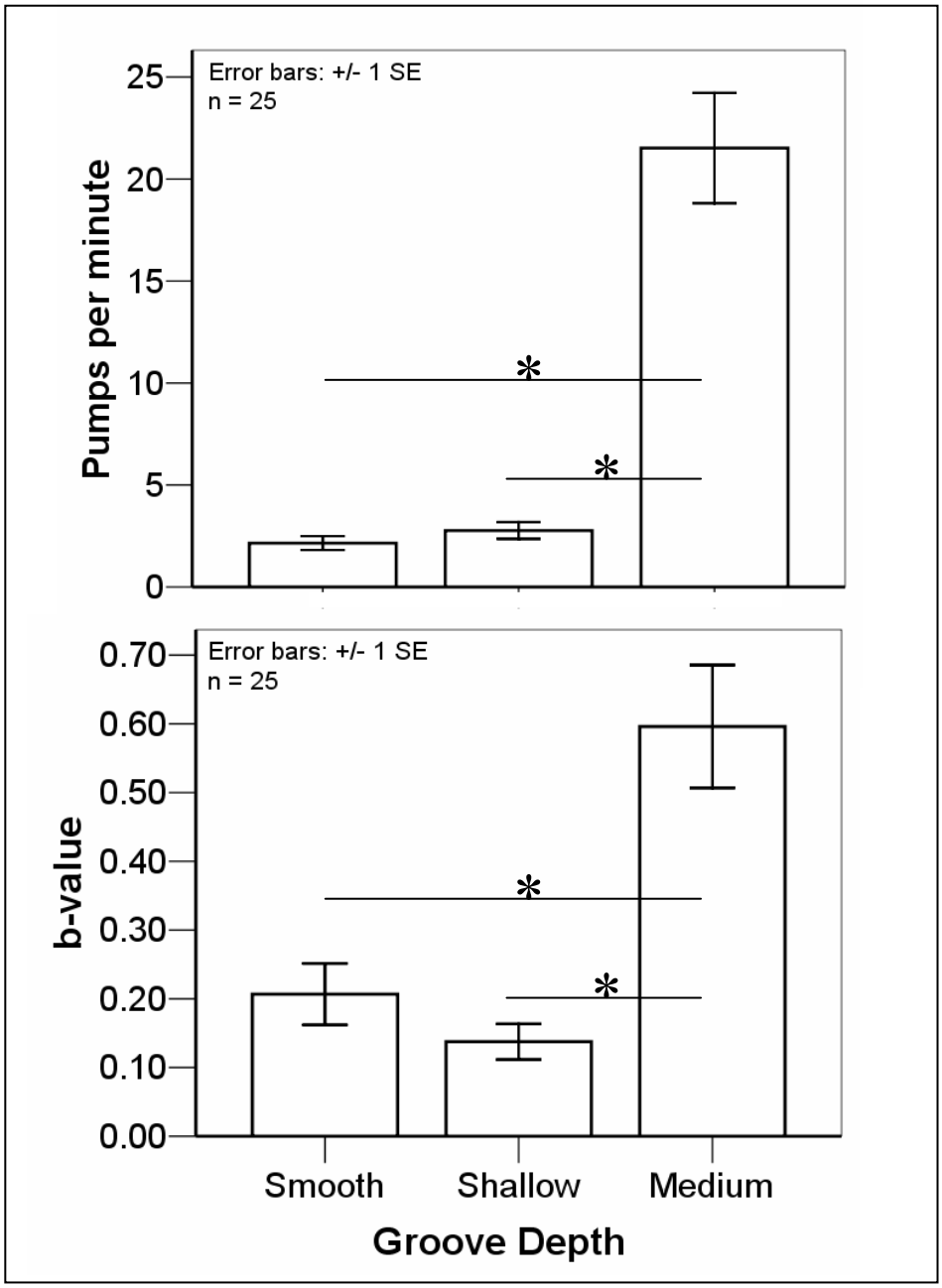

Figure 9 Results graph for Experiment 1A

Graphs of the two pressure characteristics used to quantify attachment ability. 'Pumps per minute' is the frequency of tongue movements per attachment. 'B-value' represents the average rate of pressure loss between tongue movements. Lines indicate significant differences.

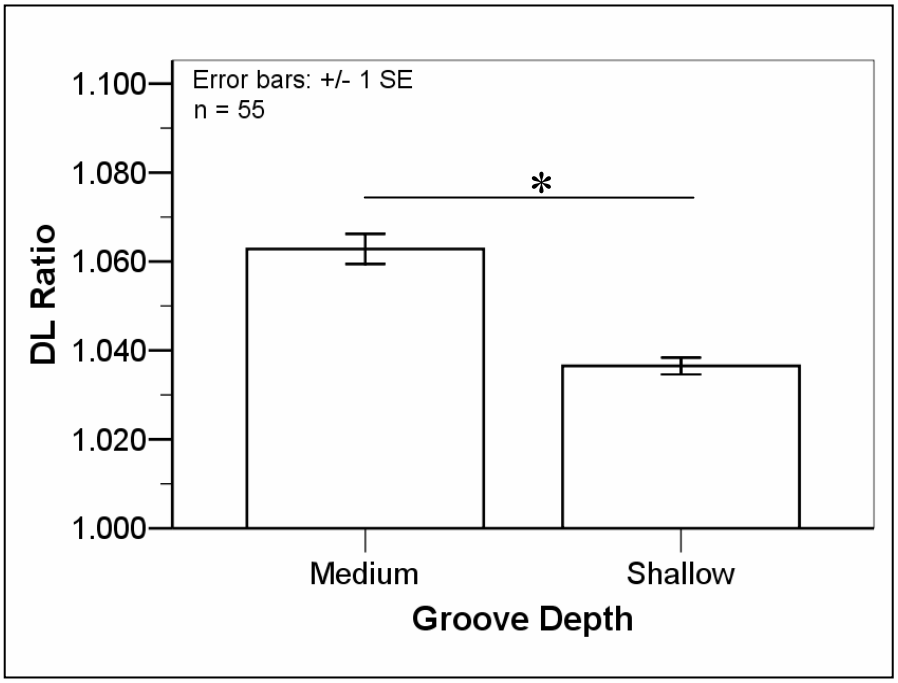

Figure 10 Results graph for the contortion analysis in Experiment 1A

Graph representing the contortion required for attachments to the medium-grooved and shallow-grooved surfaces. This ratio compares how close the oral disk shape is to a circle. The closer the ratio is to 1, the more circular the oral disk will be during attachment. The line indicates significant difference.

EXPERIMENT 1B

There were no significant effects of either simulated biofilm or orientation of grooving on the two pressure metrics (Tables 1 and 2). Results from the groove-width component of Experiment 1B showed no attachments to either the 1-mm-wide by 3-mm-deep grooved surface or the 1-mm-wide by 6-mm-deep grooved surface; however, both pump rate and pressure loss rate were significantly different between the shallow-grooved (3-mm-wide by 1-mm-deep) and medium-grooved (3-mm-wide by 2-mm-deep) surfaces [pump rate: (ANOVA on inverse square root-transformed data: $F_{0.05, 1, 10} = 24.77$, $p = 0.001$); pressure loss rate: (ANOVA on square root-transformed data: $F_{0.05, 1, 10} = 21.45$, $p = 0.001$)]. These latter results were comparable with results from Experiment 1A. In the shape component of

Experiment 1B, significant differences existed in ability to attach to the grooves of differing shapes in the comparison of pump rates (ANOVA on log-transformed data: $F_{0.05, 2, 16} = 7.45$, $p = 0.005$); however, no significant differences were found in the comparison of pressure loss rates (ANOVA on square root-transformed data: $F_{0.05, 2, 16} = 2.15$, $p = 0.149$; Power = 0.258) (Figure 11). A Tukey pairwise comparison test of the pump rate metric showed attachment to the rectangular-cut grooving to require a significantly higher frequency of pumps than an attachment to the triangular cut (Tukey HSD: MSE 0.085, df = 16, $p_{(0.05)} = 0.004$).

Table 1 Summary table for pump frequency analysis in Experiment 1B

Summary table for the pump rates characteristic used to quantify attachment ability in the pressure measurement experiments. 'Pump Rates' refers to the number of tongue pumps per minute.

Pump Rates					
Experiment	**n**	**Factors**	**mean pumps/min**	**SE**	**RB ANOVA**
Width	11	3 X 1 (shallow)	2.61	0.55	$F_{1,10} = 24.77$, $p = 0.001$
		3 X 2 (medium)	21.2	3.62	
		1 X 3	***no attachments***	--	--
		1 X 6	***no attachments***	--	--
Biofilm	13	No Biofilm	1.87	0.29	$F_{1,12} = 0.010$, $p = 0.932$
		Biofilm	2.15	0.66	
Orientation	9	Vertical	12.5	2.89	$F_{1,8} = 1.16$, $p = 0.314$
		Horizontal	8.44	2.29	

Table 2 Summary table for the pressure loss analysis in Experiment 1B

Summary table for the pressure loss rate characteristic used to quantify attachment ability in the pressure measurement experiments. 'Pressure Loss' refers to the rate of suction pressure lost between tongue pumping events.

Pressure Loss					
Experiment	**n**	**Factors**	**mean b-value**	**SE**	**RB ANOVA**
Width	11	3 X 1 (shallow)	-0.12	0.04	$F_{1,10} = 21.45$, $p = 0.001$
		3 X 2 (medium)	-0.45	0.08	
		1 X 3	***no attachments***	--	--
		1 X 6	***no attachments***	--	--
Biofilm	13	No Biofilm	-0.13	0.04	$F_{1,12} = 3.22$, $p = 0.10$
		Biofilm	-0.15	0.04	
Orientation	9	Vertical	-0.40	0.14	$F_{1,8} = 0.06$, $p = 0.385$
		Horizontal	-0.25	0.07	

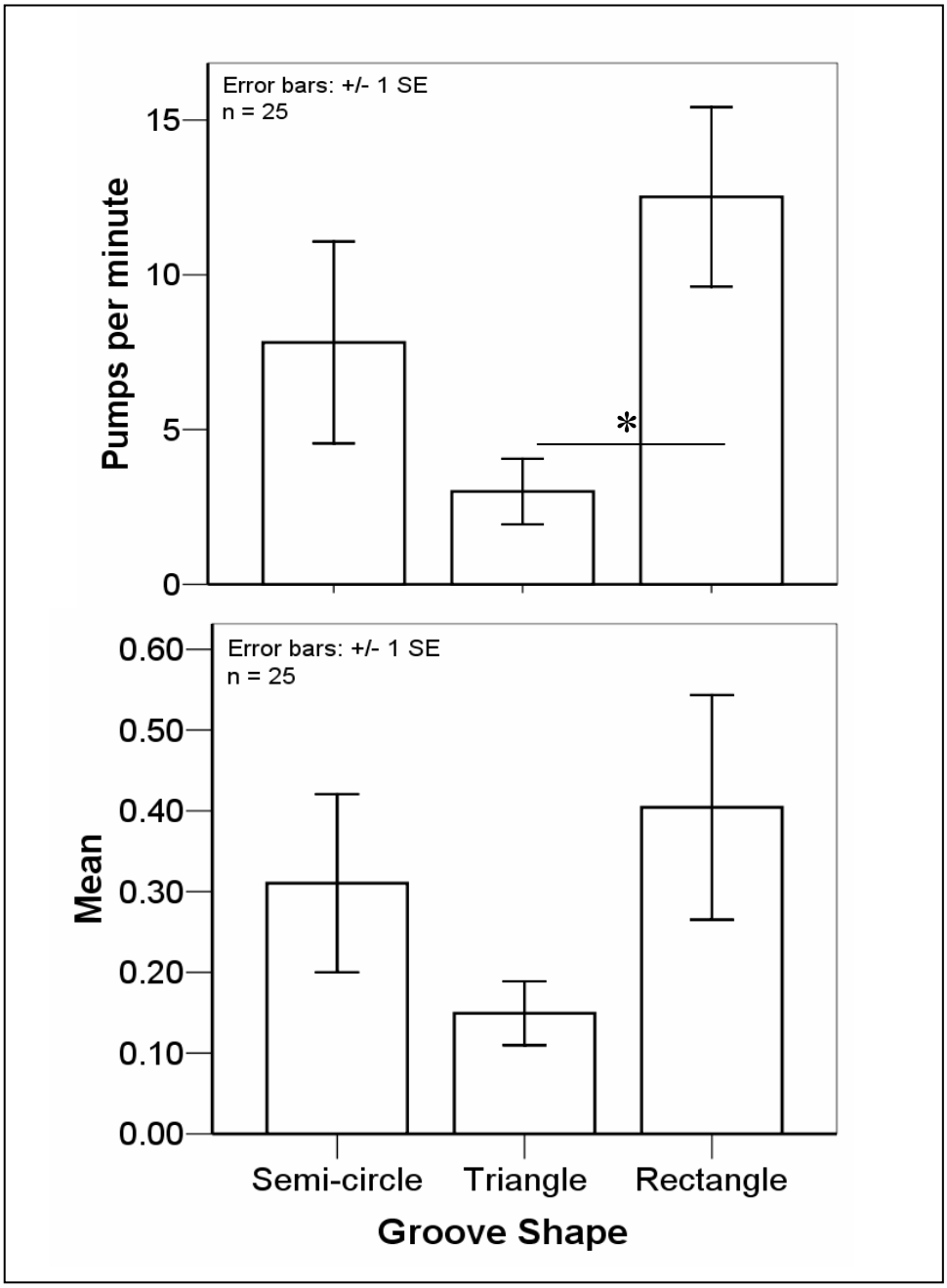

Figure 11 Results graphs for the shape of groove study in Experiment 1B

Graphs of the two pressure characteristics used to quantify attachment ability for different-shaped groove cuts. 'Pumps per minute' is the frequency of tongue movements per attachment. 'B-value' represents the average rate of pressure loss between tongue movements. Lines indicate significant differences.

EXPERIMENT 1C

The maximum pressures of 12 sea lampreys were plotted against their corresponding oral disk areas (Figure 12). The mean for the 12 maximums was –61.65 kpa and the mean oral disk area was 910 mm^2. A linear regression model was used to examine the resulting relationship. The R^2 for the regression line was 0.21, which, at a sample size of 12, was not significant ($p_{(0.05)} = 0.14$). The power of the statistical test was 0.321. On the basis of this regression equation, the sample size required for a significant result (Power of 0.80) is 37 fish.

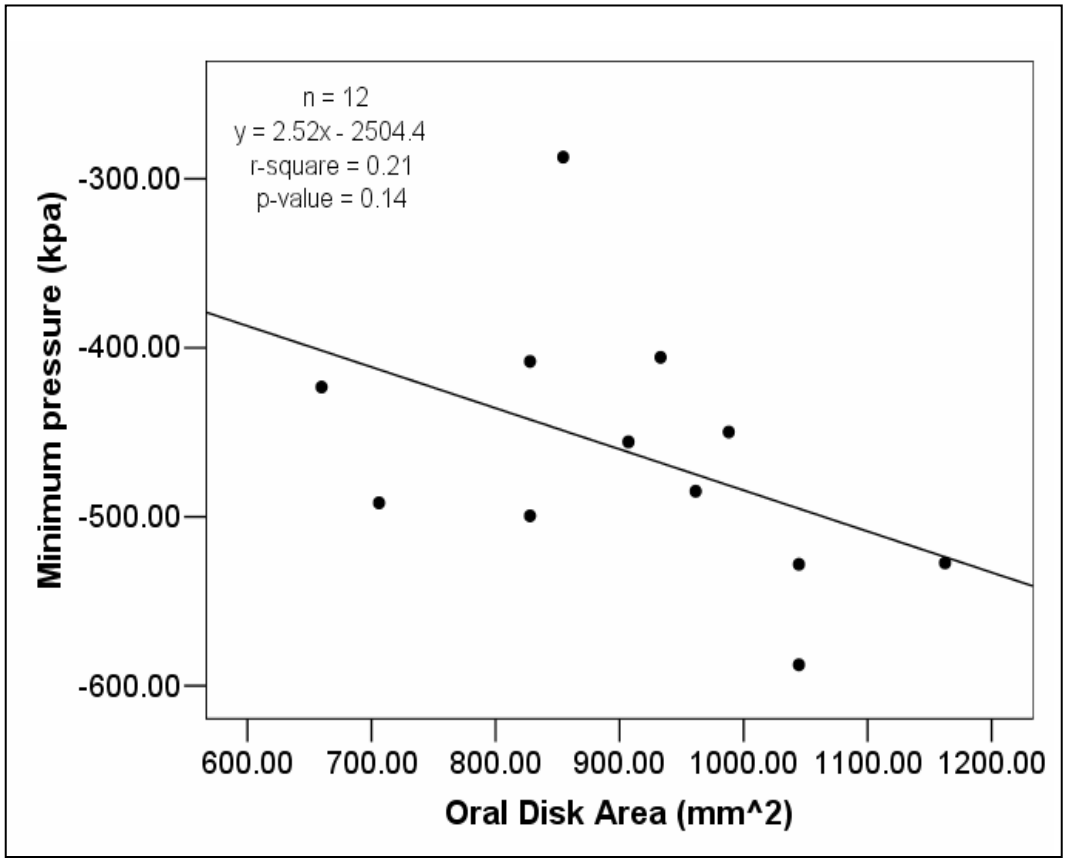

Figure 12 Results graph for Experiment 1C

The linear relationship between maximum negative pressures and oral disk areas. These pressures signify the most negative pressures exerted by test sea lamprey while manually pulled from the attachment surface. Individual oral disk areas were calculated from measurements taken of oral disk height and width.

Experiment 2: Preference of Attachment Site

EXPERIMENT 2A

Twenty-four of the 30 lampreys attached to each surface at least once. The attachment frequency and residence times of these 24 fish were further compared. On average, the lampreys made 15.79 attachments to the smooth surface, 21.88 attachments to the shallow-grooved surface, and 19.33 attachments to the medium-grooved surface. Differences were compared in a Friedman's Test and were significant ($p_{(0.05)} = 0.01$). A multiple comparison test showed the average number of attachments to the shallow-grooved surface to be significantly higher than to the average number of attachments to the smooth surface (Wilcoxon Signed-Ranks tests with a Bonferroni Correction: $Z = 2.517$, $p = 0.012$).

On average, the lampreys attached 306.89 minutes to the smooth surface, 273.05 minutes to the shallow-grooved surface, and 197.20 minutes to the medium-grooved surface. Differences were compared in a Friedman's Test and were not significant ($p_{(0.05)} = 0.75$). In addition to time spent attached, lampreys spent an average of 662.87 minutes resting at the base of the test box.

EXPERIMENT 2B

There was no significant effect of simulated biofilm or orientation of grooving on either the number of attachments or the total residence time. A summary of these results can be found in Tables 3 and 4.

Table 3 Summary table for attachment frequency in Experiment 2B

Summary table for the number of attachments characteristic used to quantify attachment preferences. 'Number of Attachments' refers to the average proportion of attachments per surface out of the total number of attachments.

Number of Attachments					
Experiment	**n**	**Factors**	**Proportion**	**SE**	**Friedman's Test**
Biofilm	12	No Biofilm	0.552	0.029	$p = 0.366$
		Biofilm	0.448	0.029	
Orientation	6	Vertical	0.548	0.044	$p = 0.564$
		Horizontal	0.452	0.044	

Table 4 Summary table for attachment time in Experiment 2B

Summary table for the attachment times characteristic used to quantify attachment preferences. 'Attachment Times' refers to the average proportion of time spent on each surface out of the total time spent attached.

Attachment Times					
Experiment	**n**	**Factors**	**Proportion**	**SE**	**Friedman's Test**
Biofilm	12	No Biofilm	0.545	0.070	$p = 0.414$
		Biofilm	0.455	0.070	
Orientation	6	Vertical	0.426	0.121	$p = 0.414$
		Horizontal	0.574	0.121	

DISCUSSION

The main finding of this study is that a lamprey's ability to attach and maintain suction depends on the interaction between the surface topography and the lamprey's ability to adjust its oral disk shape to the texture. The pressure-measurement apparatus developed for this study provided a means for quantifying this complex interaction. Limitations to the suction seal were documented on surfaces with grooves varying in depth, width, and shape. Combining these results with those by Gradwell (1972) and McAuley (1993), we now have a much larger knowledge base for understanding attachment by suction. In addition to the biological relevance, these results may also be useful in management applications in which attachment may be of concern (Ocker and others 2001; Lavis and others 2003). To hinder attachment, grooves should be placed very close together (< 8 mm apart) and cut at most 1 mm wide and at least 3 mm deep. If attachment is desired, surfaces do not necessarily have to be smooth, but they must take into account the flexibility of the fimbriae and oral disk to ensure a lasting suction seal.

Contrary to expectations, the lampreys did not prefer the smooth surface to the grooved surfaces. In fact, little preference was observed at all. Multiple reasons could account for these results; however, they are speculative because attachment characteristics such as pressure loss and tongue pumping could not be monitored in the experiment. Future preference experiments should be performed in high-velocity environments where attachment must be used to maintain position. This type of experimentation may provide insight into the ability to use attachable surfaces to direct the upstream movement of lampreys (Ocker and others 2001; Quintella and others 2004).

Experiment 1: Suction Pressure Measurement

To determine the quantifiable characteristics used in pressure analysis, our pressure records were compared with those reported by Gradwell (1972). Through visual observations of the tongue in concert with fluctuations in the pressure recordings, Gradwell's explanation of the tongue's importance to pressure oscillation was verified. The reasoning behind this observation lies in the role of the tongue in suction-pressure creation and maintenance (see Figure 2).

The tongue serves as a valve between the buccal funnel and the rest of the oral cavity. On initial contact with a surface, the tongue seals the buccal funnel from the rest of the mouth. Next, the hydrosinus forces water from the pharyngeal cavity through the velum, where it is expelled out the branchial pores. When the tongue reopens the buccal cavity to the rest of the mouth, there is an area of low pressure from the buccal funnel to the velum (Reynolds 1931; Lanzig 1958, Hardisty 1979). Once suction is established, any water leaking in through a disruption in the suction seal can be flushed out with the same process: The tongue seals the buccal funnel from the rest of the mouth, the excess water is flushed out through the compression of the hydrosinus, and the tongue reopens the buccal funnel to restore suction throughout the oral cavity. Therefore, by calculating the rate of tongue pumping as well as the rate of pressure loss between the tongue pumps, the ability of a surface to facilitate suction could be quantified.

With these metrics in place, McAuley's (1993) surface attachment study was expanded upon. Unlike McAuley's field experiment, the use of the pressure-measurement apparatus allowed a methodical, quantitative approach to the comparison of surfaces.

In Experiment 1A, drastic differences in attachment ability were documented among the grooved surfaces. The most pronounced difficulty in attachment was seen on the deep-grooved surface. Pressure records from attachments to this surface showed that a groove with the dimensions 3 mm wide by 3 mm deep was sufficient to prevent 95% of test fish from prolonged (180-second) attachments.

The best-documented disparity of attachment ability in Experiment 1A was between the shallow-grooved (3 mm wide by 1 mm deep) and medium-grooved (3 mm wide by 2 mm deep) surfaces. Due to water intrusion to the oral disk, fish lost suction pressure at a rate 4.3 times greater when attached to the medium-grooved surface than when attached to the shallow-grooved surface (see Figure 10). Additionally, the rate of tongue pumping was 7.5 times higher on the medium-grooved surface. During attachments to the shallow-grooved surface, little contortion of the mouth was necessary to maintain a suction attachment. Conversely, lampreys attached to the medium-grooved surface contorted their mouths significantly (Figure 13). Through contortion, the lampreys were able to mold their fimbriae to the grooves, which slowed water intrusion to the oral disk. Although this process allowed attachments to be established on grooved surfaces, the maintenance of the attachment required a continuous effort to remove the intruding water. For a spawning-phase lamprey with limited energy reserves, it may not be energetically efficient to maintain this type of attachment over an extended period of time.

Results from the width and shape components of Experiment 1B show that the groove shape is as important as intrusion area for the maintenance of suction. In the width component, none of the 30 experimental fish were able to attach to the surface with 1-mm-wide by 3-mm-deep grooves. This is significant as it represents the same cross-sectional area

of water intrusion as the aforementioned shallow-grooved surface, which did promote a strong suction seal. In the comparison of triangular, rectangular, and semi-circular cut grooves, major differences in attachment ability were observed between the triangular and rectangular cuts even though the areas of intrusion were the same. Lampreys attached to the rectangular grooves pumped at a significantly higher rate than when attached over the triangular grooves. These results were consistent with McAuley's (1993) findings. His test lampreys had no problem attaching to both his 11-mm wide by 6-mm deep triangular cut slab and 22-mm wide x 10-mm deep triangular cut slab. He noted that the flexibility of the oral disk allowed ease of attachment to these surfaces. The shape of the triangular cut in the current experiment allowed the lampreys to wedge their oral disks into the grooves to seal the intrusion. An illustrated representation of this ability is shown in Figure 14. Results from the orientation and biofilm components of Experiment 1B demonstrated that either these textures had no effect on attachment ability or the sample sizes were too small to yield statistically significant results.

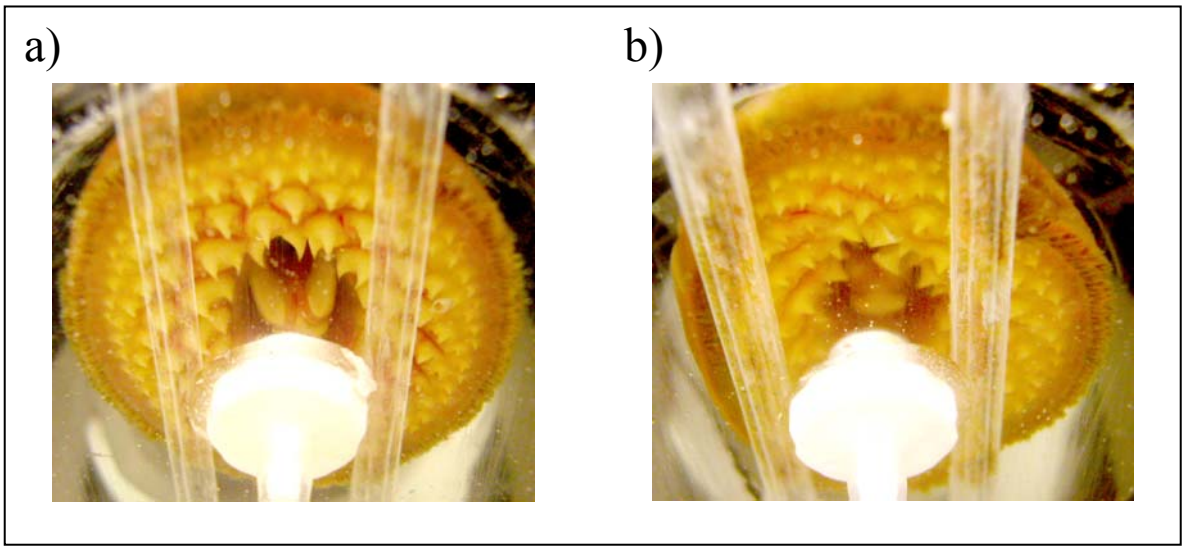

Figure 13 Pictures of two visible levels of oral disk contortion

Pictures taken of attachment events to surfaces with vertical grooving. (a) Example of an attachment requiring little oral disk contortion (b) Example of an attachment requiring a large amount of oral disk contortion.

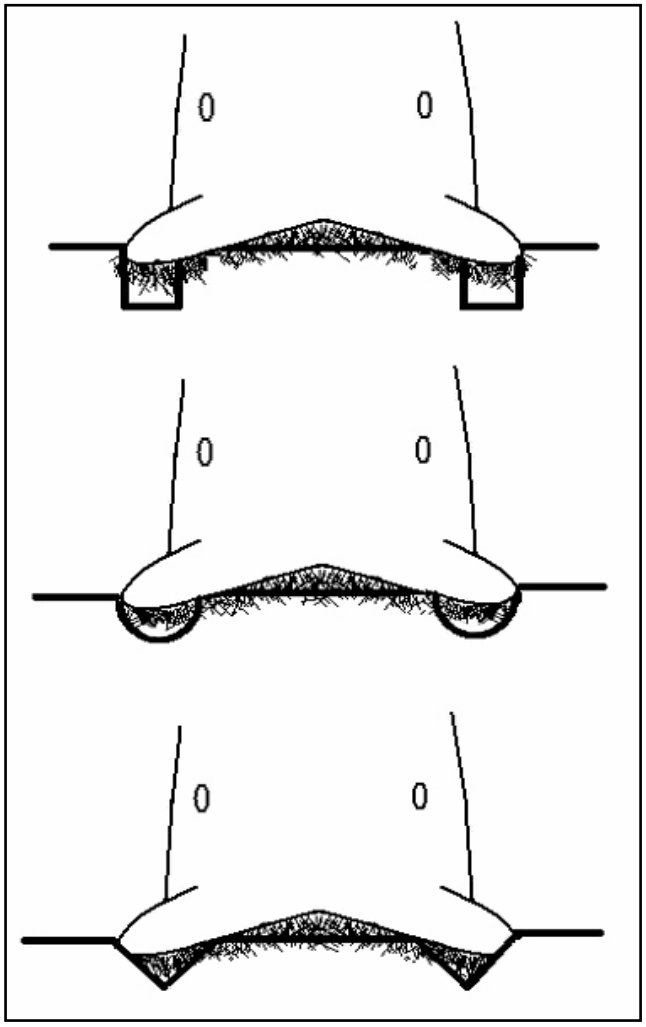

Figure 14 Schematic of attachments to the three different groove shapes

Visual representation of the different attachment abilities on rectangular, semi-circular, and triangular groove cuts.

In Experiment 1C, the average of the 12 pressure maximums was -61.65 kpa, a value lower than the most negative pressure of -11.73 kpa recorded by Gradwell in 1972. In both situations, lampreys were pulled until detached. The reason for this disparity may lie in the physical condition of Gradwell's lampreys in that his cannulation surgeries may have had an effect on suction performance. This is unlikely, however, as the lampreys used in the current maximum test had been relying on energy reserves for roughly two months. Another explanation lies in difference between the pressure-measurement devices. Gradwell mentioned that the most negative suction pressure he attained exceeded -11.73 kpa, which implies that the pressures may have surpassed the maximum pressure readings available through his device. It is also likely that the maximum pressures exerted by a lamprey should exceed this value (a tenth of ambient pressure standard = 101.33 kpa) as would be required in parasitic and substrate attachments in high-sheer environments.

Experiment 2: Preference of Attachment Site

The preference experiments were designed to complement the results found in the pressure-measurement experiments. Lampreys were expected to recognize and prefer those surfaces that best promoted attachment in Experiment 1A. Contrary to these expectations, however, the lampreys showed few signs of preferring smooth surfaces to grooved surfaces and very little preference overall. Of most interest were the results for the surface with medium grooving. In Experiment 1A, the medium-grooved surface required significantly more effort to maintain a suction attachment. Surprisingly, an average of 33% of all individual attachments were to this surface, and an average of 30% of the total attachment time was for the medium-grooved surface.

One explanation for this lack of preference is that sea lampreys may only have the ability to differentiate between attachable and nonattachable surfaces. Because the lampreys in this study were offered surfaces that all allowed attachment, none would have appeared the definitive choice. Although this is a plausible explanation, if there were constant tongue pumping on the grooved surfaces, it would have been energetically demanding and likely not preferred by the individuals.

A second explanation for the preference results may be the ability of the sea lampreys to contort their mouths. Unlike in the pressure-measurement chamber, the sea lampreys in the preference arena had more room to swim and a much larger attachment area on each surface. Combining these two factors, the lampreys may have increased their chances of success in forming a tight seal by selecting an area for attachment and forcibly filling their oral disks into the groove spaces through body propulsion. Unfortunately, tongue-pump rates and pressure-loss rates could not be monitored, so this explanation cannot be verified.

Finally, a higher flow environment may yield different results. A similar experiment should be held in a high-sheer environment, such as the one used by McAuley in 1993. The higher flow rate may force lampreys to make the most efficient attachments possible. If pressure were lost too quickly because of a leak in the suction seal, the lamprey would likely be swept downstream and forced to expend more effort to swim back to its original stream position.

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