

1 Swimming kinematics and performance of spinal transected lampreys
2 with different levels of axon regeneration

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5 Running title: Effects of regeneration on lamprey swimming

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21 Summary statement: We show that lampreys who have recovered from having their spinal cords
22 transected do not fully regain swimming abilities are not able to swim as efficiently as non-
23 transected lampreys.

24

25 **Abstract**

26 Neural and functional recovery in lampreys from spinal cord transection has been well
27 documented. However, the extent of axon regeneration is highly variable and it is not known
28 whether it is related to the level of behavioral recovery. To address this, we examined how
29 swimming kinematics were related to axon regeneration by quantifying the relationship between
30 swimming performance and percent axon regeneration of transected lampreys after 11 weeks of
31 recovery. We found that swimming speed was not related to percent axon regeneration but it was

32 closely related to body wave frequency and speed. However, wave frequency and speed varied
33 greatly within individuals which resulted in swimming speed also varying within individuals. In
34 fact, most recovered individuals, regardless of percent axon regeneration, could swim at fast and
35 slow speeds. However, none of the transected individuals were able to generate body waves as
36 large as the control lampreys. In order to swim faster, transected lampreys increased their wave
37 frequencies and, as a result, transected lampreys had much higher frequencies than control
38 lamprey at comparable swimming velocities. These data suggest that the control lampreys swam
39 more efficiently than transected lampreys. In conclusion, there appears to be a minimal recovery
40 threshold in terms of percent axon regeneration required for lampreys to be capable of
41 swimming, however, there also seems to be a limit to how much they can behaviorally recover.

42

43 **Introduction**

44 Anguilliform propulsion has been shown to be one of the most efficient forms of
45 swimming propulsion observed among animals (Van Ginneken et al., 2005). This form of
46 propulsion is characterized by a traveling wave that moves from the head to the tail with a
47 relatively short wavelength, so that about a full wave is present on the body at any time. The
48 amplitude of this traveling wave increases as it travels down the body (Lauder and Tytell, 2005).
49 These kinematics interact with the adjacent fluid to slowly build fluid vorticity and strong
50 negative pressure regions that serve to efficiently generate a suction thrust that pulls the
51 anguilliform swimmer forward (Gemmell et al., 2016).

52 Larval sea lampreys (*Petromyzon marinus*) are well characterized anguilliform swimmers
53 (McClellan et al., 2016). Healthy lampreys generate the characteristic traveling wave using
54 muscle contractions along the side of their body that are initiated just caudal to the head and
55 travel toward the tail. By alternating these contractions on each side of the body the lamprey can
56 generate successive traveling waves that make up each swimming cycle (McClellan, 1989;
57 Williams, 1989; Williams and McMillen, 2015). The speed of the observed body wave is slower
58 than that of the muscle contraction as a result of the interaction of forces acting on the body
59 which include the forces generated by the muscles and the resistive forces of the fluid acting on
60 the body (Ding et al., 2012; Tytell et al., 2010; Williams, 1989; Williams and McMillen, 2015)
61 Demonstrating the robustness of this behavior, within several months after a complete spinal
62 cord transection, lampreys are able to achieve robust recovery of swimming behaviors (Cohen et
63 al., 1999; Hanslik et al., 2018; Katz et al., 2020; McClellan, 1989; McClellan, 1994; Oliphint et
64 al., 2010; Rovainen, 1976; Selzer, 1978). Remarkably, they can also recover normal swimming
65 after a second spinal re-transection (Hanslik et al., 2018). Therefore, lampreys have served as a
66 great model for studying anguilliform swimming, as well as recovery from spinal cord injuries.
67 Lampreys spontaneously recover swimming behaviors within 8-12 weeks after their spinal cord
68 is transected rostrally at the level of the 5th gill due, in part, to long-distance regeneration of
69 descending axons (McClellan, 1994; Oliphint et al., 2010; Rovainen, 1976). Initially, such
70 transected animals are completely paralyzed (Hanslik et al., 2018; Oliphint et al., 2010). Axon
71 regeneration begins two to three weeks after spinal cord transection with axons beginning to
72 regenerate and some observed locomotor function just caudal to where the spinal cord was

73 transected. Progressively over time, locomotor function can be observed more caudally and by 8-
74 12 weeks locomotor activity, and neural activity and movement patterns can be similar to
75 normal, healthy larval lamprey (Cohen et al., 1986; McClellan, 1994; Oliphint et al., 2010).
76 Despite the ability to regenerate axons, the swimming kinematics and performance of recovered
77 lampreys still differs from non-transected lampreys (Oliphint et al., 2010). In addition, only 30-
78 70% of descending reticulospinal (RS) axons regenerate, making a few, sparse synaptic
79 connections, implicating compensatory mechanisms in locomotor recovery (Davis Jr and
80 McClellan, 1994; Oliphint et al., 2010; Shu Yin and Selzer, 1983).

81 Lampreys that have more caudal spinal cord transections (mid-body or lower) can often
82 swim immediately after transection. Under these conditions, locomotor activity is only present
83 rostral to the lesion, and the body waves are passively propagated to the caudal region (Gemmell
84 et al., 2016; McClellan, 1990). In comparison to rostral lesions, axon regeneration was reported
85 to be less robust after transection of the caudal spinal cord (Shu Yin and Selzer, 1983).

86 Despite lampreys being well documented as robust regenerators after a rostral spinal cord
87 transection (e.g. in the gill region) and progressing from paralysis to full mobility within 10-12
88 weeks post injury, the extent of axon regeneration supporting this behavioral recovery is variable
89 from animal-to-animal (Cohen et al., 1986; Hanslik et al., 2018; Oliphint et al., 2010; Rovainen,
90 1976; Selzer, 1978). Therefore, to understand the relationship between neural regeneration and
91 behavioral recovery, we quantified the kinematics and swimming abilities of larval lampreys that
92 had different levels of axon regeneration across the site of spinal transection and compared
93 performance of control lamprey (non-transected) to lamprey who recovered from having their
94 spinal cord transected 10.5 weeks prior at the 5th gill or at mid-body.

95 **Methods**

96 *Spinal cord transections:*

97 All animals used in the experiments were late larval-stage lampreys (*Petromyzon marinus*; 10–14
98 cm; M and F) that were housed at room temperature (25 °C) in 10-gallon aquariums. Fourteen
99 lamprey (treatments) underwent spinal cord transection surgery (n=11 were transected at the 5th
100 gill and n=3 at midbody) as previously described by Oliphint et al. (2010). Briefly, each lamprey
101 was anesthetized with Fiquel MS-222 (0.1 g/L tank water; Argent Chemical Laboratories) and

102 then placed in a Sylgard-lined Petri dish on a paper towel moistened in oxygenated lamprey
103 Ringers (100 NaCl, 2.1 KCl, 2.6 CaCl₂, 1.8 MgCl₂, 4 glucose, 0.5 glutamine, 2 HEPES, pH 7.4).
104 A dorsal incision was made either at the 5th gill or approximately halfway down the length of the
105 body, just above the dorsal fin, through the skin, musculature and fat tissue in order to expose the
106 spinal cord. Then, the spinal cord was completely transected either at the 5th gill or the mid-body
107 with a single horizontal cut made with fine iridectomy scissors (Fig. 1A). The incision was
108 closed with a single suture (Ethilon 6-0 black monofilament nylon; Johnson & Johnson,
109 Langhorn, PA). Three control animals received a sham treatment in which they underwent the
110 same surgical procedures, but the spinal cord was not cut (Table 1). All animals were then
111 returned to their home tanks for 11 weeks post-injury until they were video recorded. All
112 procedures were approved by the Institutional Animal Care and Use Committee at the Marine
113 Biological Laboratory in accordance with the standards set by the National Institutes of Health.

114 *Video recording and Kinematics Calculations:*

115 At 11 weeks post-injury, videos were taken of the lampreys as they were prompted to swim
116 through a 1.5 X 5 m acrylic aquarium that was filled with 5 cm of lamprey tank water. Video
117 was captured at 1000 fps using a Photron Fastcam 1024 PCI video camera positioned below the
118 lampreys (as in Gemmell *et al.*, 2016).

119 To compare the kinematics and swimming of the lamprey, each animal was video recorded
120 during steady state swimming according to Gemmell *et al.* (2016) and (Du Clos *et al.*,
121 2019)DuClos *et al.* (2020). Accordingly, lampreys were placed at one end of long (1.5 meter)
122 tanks where swimming was initiated by touching the individual gently at the tail. Swimming and
123 kinematics was videoed as the lamprey passed the middle of the tank (no longer accelerating and
124 swimming in steady state). Their bodies were illuminated with a light sheet that was oriented
125 horizontally and directed perpendicular to the camera angle, and was generated using two lasers
126 (532 nm, 600 mW continuous wave per laser) placed on opposite sides of the aquarium. Using
127 two lasers eliminated shaded regions around the swimming lampreys and enabled us to
128 thoroughly illuminate the outline of the lamprey. The laser light did not affect the lampreys'
129 swimming behaviors as the animals were larvae and thus still retained tissue covering their eyes.
130 Only video sequences where the velocity averaged over the entire sequence remained constant
131 were used in the analysis.

132 Swimming kinematics were quantified manually using ImageJ (NIH) software and an in-house
133 MATLAB program (<https://github.com/tytell/neuromech.wiki.git>). Raw images of the freely
134 swimming animals were input to a custom program in MATLAB that identified and tracked the
135 midline of the lampreys as they swam. Based on the X, Y coordinates of the lamprey midline the
136 max amplitude, wavelength, and frequency was calculated over time. Maximum amplitude was
137 calculated at the highest point in the wave along the body. Wavelength was done similarly but
138 between wave peaks or troughs.

139 To arrive at estimates of relative efficiency based on kinematics we calculated Strouhal number
140 (St) as $St = 2fA/U$, where A is the maximum amplitude, f is the frequency and U is the
141 swimming speed (Triantafyllou et al., 1993; Tytell, 2004). We also calculated the stride length,
142 the distance traveled per body wave, by dividing swimming speed by wave frequency to get an
143 estimate of how effective each body wave was at propelling the lamprey forward.

144 *Axon labeling, Imaging and Regeneration analysis:*

145 Following video recording at 10.5 weeks post injury, the descending reticulospinal axons were
146 bulk labeled in order to assess the extent of axon regeneration, as previously described
147 (Armstrong et al., 2003; Hanslik et al., 2018; Lau et al., 2013). Briefly, animals were re-
148 anesthetized in MS-222, and a second spinal lesion was made 0.5 cm rostral to the original
149 transection site. A 1x1x1 mm cube of Gelfoam (Pfizer; New York, NY) soaked in 5 mM Alexa
150 Fluor® 488-conjugated dextran (10kDa; Thermo Fisher, Inc. Waltham, MA), diluted in lamprey
151 internal solution (180 mM KCl, 10mM HEPES, pH7.4), was placed in the lesion which was then
152 closed with a single suture. Spinal cords were harvested three days after labeling to allow for
153 maximum transport of dye. The anterograde-labeled, regenerating axons were imaged live within
154 whole mounted spinal cords submerged in oxygenated lamprey ringer. Imaging was performed
155 using a Zeiss LSM 510 laser scanning confocal on an Axioskop 2FS upright microscope (10x,
156 0.3 NA Zeiss EC Plan-Neofluar objective). Z-stacks of spinal cords were acquired at distances
157 ranging from 2 mm proximal to the original transection site to 5 mm distal. Maximum intensity
158 projections were made using the Zeiss LSM software and stitched together in Photoshop. For
159 fifth gill and mid-body transections, the number of labeled axons crossing fiducial markers
160 positioned at 1-1.5 mm proximal and 1.0 mm distal to the transection site were counted. Percent
161 axon regeneration was calculated as the number of labeled axons distal to the transection site,

162 divided by the number of labeled axons at rostral, though we acknowledge that this is a semi-
163 quantitative estimate that may include some axon branches. Controls spinal cords were imaged
164 and analyzed the same way, except without an intervening lesion site.

165 *Statistics:*

166 All comparisons were tested to determine if they complied to the assumptions of parametric
167 tests. Wave kinematics were compared among treatments (5th gill transected, mid-body
168 transected and sham) using One-way ANOVAs. The relationships between axon regeneration
169 and swimming performance and kinematics were examined using regression analyses. We
170 additionally examined swimming speed as a function of axon regeneration and tail beat
171 frequency using a mixed model multiple regression, including regeneration, tail beat frequency,
172 and their interaction as fixed factors and individual animal as a random effect. This statistical
173 model was implemented using R 4.0.2 and nlme 3.1-148 (Pinheiro et al., 2007)

174 **Results**

175 *Axon regeneration after spinal cord injury in lampreys*

176 For this study, we examined and compared axon regeneration, kinematics, and performance in
177 lampreys that underwent spinal cord transection at the level of the 5th gill or the mid-body
178 (Fig.1A). In untransected control spinal cords, RS axons generally projected in relatively straight
179 patterns within the ventromedial and ventrolateral tracts (Fig. 1B). In contrast, at ~10.5 weeks
180 post-injury, RS axons proximal to the spinal lesion can be straight, curved, or branching within
181 the spinal cord (Oliphint et al., 2010), and only a subset of RS axons regenerated distal to the
182 lesion (Fig. 1C). Similar amounts of RS axon regeneration were observed in spinal cords that
183 were transected at the mid-body (Fig. 1D), a perturbation that does not result in paralysis of the
184 animal due to preservation of the rostral spinal circuits that initiate swimming (Gemmell et al.,
185 2016). To estimate the extent of axon regeneration, we counted the number of Alexa-Fluor®
186 488-labeled RS axons 1 mm distal to the lesion center and divided this by the number of labeled
187 axons 1-1.5 mm proximal to the lesion. In this cohort of animals, the percent axon regeneration
188 in spinal cords was between 33.3 and 84.2 percent with a median of 58.6 percent regeneration,
189 which is similar to that reported in previous studies (Lau et al., 2013; Oliphint et al., 2010; Shu

190 Yin and Selzer, 1983), thus providing a range of neural regeneration to compare to behavioral
191 performance.

192 *Swimming performance and kinematics*

193 All of the lampreys examined recovered sufficiently to be able to swim. In fact, we found that
194 how fast the lampreys were capable of swimming was not related to the percent of axon
195 regeneration within their spinal cords (Fig 2A; Regression analysis, $df = 1$, $F = 2.02$, $P = 0.2$).
196 Comparison of swimming speeds among treatments (5th gill transected, mid-body transected and
197 sham control) suggests that the control sham lampreys swam faster than transected individuals
198 but the differences were not significant (Fig. 2B; ANOVA, $df = 2$, $F = 2.72$, $P = 0.09$). A more
199 quantitative comparison of body kinematic variables to percent regeneration shows that none of
200 the wave kinematics were significantly related to axon regeneration for the recovered transected
201 lampreys (Fig. 2C-E, Regression analysis, $df = 1$, $P > 0.07$). However, the control sham lampreys
202 had significantly longer wavelengths and higher amplitudes than transected lampreys (Holm-
203 Sidak post-hoc comparison, $p < 0.05$), but their wave frequencies were not different than
204 transected lampreys (ANOVA, $df = 2$, $p > 0.1$).

205 Despite the lack of correspondence between spinal cord regeneration and swimming
206 performance, a visual comparison of representative recovered individuals to a control (or sham)
207 lamprey illustrates that there were important differences in the body and swimming kinematics
208 that swimming speed did not capture. Sequential images of the lampreys (Fig. 3A) reveals that
209 the wavelength of the body wave of the control lamprey was large compared to the recovered,
210 transected lampreys. As such, more waves occurred along the bodies of the transected lampreys
211 (1.9 ± 0.2 waves per body) at any one time than the control lampreys (1.2 ± 0.01). The
212 swimming kinematics of the control lamprey were also very regular during consecutive
213 swimming cycles, while the swimming kinematics of the transected lampreys were much more
214 irregular (seen in the motion of the head and swimming velocity (Fig. 3B and D)). A fast
215 swimming 5th gill transected individual was included in the comparison to illustrate the
216 differences in the kinematics between the fast transected and the control (Fig. 3). Despite
217 traveling a similar distance as the control (Fig. 3C), the transected lamprey still had a smaller
218 wavelength (Fig. 3A), the head moved back and forth much more frequently (Fig. 3B) and the
219 swim pattern was much more erratic than the control (Fig. 3D).

220 A closer look at the wave amplitudes among the groups revealed that the larger amplitudes
221 observed for the control lampreys were achieved by the lamprey increasing amplitude as the
222 wave traveled head to tail (Fig. 4). In contrast, the wave amplitudes of the 5th gill and mid-body
223 transected lampreys did not change as much as the waves moved head to tail.

224 While average swimming speed was not significantly related to axon regeneration, swimming
225 speed was directly related to the body wave characteristics of beat frequency, wavelength and
226 wave speed (Fig. 5; Regression analysis, $df = 1$, $P < 0.01$). Multiple regression indicates that
227 swimming speed depends on tail beat frequency ($P < 0.001$), but not on regeneration percentage
228 ($P = 0.65$) or its interaction with tail beat frequency ($P = 0.88$) (Table S1, Fig. S1). Wave
229 amplitude did not have a significant effect on swimming speed (Fig. 5D; Regression analysis, df
230 $= 1$, $P > 0.05$). The beat frequency of the control sham lampreys was low compared to transected
231 lampreys swimming at a similar speed; therefore, the control lampreys were able to achieve
232 higher swimming speeds at lower beat frequencies and wave speeds than the transected lampreys
233 (Fig. 5A and C; Oliphint et al. 2010).

234 *Kinematic indicators of swimming efficiency*

235 In order to examine how the differences in kinematics and performance may translate into
236 efficiency, we calculated Strouhal number and stride length, indices that can be used as
237 indicators of efficiency (Fig. 6). The Strouhal numbers (St) of the control lampreys (and one
238 mid-body lamprey) fell within the range ($St = 0.25 - 0.35$) that has been shown to provide the
239 maximum propulsive efficiency (Fig. 6A; Taylor et al. 2003, Eloy 2012) and were significantly
240 lower than the Strouhal of the 5th gill transected lampreys (Fig. 6B; Holm-Sidak post-hoc
241 comparison, $p < 0.05$). However, the controls did not significantly differ from the mid-body
242 transected lampreys (Holm-Sidak post-hoc comparison, $p > 0.05$). The control lampreys also
243 swam further with each tail beat (Fig. 6C; One-way ANOVA, $F = 39.8$, $p < 0.001$). Therefore,
244 both of these indices suggest that even when transected lampreys swim as fast as controls, they
245 do not swim as efficiently.

246 *Comparison between 5th gill and mid-body transected lampreys*

247 The 5th gill transected lampreys and the mid-body transected lampreys did not differ in any of
248 measured performance or kinematic parameters (Figs. 1, 4, 5; Holm-Sidak post-hoc comparison,

249 $p > 0.05$). However, the mid-body transected lampreys had significantly lower Strouhal numbers
250 and stride lengths, compared to controls (Fig. 6D; Holm-Sidak post-hoc comparison, $p > 0.05$).

251 **Discussion**

252 One of the primary goals of this study was to examine how swimming performance was related
253 to degree of axon regeneration in lampreys recovering from spinal cord transection. We
254 hypothesized that a larger fraction of axons regenerated would lead to more complete activation
255 of the spinal locomotor circuits below the lesion. Based on this hypothesis, we predicted that
256 animals with a greater fraction of regenerated axons would swim faster and more efficiently than
257 those with fewer regenerated axons. But that is not what we observed. Swimming speed was not
258 related to the percent axon regeneration of lampreys recovered from spinal cord transection (Fig.
259 2). However, individuals swam at highly variable speeds, whereby, most individuals could swim
260 both rapidly and slowly (Supplemental fig. 1) and this variability may have obscured our ability
261 to see a statistical relationship between swimming speed and axon regeneration. Basically, all the
262 individuals, which had recovered for 10.5 weeks, had the ability to swim at variable speeds and
263 modulated their swimming speeds by changing their wave frequency and shape (Fig. 5). Despite
264 being able to swim, and, at times, swim relatively fast, transected individuals did not produce
265 body waves as large as the control lampreys (having significantly lower wavelengths and
266 amplitudes). As a result, in order to swim, fast transected individuals had to produce body waves
267 very rapidly – i.e. high wave frequency – much higher frequency than control animals required
268 to swim at the same speed. This suggests that the swimming efficiency, as indicated by Strouhal
269 number and stride length was lower in the transected lampreys (both 5th gill and mid-body)
270 compared to control.

271 There appears to be a minimal recovery threshold required for lampreys to be capable of
272 swimming, but there also seems to be a limit to how much they can recover. All the transected
273 lampreys in this study, regardless of percent axon regeneration, which ranged from 33-84%, had
274 a similar relationship between their wave frequency and swimming speed (i.e., similar stride
275 length (Fig. 6B)). In fact, the wave kinematics and swimming performance of the 10.5 week
276 recovered lamprey in this study were not much different than the 2 week recovered mid-body
277 transected lamprey reported in Gemmell et al. (2016). It has been shown that spinal cord
278 transected lamprey recover some locomotor function at 2 weeks, albeit with aberrant movements

279 and locomotor activity and appear to increase their locomotor activity after that (Davis et al.,
280 1993; McClellan, 1994). By 8 weeks recovered, transected lamprey have near normal locomotor
281 movement and muscle activity patterns (McClellan, 1994). However, others have shown that
282 even after 10 weeks, recovered lampreys need to use higher wave frequencies than control
283 lamprey to reach similar swimming speeds (Oliphint et al., 2010). Likewise, we found that the
284 recovered, transected lampreys in this study also swam significantly shorter distances per tail
285 beat than the control lampreys. This suggests that recovered, transected lamprey are not capable
286 of coordinating the kinematics necessary to generate swimming thrust as efficiently as non-
287 transected lampreys.

288 Why are control lampreys able to swim better than transected lampreys? While all the lampreys
289 in this study generated body waves that travel head to tail produced by waves of muscle
290 activation on alternating sides of their body (McClellan et al., 2016; Williams, 1989), the shape
291 and kinematics of these waves differ considerably between transected and control lampreys (Fig.
292 3). The body waves of the control lampreys are larger (longer wavelength and higher amplitude)
293 and they develop more gradually resulting in amplitude increasing as each wave travels along the
294 body (Fig. 4). The wave amplitudes of the transected lampreys did not increase gradually as the
295 waves travelled along their bodies (Fig. 4). The gradual build-up of the wave amplitude has been
296 shown to be essential for efficiently building and steering vortices for thrust generation
297 (Gemmell et al., 2016). A comparison of the hydrodynamics generated by transected versus non-
298 transected lampreys showed that the increase in wave amplitude gradually built up vorticity
299 adjacent to the wave. The gradual build-up of vorticity lead to the non-transected lampreys
300 generating suction thrust consistently along most of the body (Gemmell et al., 2015; Gemmell et
301 al., 2016). In contrast, the body waves of the transected lampreys did not increase in amplitude or
302 build vorticity along the body and thrust was inconsistent and primarily generated at the tail by
303 positive pressure fields (Gemmell et al., 2016). Consequently, the non-transected lampreys get
304 more thrust out of each body wave more efficiently (Fig. 6).

305 We speculate that transected animals, while they are able to produce muscle activity, are not able
306 to produce as forceful contractions as control animals. Lower muscle forces would result in
307 lower amplitude body waves, as we observed (Fig. 2E). Similarly, computational work has
308 suggested that, when muscle forces are low compared to fluid forces, the body wavelength

309 shortens (Tytell et al., 2010). If the wavelength of neural activity is similar in control and
310 transected animals (as observed in vitro by McClellan, 1990), then the shorter mechanical body
311 wavelength we observed would result in muscle activation earlier in the tail beat cycle relative to
312 muscle shortening, and thus more eccentric activity, particularly toward the tail. Such eccentric
313 muscle activity does not produce propulsive power, but instead may stiffen the caudal region to
314 more effectively transmit muscle force from the anterior body to the fluid (Blight, 1977; Tytell et
315 al., 2010). However, if the anterior body is not producing force effectively, as seems to be
316 occurring in transected animals, the body stiffening may not be useful and may instead reduce
317 the total power produced, decreasing swimming efficiency.

318 That lampreys can regain swimming behaviors post-recovery, despite incomplete axon
319 regeneration, implies that other compensatory mechanisms are in play to restore locomotor
320 behavior. In addition to RS axon regeneration, regeneration of other neuron types, as well as
321 altered synaptic properties, has been observed within the lamprey spinal cord post-injury, which
322 contribute to locomotor recovery (Becker and Parker, 2019; Cooke and Parker, 2009). Thus, the
323 regenerated lamprey spinal cord is likely a “new” locomotor network (Parker, 2017).

324 In conclusion, just as there appears to be more than one way to “skin a cat” there appears to be
325 more than one way for lampreys to swim. Recovered, transected lampreys clearly have the
326 ability to swim and swim at high speeds. However, they have to produce many small body waves
327 to achieve high swimming velocities which control lampreys achieve using less frequent, larger
328 waves. The differences in wave kinematics rely on different thrust mechanisms (Gemmell et al.,
329 2016) and ultimately result in different swimming efficiencies.

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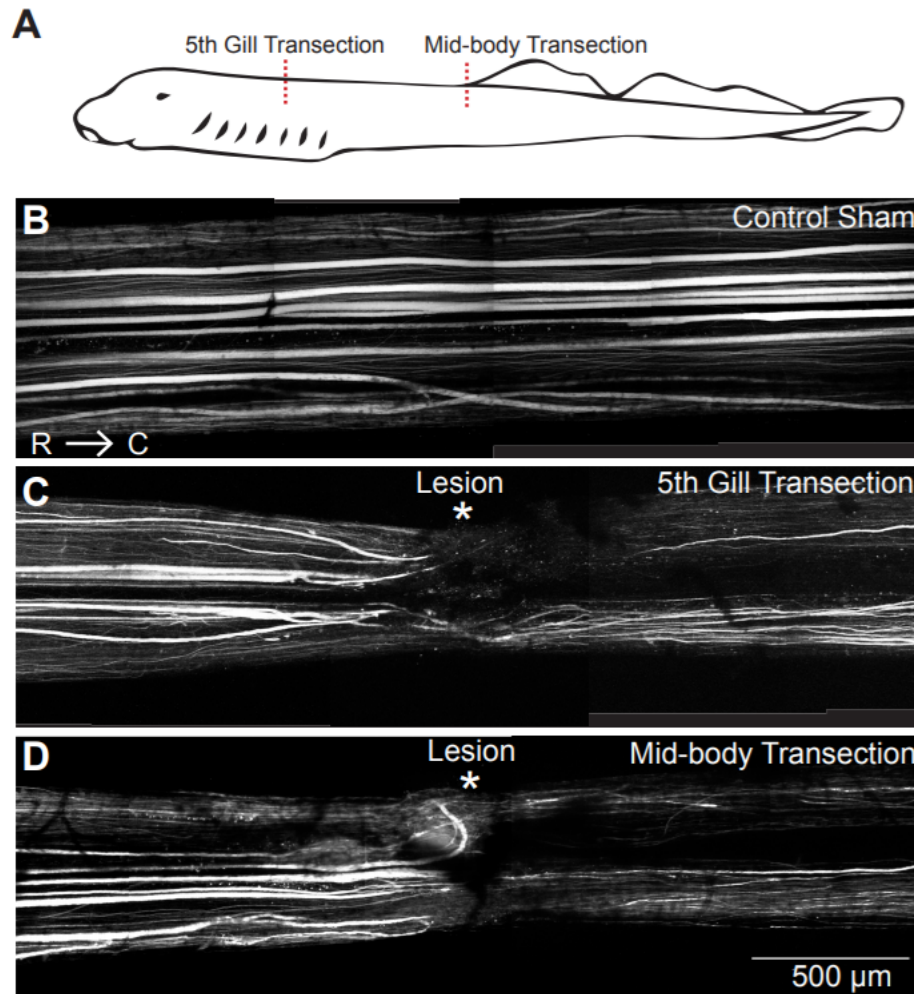
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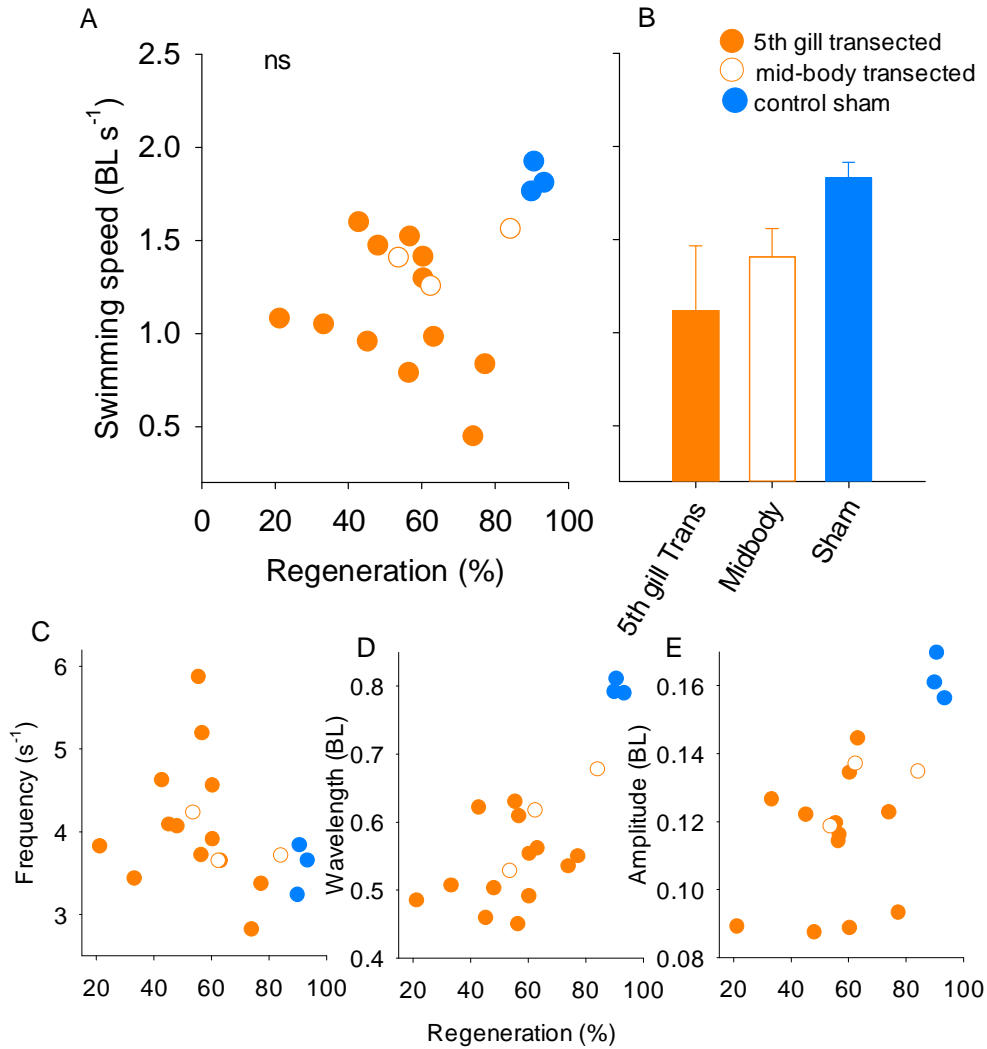
413 Figures



414

415 Fig. 1. Bulk labeling of regenerating axons ~10.5 weeks following a full spinal cord transection.
416 A) Schematic of a larval lamprey with the site of spinal cord transection indicated with a red
417 dashed line for a 5th gill or mid-body transection. B) A montage of confocal z-projections
418 stitched together of a control, sham uninjured spinal cord with axons labeled by a 10kDa Alexa
419 Fluor 488 dextran, showing fairly uniform labeling along the length of the spinal cord. C-D)
420 Labeling of axons ~ 10.5 weeks post injury in a 5th gill transected and a mid-body transected
421 animal shows sparser axon labeling in the region caudal to the lesion site in comparison to the
422 rostral region, indicating the amount of axon regeneration. Note that the amount of axon
423 regeneration is comparable between the 5th gill and mid-body transected spinal cords. Scale bar
424 in D applies to panels B-D. Rostral (R) is to the left and caudal (C) is to the right.

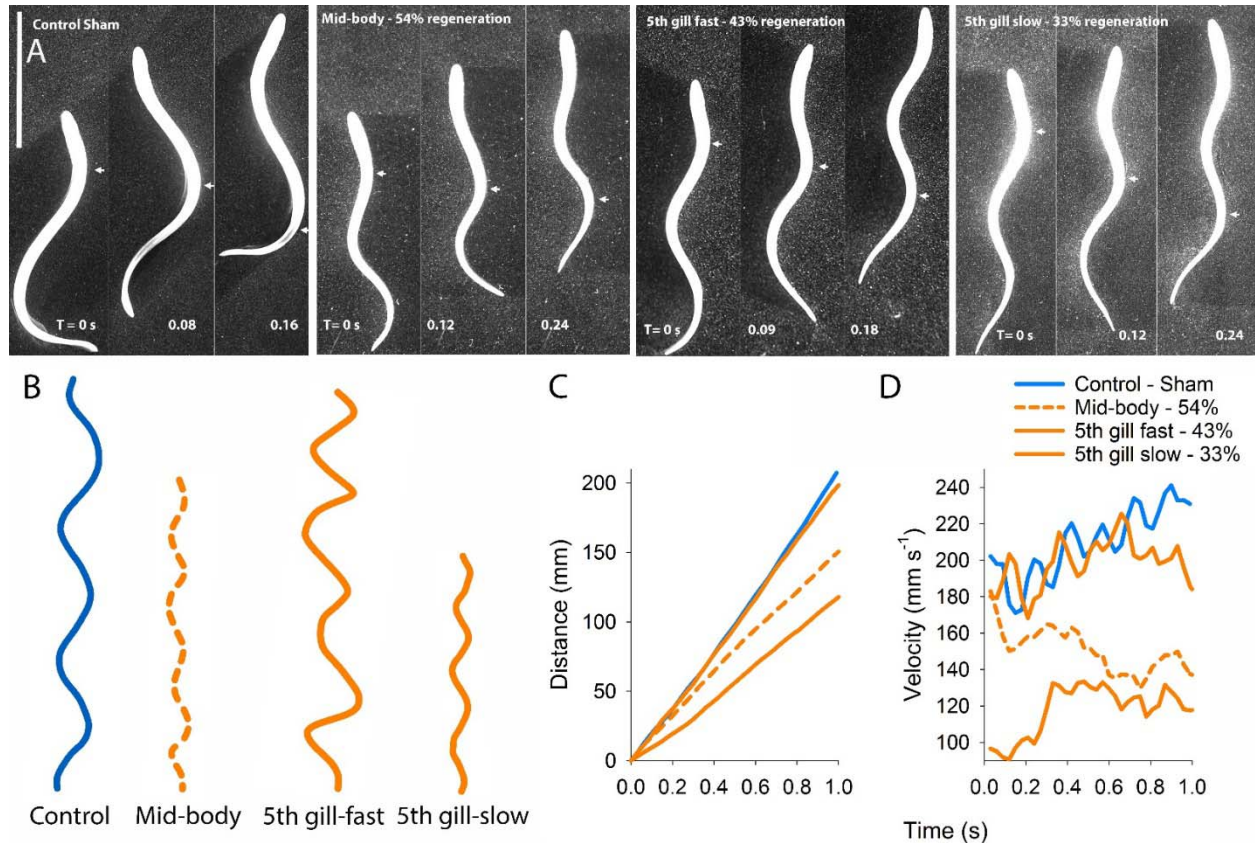
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427 Fig. 2. A) Swimming speed of lampreys versus their degree of spinal cord regeneration (%) after
428 recovering for 10.5 weeks from having their spinal cord transected (Regression analysis, $df = 1$,
429 $p > 0.05$). B) Comparison of mean swimming speeds among treatments (ANOVA, $df = 2$, $p >$
430 0.05). C-E) Comparison of A) wave frequency, B) wavelength and C) wave amplitude versus the
431 degree of spinal cord regeneration (%; Regression including 5th gill and midbody, $df = 1$, $p >$
432 0.05).

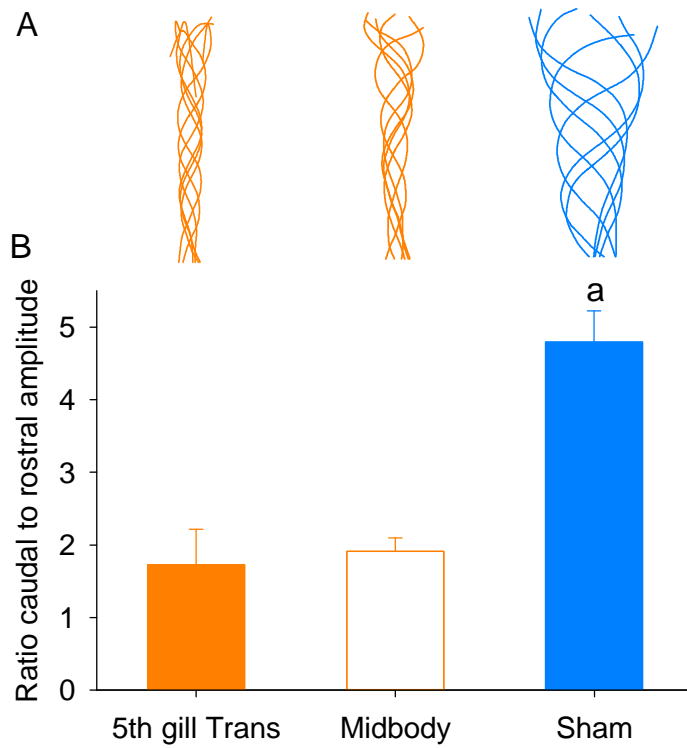
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435 Fig. 3. Comparison of body and swimming kinematics of lampreys. A) Sequential images of
436 different lamprey showing the progression of a body wave (indicated by white arrow) moving
437 from head to tail. Notice the control lamprey has only one large wave traveling along the body at
438 a time while all the transected lampreys, regardless of swimming velocity (D), have multiple
439 smaller waves moving along the body. B) Tracking of the movement of the head of the lamprey
440 for 1 second. Notice the distance traveled and the evenness vs. unevenness of the lateral motion of
441 the heads through time. C) Distance the different lamprey traveled over a second. D) Velocity of
442 the different lamprey over a second. Notice the regular swim cycles of the control lamprey (blue)
443 versus the more erratic motion of the lampreys transected (orange).

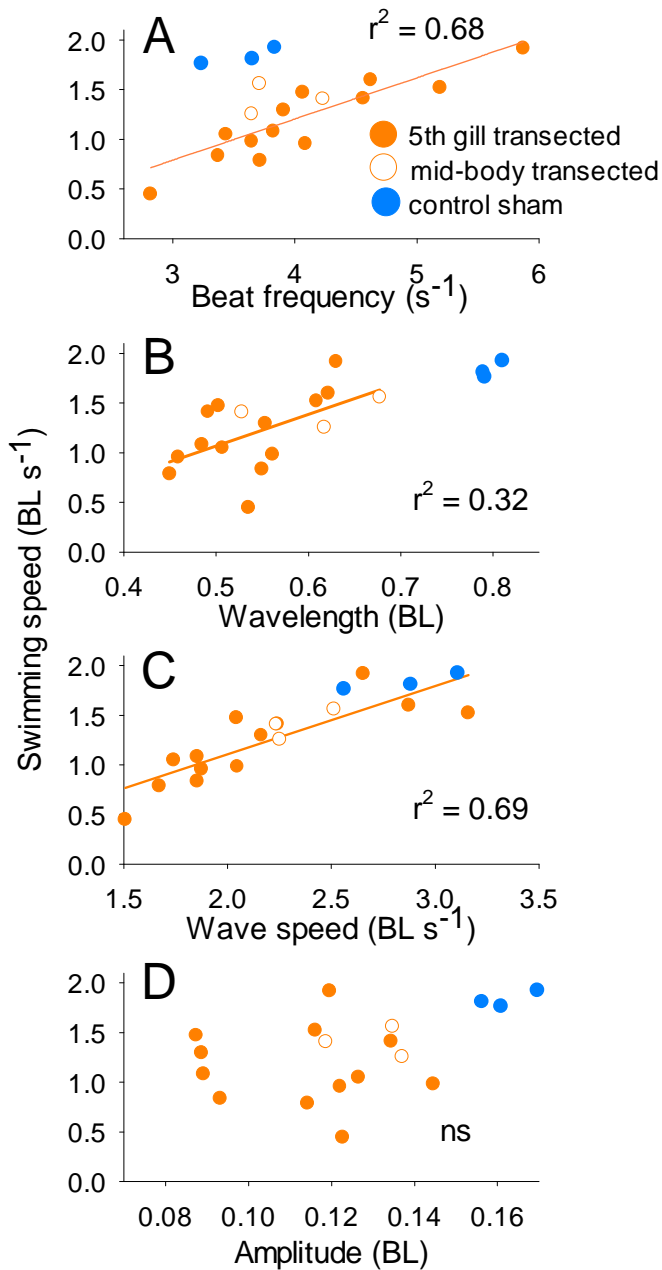
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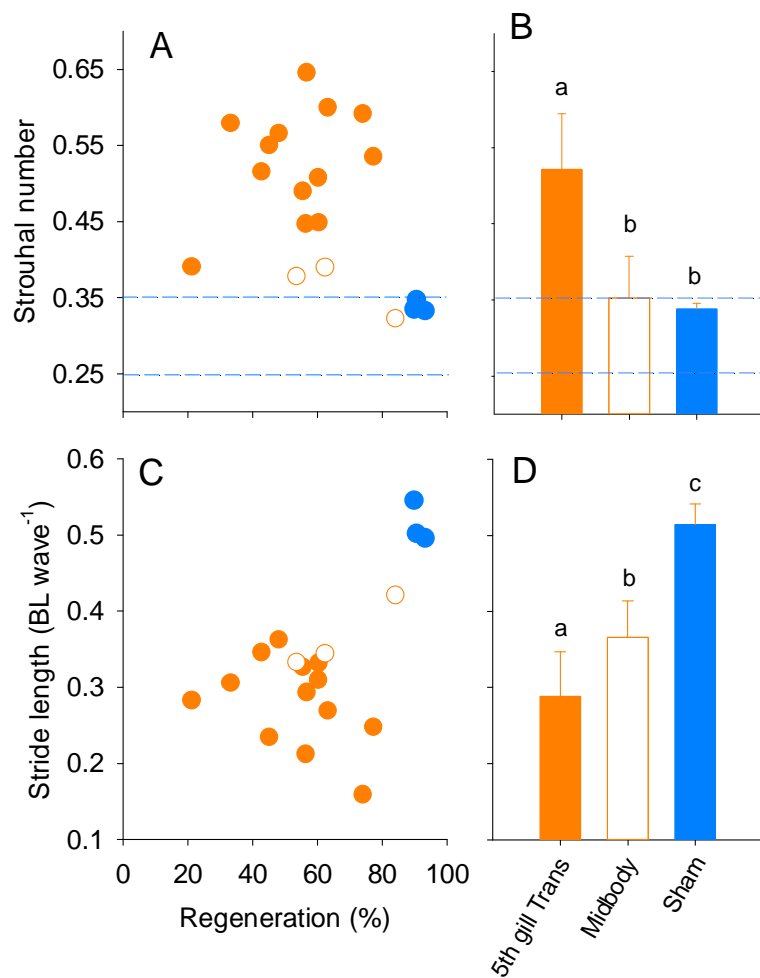
446 Fig. 4. Change in body wave amplitude as wave travels from head to tail. A) Change in midline
447 of representative lampreys over time. B) Mean change in amplitude (as ratio of amplitude at the
448 tail (caudal) and the head (rostral)) among treatments. Lower case letters significantly different
449 treatment groups (Holm-Sidak post-hoc comparison, $p < 0.05$).

450



451

452 Fig. 5. Body kinematic variables versus swimming speeds of different lampreys of the 5th gill
453 transected (filled orange circles), the mid-body transected (open circles) and control lampreys
454 (filled blue circles). A) beat frequency, B) wavelength and C) wave speed were all positively
455 related to swimming speed for the transected lampreys (Regression analysis, $p < 0.01$). Wave
456 amplitude of the traveling body waves was not significantly related to swimming speed
457 (Regression analysis, $p > 0.1$).



458

459 Fig. 6. Effects of percent regeneration on the Strouhal number and stride length (BL traveled per
460 wave) for the transected and control lampreys. A) Strouhal number of lamprey with different
461 levels of regenerated spinal cord. Dotted blue lines highlight region where studies have shown
462 animals and flapping foils to have the highest propulsive efficiency. The 5th gill lampreys fell
463 outside the optimal range of Strouhal while the control and the one mid-body lamprey fall within
464 the optimal range. B) The 5th gill (filled orange circles) transected lampreys had significantly
465 higher Strouhal numbers than the mid-body (open circles) transected and control lampreys
466 (Holm-Sidak post-hoc comparison, $p < 0.05$). C) Stride length of lampreys with different levels
467 of regenerated spinal cord. D) Comparison of the stride lengths among treatments, letters
468 designate significantly different groups (Holm-Sidak post-hoc comparison, $p < 0.05$).