The physiology and mechanics of undulatory swimming: a student laboratory exercise using medicinal leeches

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Ellerby DJ. The physiology and mechanics of undulatory swimming: a student laboratory exercise using medicinal leeches. Adv Physiol Educ 33: 213–220, 2009; doi:10.1152/advan.00033.2009.—The medicinal leech is a useful animal model for investigating undulatory swimming in the classroom. Unlike many swimming organisms, its swimming performance can be quantified without specialized equipment. A large blood meal alters swimming behavior in a way that can be used to generate a discussion of the hydrodynamics of swimming, muscle mechanics, hydrostatic skeletons, and the physiological features that allow leeches to deal with the volume increase and osmotic load imposed by the meal. Analyses can be carried out at a range of levels tailored to suit a particular class.

Hirudo verbana; Hirudo medicinalis; feeding; locomotion

SWIMMING ANIMALS use muscle power to increase the kinetic energy of the surrounding water, generating movement. This can be achieved using propulsive appendages like fins or paddle-shaped limbs (e.g., Ref. 29) or via undulations of the body axis, as in many fish (e.g., Ref. 1). Analysis of swimming behavior in many animals is not feasible as part of a teaching exercise. Rapid propulsive movements require a high-speed camera for accurate analyses, and, particularly in larger organisms, a swimming flume is needed to maintain the animal’s position relative to the camera as it swims against a controlled current. The swimming movements of a sufficiently small and slow experimental organism, for example, the medicinal leech (Hirudo verbana or H. medicinalis), can be quantified without these types of specialized equipment. Medicinal leeches are therefore a suitable model organism for a teaching exercise investigating the physiology and mechanics of swimming.

Swimming in leeches is powered by dorsoventral undulations of the body axis. These are created by longitudinal body wall muscles contracting against an incompressible, fluid-filled, hydrostatic skeleton (10, 39, 41). The swimming rhythm is generated by neuronal oscillators in the ventral nerve cord (9, 24) that trigger waves of muscle contraction moving from anterior to posterior along the body. Adult medicinal leeches with a body length of 10 cm have a swimming cycle frequency of 2–3 Hz at 20°C (6, 14), allowing their swimming movements to be accurately quantified in an aquarium using a standard video camera.

The body undulations transfer kinetic energy to the water, creating a wake behind the animal (7). This typically consists of a series of spinning vortexes that are periodically shed from the trailing edge of the propulsor when it changes direction during the propulsive cycle (7, 30, 45). Organisms that move through fluids by undulation of the body, or oscillation of wings or fins, do so in such a way that the relationship between their forward velocity (U), propulsor cycle frequency (f), and amplitude of propulsor movement (A) is relatively constrained. This relationship is summarized by the Strouhal number (St), which is calculated as St = fA/U (48). St describes the properties of the wake in terms of the rate of vortex formation and vortex spacing (48). St for most swimmers and fliers falls within the range of 0.2 < St < 0.4 (31). Leeches are no exception, typically swimming with a St of 0.25–0.37 (6, 14).

Propulsive efficiency and thrust production both peak within the same St range favored by swimmers and fliers (2, 44), leading to the suggestion that natural selection has tuned St to maximize locomotor performance (31).

The consequences of adopting a suboptimal St are not usually observable as most locomotor systems cannot be manipulated to alter St. Undulatory swimming in medicinal leeches is an exception. Their feeding behavior is associated with a marked change in swimming performance. This feeding effect is due to the extreme size of the blood meal, which can increase body mass to 900% of the prefeeding value (21). This alters the function of the body wall muscles and hydrostatic skeleton with consequent effects on swimming ability (6). An investigation of swimming kinematics before and after feeding provides a framework for discussing the links between animal swimming movements, fluid dynamics, and swimming performance.

Here, I describe a teaching laboratory that was developed as part of an upper-level undergraduate physiology and biomechanics course. The laboratory is designed to complement lecture and discussion sessions focused on muscle as a power source for swimming and flying and how this power is used to increase the kinetic energy of the surrounding fluid. The objective of the laboratory is not only to reinforce lectures but also to train students in a range analytic skills and generate discussion about the physiology and mechanics of movement and feeding in medicinal leeches. More broadly, the hydrodynamic principles discussed can be applied to all organisms that move through fluids.

METHODS

Equipment

The following equipment is needed:

- 10-gallon holding aquarium (1 per class)
- 37°C water bath for warming blood (1 per class)
- Video camera (1 for the class, more if available)
- 15-gallon swimming aquarium with a lane created along one side (1 for each video camera)
- Ruler or tape measure (1 per class)

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Teaching In The Laboratory

LEECH SWIMMING LABORATORY PROJECT

- PC or Mac for video analyses (1 per group) with the following software: 1) video editing software (e.g., iMovies or Windows Movie Maker) and 2) video analysis freeware (e.g., ImageJ or NIH Image).
- Clamp stands for holding the feeding apparatus (1 per group)
- 500-ml beaker (1 per group)
- Small, fine-mesh aquarium nets (1 per group)

Disposable Supplies

The following disposable supplies are needed:

- Difibrinated mammalian blood (50 ml per group)
- Parafilm or sausage casing
- 15-ml polystyrene centrifuge tubes with a conical end cutoff (1 per group)
- Laboratory tape and a marker pen for calibration marks
- Solution of 0.75 mg/l aquarium salt in deionized water (see below)
- 250-ml jars with screw caps (1 or 2 per group)
- Disposable gloves (1 pair per student)
- 8% and 70% ethanol solutions if euthanasia for dissection specimens is required

Animals

Medicinal leeches can be purchased from commercial suppliers, and renewed interest in their medical usage has ensured their wide availability (Leeches USA, Westbury, NY; Carolina Biological Supply, Burlington, NC; Biopharm UK, Hendy, Carmarthenshire, UK; and additional worldwide distributors via Biopharm). Although typically listed as *H. medicinalis*, they are likely to be *H. verbana* (38).

They are maintained at room temperature in an aquarium containing deionized water with 0.75 g/l aquarium salt (e.g., Doc Wellfish, Chalfont, PA) added. The aquarium should be covered with a fine mesh or gauze such as mosquito netting to prevent escapes, and the water should be changed every 2–3 days. Depending on the experimental design, either 1 or 2 leeches are needed per group, plus some spares in case of refusal to feed (see below). Additional leeches can be provided for dissection so that students can examine the morphological features mentioned in subsequent discussions. If so, leeches are initially provided for dissection so that students can examine the morphological features mentioned in subsequent discussions. If so, leeches are initially anesthetized in 8% ethanol and then, after the cessation of movement, transferred to 70% ethanol for euthanasia. During the laboratory, individual leeches are kept in 250-ml jars of aquarium salt solution with screw closures. These should be labeled by the student groups to identify their leeches. Leeches are primarily moved using aquarium nets. Students should use gloves if handling leeches directly as the unfed leeches will appear to have warm skin.

Video Analyses of Swimming

The primary experimental aim is to obtain and analyze video clips of swimming leeches before and after feeding. The methods described here have been adapted from previously described experiments (6). The video recording setup and video analyses have been simplified so that they can be completed as part of a teaching exercise.

Leeches swim in a 10-cm wide lane created along the long edge of a 15-gallon aquarium tank (typical length: ~60 cm) with a plastic barrier the same length as the tank. The water depth should be ~6 cm, and the barrier height should be ~10 cm. The barrier, cut from an acrylic sheet or a similar material, should fit closely to the bottom and end walls of the tank and be held in place with silicone aquarium sealant to prevent escapes from the swimming lane. A light-colored barrier or dark matte barrier and strong lighting will maximize background contrast with the leeches. Leeches swim with dorsoventral undulations, so a video camera placed horizontally perpendicular to the center of the swimming lane will capture the frequency and amplitude of the swimming cycles. Calibration marks placed a known distance apart and visible in the camera field of view should be made on the tank wall with tape or a marker pen. To minimize the appearance of the camera as a reflected image on the aquarium wall, a dark cloth with a hole cut for the lens can be draped over the camera.

Individual leeches are moved from their holding container to the swimming lane with a small aquarium net. Swimming is initiated by touching the posterior end of the animal with the net handle or a ruler. Leeches stop swimming if allowed to contact a surface and attach with their suckers. If this happens, the leech should be lifted away from the lane walls with the net handle, ruler, or a gloved hand to initiate swimming.

Several video clips should be obtained for each leech to ensure that a bout of relatively steady swimming is recorded. Clips of leeches before and immediately after feeding (see below) are required. These are downloaded to a computer hard drive for analysis. Downloads from a digital video camera are typically made via a FireWire (IEEE 1394) connection and can be saved using iMovie (Mac), Windows Movie Maker (PC), or a similar video capture and editing application. PCs will likely require a FireWire adapter. Older cameras with analog S-Video or composite cable connections will require a compatible hardware device such as a video capture card. Clips used for further analysis should include three or more propulsive cycles with the posterior end of the leech and calibration markers clearly visible. Ideally, analyses should be confined to video segments where the leech is swimming horizontally and not in contact with the aquarium walls. After the completion of filming, the body mass of each leech is recorded.

Analyses are carried out using NIH Image or ImageJ (on Mac or PC, respectively). These are freeware programs available from http://rsb.info.nih.gov/ij/download.html (ImageJ) and http://rsb.info.nih.gov/nih-image/download.html (NIH Image). ImageJ and NIH Image work with AVI and Quicktime movie files, respectively. Both programs have similar analysis functions. The point-selection tool is used to mark and record the horizontal (x) and vertical (y) coordinates of a point on a frame selected with a mouse click. The line-selection tool is used to measure the lengths of segments traced on a frame. This tool is also used to spatially calibrate the image using the calibration markers on the aquarium. Any similar video analysis application capable of recording calibrated coordinates and segment lengths could also be used, e.g., VideoPoint (Lenox Softworks, Lenox, MA).

The majority of the kinematic parameters can be calculated by tracking the movements of the trailing edge of the leech across one or more locomotor cycles. The start of a propulsive cycle is taken as the dorsal or ventral extreme of trailing edge displacement. Mean propulsive cycle frequency (f; in Hz) can be calculated as follows: $f = N/(FR \times NF)$, where FR is the frame rate (in Hz; 29.97 NTSC or 25 PAL digital video), N is the number of complete, consecutive propulsive cycles, and NF is the number of video frames spanned by the cycles. Mean forward swimming velocity (U; in m/s) can be calculated as follows: $U = D/(FR \times NF)$, where D is the distance traveled in the x-direction during the given number of frames. Stride length (SL) is the distance traveled during a propulsive cycle and is equal to U/F. Trailing edge amplitude (A) is estimated from the difference in the y-coordinates of the trailing edge position between the dorsal and ventral extremes of a locomotor cycle. $St$ is calculated as follows: $St = fA/U$.

Feeding Apparatus

The leeches are fed warmed, defibrinated mammalian blood readily available from commercial suppliers. The data presented were obtained using a water-jacketed tissue bath (Radnoti 1583, 50 ml, Radnoti Glass Technology, Monrovia, CA) containing defibrinated sheep blood (product no. DSB500, Hemostat Laboratories, Dixon, CA) maintained at 37°C. The leeches were fed via a lateral 1-cm-diameter access port covered with Parafilm (sausage casing can also be used) and presented to a leech in a container of aquarium salt solution. A simpler feeding apparatus can be constructed from a 15-ml polystyrene centrifuge tube with the conical end removed to form a cylinder. This is placed vertically in a clamp, with the lower opening covered with Parafilm and the tube filled with 37°C...
blood. The Parafilm is immersed in a 500-ml beaker of room temperature aquarium salt solution containing a leech (see Fig. 2) (21). For this setup, occasional blood top ups may be needed to maintain the blood temperature. The initiation of leech feeding behavior is stimulated by chemical and thermal cues; a small hole made in the Parafilm with a pin to release blood into the outer water container therefore acts as an attractant in addition to the warmth of the feeder. Care should be taken not to release a large amount of blood that accumulates at the bottom of the beaker as the leech may move toward this rather than the Parafilm. A collar made from laboratory tubing can be used to direct the leeches toward the Parafilm as they tend to indiscriminately bite any part of the warm feeding apparatus. If necessary, the leech can be lifted with a gloved hand so that its searching anterior end will encounter the Parafilm. After attachment to the Parafilm with the anterior sucker, a leech will feed until sated and then spontaneously detach. This typically takes 20–30 min. If a leech does not start to feed within 5 min, replace it with an alternate leech. Fed leeches should not be kept in the same container as unfed leeches as cannibalism may occur (18).

Practical Considerations

The exercise is designed to be carried out within a 3-h laboratory period. Students work in groups of three or four to collect and analyze data. Data from all class groups are collated for statistical analyses and report writing. A number of factors determine the time needed to complete the exercise. The steps with the most potential to delay the analyses are video recording, video download, and leech feeding. The extent of these time constraints is determined by the number of video cameras and swimming tanks available, the speed of the video download (maximized by recording and selecting short clips), the number of feeding setups (ideally 1 per group), whether the same leeches are used for pre- and postfeeding measurements, and the number of student groups.

Assuming a single camera is available, the unfed leeches should be video recorded sequentially in a single session at the start of the laboratory. A Post-it note with an identifier for each group’s leech should be placed in the field of view. These sequences are downloaded, and the analysis started while the leeches are being fed. Time can be saved by using separate groups of leeches for the unfed versus fed comparison. This means that feeding for the latter group can be started immediately at the start of the laboratory period while video recording of the unfed group is underway. Division of labor within laboratory groups (leeching swimming for video recording, video analysis, leech feeding, and calculations based on kinematic data) will minimize the time taken for data collection and analyses by allowing several tasks to be performed concurrently. Some leeches will not swim immediately after feeding, but the rate of fluid and mass loss from the leech is so rapid that swimming ability will be regained if the leech is left for 5–10 min.

Statistical Analyses

If two swimming conditions (prefeed and immediately postfeed) are recorded, then t-tests are used to test for significant differences in the various kinematic parameters between the two conditions. The experimental design will determine the type of t-test used: paired if the same group of leeches were recorded before and after feeding, and unpaired if separate groups were used. The recovery of swimming ability is relatively rapid (6). For an assessment of recovery, a group of leeches fed 24 h before the laboratory can be provided as an additional study group. If so, ANOVA is used to test for significant differences in kinematic parameters between groups. Alternatively, a 24 h postfed leech can be provided for a qualitative demonstration of fluid and mass loss and associated swimming recovery.

RESULTS

Typical results are shown in Fig. 1. Figure 1 shows representative images of swimming leeches before and after feed-
propulsive movements and the resulting forward velocity of the animal. This analysis can be taken further to illustrate how these kinematic data can be used to estimate the efficiency of locomotion and the properties of the wake generated by a swimmer or flier. The data set can also be used to teach students the appropriate statistical analyses to test for significant differences in kinematic and performance variables between two or three experimental conditions depending on the experimental design used.

Quantifying Swimming Performance

Many organisms, both vertebrate and invertebrate, move through fluids. While spanning a vast range of sizes, and adopting a variety of locomotor modes, movement is achieved through the same basic mechanism: muscle power is used to increase the kinetic energy of the surrounding fluid. This applies to the flapping wings of birds and insects in air and to the flapping fins and paddles or undulating bodies of aquatic animals. The principles demonstrated in the present exercise can therefore be applied widely in discussing both swimming and flight.

A further common feature of swimming and flying in many organisms is that the transfer of energy to the fluid results in the formation of a wake. The nature of the wake is determined by three factors: the viscosity of the fluid, the velocity of movement, and the size of the organism. These are the basis for calculating what is perhaps the most useful descriptor of fluid flow, the Reynolds number \( Re \). \( Re \) can be calculated as follows:

\[
Re = \frac{UL}{v},
\]

where \( v \) is the kinematic viscosity of the fluid (in the present example \( 1.0 \times 10^{-6} \text{ m}^2/\text{s} \) for water at \( 20^\circ\text{C} \)) and \( L \) is the leech length (in m; estimated from the video images using the NIH Image/Image J line-selection tool). \( Re \) represents the ratio of inertial to viscous forces in a flow regime (48). Inertial forces are those associated with fluid momentum, and viscous forces are those associated with a fluid’s internal resistance to flow. High \( Re \) favor the formation and persistence of vortexes and therefore a detectable wake behind swimming animals (7, 45). At low \( Re \), the viscous forces tend to “smooth out” the flow, inhibiting vortex formation and favoring smooth, laminar flows (48).

The \( Re \) for leeches before feeding is \( \sim 18,000 \) and falls to \( \sim 6,000 \) after feeding. This places leeches in an inertially dominated flow regime where vortexes will be shed into the leech’s wake during swimming (49). This allows for further hydrodynamic analyses based on the links between kinematics, wake properties, and swimming performance.

A number of factors underlie the change in performance associated with feeding. Decreased speed is partly explained...
by the drop in propulsor cycle frequency (Table 1). Other factors must be considered, however, as the relative decrease in speed (78%) is greater than the relative decrease in cycle frequency (33%). This is apparent as a decrease in SL after feeding (SL falls from 0.065 to 0.023 m; Table 1), indicating that a given propulsive cycle is less effective at creating forward velocity than before feeding. The average speed of the mechanical wave of undulation on the body (V) can be calculated as follows: V = fL. U/V is termed the slip factor (50). If the backward traveling mechanical wave pushed the animal forward with no energy losses, then U and V would be equal, giving a slip factor of 1. The prefeeding slip factor is 0.55 ± 0.05 and falls to 0.16 ± 0.03 after feeding (mean ± SE, n = 5). A longer SL and a higher slip factor are assumed to indicate a high efficiency. Neither stride length nor slip factor are, however, direct measures of swimming efficiency, although they do provide useful relative measures of the effectiveness of mechanical power transfer from the swimming muscles to the surrounding water to generate thrust.

A more direct measure of swimming efficiency can be calculated from basic kinematic parameters. Unfortunately, the terminology describing swimming efficiency is confusing. Many terms, such as slip factor, propeller efficiency, and Froude efficiency, have been used interchangeably. Efficiency is also used to refer to the cost of transport of swimming animals, the metabolic energy used to travel a unit distance (e.g., Ref. 46). Of the total amount of mechanical power used by the leech to increase the kinetic energy of its wake, only a portion is used to generate thrust. The remainder is essentially wasted in imparting lateral momentum to the water. For the purposes of this laboratory exercise, I suggest confining the term “efficiency” to the ratio between the “useful” thrust power and total wake power. This can be calculated using elongated body theory (EBT) (22). EBT is a mathematical model that uses the kinematics of an undulating body to estimate its transfer of power to the surrounding fluid. Propulsive efficiency equals thrust power divided by total wake power. Propulsive efficiency using EBT (\( \eta_{\text{EBT}} \)) can be calculated as follows: \[ \eta_{\text{EBT}} = 1 - 0.5(V - U)/V. \] Prefeeding \( \eta_{\text{EBT}} \) for the data presented is 0.78 ± 0.03 and falls to 0.58 ± 0.01 after feeding (means ± SE, n = 5). The prefeeding value is in the range previously calculated for undulatory swimmers (45, 51, 52), but the postfeeding value is below the typical range.

These performance and efficiency changes are associated with a feeding-related shift in St (Table 1). Like Re, St is a useful summarizer of flow characteristics, specifically the properties of a vortex wake. St, which is calculated as \( S_t = f/U \), describes the properties of the wake in terms of the rate of vortex formation and vortex spacing (48). A striking observation concerning St is the narrow range (0.2 < St < 0.4) adopted by both fliers and swimmers spanning a size range from moths to dolphins (31). Before feeding, leeches also have an St within this range (0.35; Table 1). This corresponds to the St range at which the propulsive efficiency and thrust production of oscillating propulsors are maximized (2, 44). The apparent convergence on this narrow St range suggests tuning of flight and swimming kinematics through natural selection to maximize performance. After feeding, leech St shifts outside the typical range (0.74; Table 1), coinciding with the calculated drop in swimming efficiency and performance.

Further Analyses

Further analyses and points for discussion are outlined below. The provision of additional leeches for dissection is useful if the discussion is to focus on muscle function and neural control of movement as this will allow students to examine the overall structure of the hydrostatic skeleton (39), the muscle layers in the body wall (35), and the segmented nervous system (16, 17).

Temperature and swimming performance. An alternate or further set of experiments could investigate the effects of changing water temperature on leech swimming performance. Environmental temperature changes profoundly affect the locomotor performance of ectotherms (e.g., Refs. 13 and 25). The experiment could generate discussion of the many physiological processes and physical factors that change with temperature and how these can interact to affect swimming performance.

The effects of temperature change on physiological processes are typically summarized by the temperature coefficient (\( Q_{10} \)), which can be calculated as follows: \( Q_{10} = (R_2/R_1)^{10/(T_2 - T_1)} \) where \( R_1 \) and \( R_2 \) are the rates of a given process at temperatures \( T_1 \) and \( T_2 \), respectively. Leech swimming cycle frequency has a \( Q_{10} \) of 1.3 between 15 and 35°C [acclimation temperature not stated (27)]. Several interacting physiological systems may play a role in this observed change. The \( Q_{10} \) values for the neuronal oscillators that control the swimming rhythm range from 2 to 3 between 21 and 24°C [acclimation to “room temperature” (32)]. No data are available for the effects of temperature on leech muscle contractile properties, but the rates of force generation and relaxation of the body wall muscles are likely to have a \( Q_{10} \) of 2.0 or greater, in line with those typical for the processes underlying contraction and relaxation, e.g., Ca\(^{2+}\) release, actomyosin ATPase activity, and Ca\(^{2+}\) uptake by the sarcoplasmic reticulum (4). The processes that support muscle metabolic activity are also temperature dependent, for example, the neuronal oscillators that control the leech heart rate have a \( Q_{10} \) of 2.2 between 7 and 25°C [acclimation temperature of 15°C (3)].

Temperature-related changes in swimming cycle frequency (\( Q_{10} \) of 1.3) are less pronounced than those in the individual physiological processes that may influence swimming performance (\( Q_{10} \) range of 2–3). Similar mismatches have been observed in other ectotherm locomotor systems, particularly at high temperatures, where the frequency with which a muscle can move a propulsor in vitro can exceed that observed in the intact animal (15, 25). This may be due to performance limits imposed by passive components in the locomotor system such as connective tissue, skeletal elements, and the external fluid or substrate. A prime example that has already been discussed is St, which indicates that fluid dynamic factors may constrain propulsive movements in a fluid independently of physiological factors (31).

In some circumstances, temperature-related changes in fluid properties may also affect locomotion. The kinematic viscosity of water is temperature dependent, falling from \( 1.3 \times 10^{-6} \) m\(^2\)/s at 30°C to 0.8 × 10\(^{-6}\) m\(^2\)/s at 10°C. This can alter the balance between inertial and viscous forces summarized by Re. Determining whether this affects swimming performance is complicated by the interacting physiological effects of a given temperature change. Physical and physiological factors can
potentially be partitioned out. For example, temperature can be held constant and viscosity changed by the addition of inert thickeners such as polyvinyl pyrrolidone to the water (33). Such changes, however, are only likely to be significant for microscopic aquatic organisms swimming at low Re (33). In the inertia-dominated Re range experienced by leeches, the viscosity changes within the physiological temperature range are unlikely to significantly affect swimming performance (49).

Sensory cues for feeding initiation and cessation. The design of the feeding apparatus and leech behavior during feeding can be used to generate a discussion of the sensory cues that initiate and terminate feeding behavior. Heat is an important feeding stimulus. Warming the prostomial lip adjacent to the mouth by 3–5°C above the ambient temperature initiates feeding behavior (21). Thermal cues are not essential for feeding, however, as medicinal leeches also feed on amphibians (26). Nonthermal stimuli include water movements [leeches move toward ripples in water that indicate prey movement (20)] and chemical stimuli [leeches are attracted to low-molecular-weight blood components, primarily NaCl and arginine (8)]. Leech feeding is terminated in response to the activation of stretch receptors in the body wall. Feeding requires active pumping by the muscular pharynx, which is visible as rhythmic contractions in the anterior of the body. Pharynx muscle fatigue has also been suggested as a factor in feeding cessation. This can be discounted, however, as leeches filled with fluid via a tube in the absence of pharyngeal pumping cease feeding once distended, and pharyngeal pumping persists for much longer than the typical feeding duration in leeches with their posterior half removed so that they cannot retain blood (20). There is potential to perform further experiments to isolate the necessary stimuli by presenting leeches with a range of feeding options (e.g., room temperature blood, 37°C deionized water, and room temperature and 37°C solutions of 150 mM NaCl and 90 μM arginine).

Recovery of swimming ability. Rapid volume reduction. Reduced swimming performance after feeding is likely to increase vulnerability to cannibalism (18) and predation by vertebrates (23). This may create selection pressures for mechanisms that favor postfeeding recovery and/or retention of swimming abilities. Rapid mass and volume reduction immediately after feeding (Fig. 3) aids in the restoration of a flattened body cross section and effective swimming mechanics (Fig. 1 and Table 1). This is primarily achieved through fluid loss via paired nephridia (55). The process of fluid loss starts during feeding (21). In unfed leeches, urine production approximately matches body mass over a 24-h period, primarily to offset osmotic water gains. Urine production increases eightfold within an hour of feeding (54). This removes plasma from the crop, concentrating the ingested blood and increasing its hematocrit to 100% over the 4- to 6-day period postfeeding (6, 21). If students record leech mass periodically after feeding, then they can calculate the net rate of fluid loss.

Maintenance of muscle function. After plasma removal, body mass stabilizes and digestion of the erythrocytes takes several months (12, 42). Consequently, after the initial period of rapid fluid loss, the body wall musculature must be able to power swimming despite a prolonged increase in body mass and volume relative to the prefeeding level. The leech body wall contains three layers of muscle fibers: an outer layer of circular muscle, an intermediate layer of obliquely oriented muscle, and an inner layer of longitudinal muscle fibers (37). The longitudinal layer is the thickest and is the primary source of power during swimming (40).

The leech body wall is quite plastic. When stretched and held at the increased length, the residual stress in the tissue declines as it plastically deforms (43, 52). This is termed stress relaxation. The resistance of the body wall to being stretched is further decreased by serotonin (43, 52), a hormone that stimulates and coordinates feeding behavior in medicinal leeches (20). These properties of the body wall allow the impressive expansion in body volume during feeding.

This extensibility means that the longitudinal muscles of leeches can undergo a threefold change in length in vivo (43, 52). Leech muscle is obliquely striated (35, 37). This type of muscle is characterized by an offset arrangement of adjacent thick myofilaments within the myofibrils. Obliquely striated muscles are more extensible and have broader length-tension curves than vertebrate cross-striated muscle (28, 34). This extensibility appears to be due to the shearing of adjacent thick filaments (36). When the longitudinal muscle is plastically deformed, as during feeding, this changes the shape of its length-tension relationship, shifting maximal force production to a greater muscle length (28). These features of the musculature and body wall contribute to the ability of leeches to maintain longitudinal muscle contractility after feeding and allowing the resumption of swimming immediately after feeding and the restoration of swimming performance to prefeeding levels despite a prolonged increase in body mass and volume (6).

Neural control of movement. Body curvature and changes in body shape are generated by contraction of the body wall muscles against a fluid-filled hydrostatic skeleton. The hydrostatic skeleton within each body segment is isolated from its neighbors so the effects of muscle contraction in a given segment are localized. The two locomotor modes, swimming and crawling, are both driven by very different rhythmic patterns of muscle contraction (10). The contrasting modes allow students to observe the differing effects of waves of contraction in a single muscle layer and alternating contraction of antagonistic muscle layers.

The swimming rhythm in leeches is generated by a neuronal swim oscillator circuit in the central nervous system (16, 17, 24). The circuit consists of a chain of coupled oscillators in the ventral nerve cord (9, 11), which, during swimming, generate waves of longitudinal muscle contraction moving from anterior to posterior along the body of the leech. The timing of the wave is offset between the dorsal and ventral musculature, generating local shortening and curvature of the body. The change in cycle frequency after feeding is supportive of observations that the centrally generated rhythm is subject to modulation by sensory input from stretch receptors in the longitudinal muscle layer that senses tension in the body wall (53).

An important property of the hydrostatic skeleton is that it is incompressible, and the volume of each body segment therefore remains constant (39). This means that contraction of the dorsal and ventral longitudinal muscle layers will shorten a segment with a consequent increase in width, and contraction of the circular muscle layer will decrease its circumference with a consequent increase in length. This antagonistic action of the longitudinal and circular muscle layers is most evident.
during crawling. As with swimming, rhythmic muscle contractions generate crawling movements. To start the “step,” the leech releases its anterior sucker, and an anterior to posterior wave of circular muscle contraction elongates the body forward from the still-anchored posterior sucker. The anterior sucker attaches, and, after release of the posterior sucker, an anterior to posterior wave of longitudinal muscle contraction then shortens the body, pulling it forwards toward the anterior sucker (5).

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