SENSORY CAPABILITIES OF *POLYPTERUS SENEGALUS* IN AQUATIC AND TERRESTRIAL ENVIRONMENTS

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LIST OF ABBREVIATIONS

4-Di-2-ASP	4-(4-diethylaminostyryl)-1-methylpyridinium iodide
ANOVA	analysis of variance
B	Bartlett's statistic
BL	body length
CC	cobalt-treated
cpd	cycles per degree
CPG	central pattern generator
deg	degree
DK	filmed in dark
EOD	electric organ discharge
Fig.	figure
HSD	honest significant difference
LT	filmed in light
MS-222	tricaine methanesulfonate
obs.	observation
OKR	optokinetic response
OMR	optomotor response
P_{crit}	critical p-value for null hypothesis rejection
Q-Q plot	quantile-quantile plot
s.e.m.	standard error of the mean
SH	sham

ABSTRACT

In the amphibious fish *Polypterus senegalus*, focussing on lateral line, vision and electrosensation, we investigated sensory abilities, their interactions, and changes in their effects on locomotor behaviour between aquatic and terrestrial environments. First, we blocked lateral line, vision, or both, and examined effects on locomotion in both environments. Both senses affected both types of locomotion. When fish could see but not feel, variation in several kinematic variables increased, suggesting that sensory integration may affect locomotor control. Next, we assessed response to optokinetic stimuli of varying size and speed. Temporal and spatial visual acuity were both low, as expected in a nocturnal ambush predator. Visual ability in air was much reduced. Finally, we attempted to record electrogenesis in *Polypterus*, but did not observe the electric discharges reported in a previous study. Future studies might examine changes in sensory function, interaction and importance in behaviour in *Polypterus* raised in a terrestrial environment.

RÉSUMÉ

Chez le bichir amphibien *Polypterus senegalus*, nous avons étudié leurs capacités sensorielles, et les changements dans leurs effets sur la locomotion entre milieux aquatiques et terrestres. Premièrement, nous avons bloqué, soit la ligne latérale, la vision ou les deux simultanément, et examiné les effets sur la locomotion. Chaque sens a affecté la locomotion en chaque milieu. Lorsque les bichirs pouvaient voir mais pas sentir, la variation de plusieurs variables cinématiques a augmenté, suggèrant qu'une intégration sensorielle aurait un effet sur le contrôle locomoteur. Deuxièmement, nous avons évalué la réponse aux stimuli optocinétiques. L'acuité était faible, comme il est attendu pour un prédateur d'embuscade nocturne. La vision était réduite dans l'air. Finalement, nous avons tenté d'enregistrer l'électrogenèse, mais n'avons pas pu observer les décharges électriques constatées dans une étude précédente. À l'avenir, nous pourrons examiner les changements dans la fonction et l'importance des sens chez les bichirs élevés sur terre.

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INTRODUCTION

Sensory systems gather information about the surrounding environment, which can then inform behaviour. As the environment changes, for example, as an organism moves from water to land, the relative importance of different sensory modalities may change. *Polypterus senegalus* is a basal ray-finned fish, capable of survival in a moist terrestrial environment for an extended period. Its propulsion strategy differs between land, where it moves using alternating pectoral fin plants in coordination with large axial undulations, and water, where it primarily uses fin oscillation, sometimes accompanied by smaller body undulations (Standen et al., 2016). These differences raise questions about how the control of locomotion changes between environments.

Sensory systems such as vision and the lateral line system might influence the descending inputs to the spinal central pattern generators controlling locomotion. The interactive effects of visual and lateral line systems on locomotor behaviour have received limited attention (Liao, 2006), and have never been assessed in an amphibious fish. We examined the effects of impairing vision, the lateral line, or both, on swimming and walking behaviour. Based on previous studies (Dijkgraaf, 1963; Sutterlin and Waddy, 1975), we expected at least partial compensation for the loss of one sense when the other remained intact. Both vision and the lateral line system were expected to function poorly in air (Fritzsch, 1989; Kröger et al., 2014), and thus their impairment was expected to have a smaller effect on walking than on swimming.

After finding that loss of visual input affected locomotor behaviour, we next tested the visual abilities of *Polypterus*, since acuity had not been quantified in the species. *Polypterus* are nocturnal and have relatively small eyes, so their vision was suspected to be poor. As they do not possess adaptations to improve aerial vision (Kröger et al., 2014; Rochon-Duvingneaud, 1943, in

Pfeiffer, 1968), visual acuity on land was expected to be worse than in water. We used a rotating striped drum, designed to stimulate the optokinetic, or stimulus-tracking, response in vertebrates, to determine the limits to the size and speed of stimuli that could be perceived by the fish in both environments.

Polypterus, long known to be electroreceptive (Jørgensen, 1982; Roth, 1973), had more recently been found to be electrogenic, capable of producing sporadic, weak electric discharges similar to those generated by some catfishes (Baron and Pavlov, 2003). Although electrogenesis has not been observed in tetrapods, with the exception of the Chinese giant salamander (Olshanskii et al., 2016), and is unlikely to be useful on land, in water, it can be important in behaviours such as communication and navigation (Kalmijn, 1982; Metzner and Heiligenberg, 1991). Its purpose had yet to be determined in *Polypterus*, and we aimed to record the electric discharges of the fish and determine the behaviours with which they were associated.

Overall, we sought to gain a better understanding of the sensory abilities of *Polypterus*, by determining the limits of sensory perception, by examining the influence of various inputs, and their interaction, on behaviour, particularly locomotion, and by assessing how function might be altered in the terrestrial environment. The thesis is presented in three chapters, formatted as journal articles. The introduction to each chapter may therefore repeat some of the background information outlined here.

CHAPTER 1.

Changes in aquatic and terrestrial locomotion due to sensory deprivation in *Polypterus senegalus*

ABSTRACT

The amphibious fish *Polypterus senegalus* is capable of locomotion both in water and on land, but its aquatic and terrestrial movements differ substantially. Sensory feedback is important in the control of locomotion, and the sensory information available to *Polypterus* changes between environments. We removed lateral line and visual input, both independently and in combination, to examine the relative importance of each sense to locomotor control in both environments. We found that, with regard to general exploratory behaviour, lateral line sense could compensate entirely for the loss of vision, while vision could only partially compensate for lateral line block, which might be expected in a nocturnal fish like *Polypterus*. Interestingly, when the lateral line was blocked but the fish could see, several kinematic variables, including body wavelength in both swimming and walking, showed large, significant increases in variability, and small, non-significant increases in mean. This may be due to the discord between the visual information received and the lack of corresponding lateral line input; the integration of these two sources of sensory information appears to affect locomotor control. As these kinematic changes were observed in both environments, it seems that both senses also affect the control of locomotion on land, despite predicted functional impairment in air.

1.1 INTRODUCTION

Information collected by the senses allows modification of motor output in response to changes in the surrounding environment. In amphibious fishes, movement strategies employed in terrestrial environments often differ substantially from those used in aquatic environments. While aquatic locomotion of *Polypterus senegalus* is driven by fin movement and small lateral oscillations of the tail, terrestrial locomotion is driven by alternating placement of the pectoral fins on the terrestrial surface, coupled with much larger axial oscillations (Standen et al., 2016).

In most fishes, the two sensory systems most important to the control of locomotion are vision and the mechanosensory lateral line (Liao, 2007), although the extent of reliance on each depends on the ecology of the species (Hobson et al., 1981). Given that they are nocturnal, swimming *Polypterus* may depend less heavily on vision than on other sensory inputs. When *Polypterus* move into a terrestrial environment, the relative importance of each sensory modality is likely to change. Some senses may become non-functional in a terrestrial environment, or may retain their function but contribute less to the control of locomotion than they do in water. In order to understand the role of sensory feedback in controlling the terrestrial and aquatic locomotion of *Polypterus*, we need to determine which sensory inputs are pertinent in each environment.

Most fishes would have myopic vision in an aerial environment as a result of the difference in refractive index between air and water, but some amphibious fishes possess adaptations which improve their aerial vision. For example, the lenses of many mudskippers are slightly flattened, which limits refraction in air, and improves acuity (Sayer, 2005). *Polypterus* are not known to have such adaptations (Kröger et al., 2014; Rochon-Duvingneaud, 1943, in Pfeiffer, 1968), and have poor visual acuity both in air and in water (see Chapter 2). The aerial

vision capabilities of an amphibious species are likely related to the relative amount the time spent in the terrestrial environment (Sayer, 2005). Terrestrial excursions in *Polypterus* may be infrequent, as efforts to observe voluntary emergence onto land in the natural environment have been unsuccessful (Du et al., 2016), although historical anecdotes suggest it may occur.

The lateral line system detects hydrodynamic disturbances. The gelatinous cupula of a lateral line neuromast is deflected by water movement, and this deflection is transmitted to underlying mechanosensory hair cells (Moyes and Schulte, 2008). Superficial neuromasts, found on the surface of the fish, are thought to transduce velocity, while canal neuromasts transduce acceleration (Kalmijn, 1989; Munz, 1989). *Polypterus* have both superficial and canal neuromasts on the head, as well as lateral line canals down both sides of the body (Jollie, 1984).

As they move through the water, fish generate hydrodynamic disturbances within the range of those detectable by their lateral line. Previously, it was thought that lateral line input was not used in control of steady swimming, as its removal did not affect swimming performance in cyprinids (Dijkgraaf, 1963). In order to be able to detect stimuli not generated by self-motion, fish might disregard hydrodynamic disturbances produced by their own movement, either through efferent suppression (Russell and Roberts, 1974) or higher-order processing (Montgomery and Bodznick, 1994; Palmer et al., 2005), as they often disregard self-generated noise when processing other sensory inputs (Bell, 2001). For example, in electroreceptive fishes, electrical signals generated by the fish's own muscle contraction, predictable based on proprioception and other cues, can be separated from the external electrical signals of interest (Bell, 2001). However, more recently, various changes in steady swimming kinematics as a result of lateral line block have been reported (Ayali et al., 2009; McHenry et al., 2010; Yanase

et al., 2014). Although results differ substantially between studies, they suggest a role for the lateral line in locomotor control.

The lateral line has regressed in the vertebrate transition to terrestrial environments (Fritzsch, 1989). It is lost at metamorphosis in most amphibians, and is not present at all in more derived tetrapods. The usefulness of the lateral line in the terrestrial locomotion of amphibious fishes is unknown (Gordon, 1995). As the densities of air and water differ so greatly, pressure gradients in air may not result in cupula deflection as they do in water. During temporary emersion, the lateral line canals might be sufficiently fluid-filled to retain their function, allowing the lateral line system to contribute to motor control on land. However, the neuromast cupula is likely to become desiccated and less functional when exposed to air for prolonged periods (Fritzsch, 1989).

Many fish behaviours are modulated by inputs from both visual and lateral line systems. Although loss of either modality by itself has been known to affect behaviour, there is often an interactive effect between the two, wherein the effect of the loss of one modality is dependent on whether or not the other remains intact. The result is sometimes a compensatory effect, in which the presence of one sense mitigates, either in part or in full, the effect of the loss of the other. In terms of locomotor behaviour, the effects of lateral line loss are often not apparent while vision is available to the fish. Fish can swim in uniform flow (Dijkgraaf, 1963), or entrain behind an obstacle (Sutterlin and Waddy, 1975), without a lateral line, so long as they can see.

In order to assess the relative importance of vision and the lateral line in the terrestrial and aquatic locomotion of *Polypterus*, we filmed the fish in the dark, and applied a pharmacological block of the lateral line neuromasts. We expected both sensory modalities to be operational in swimming fish, and to contribute to motor control. We predicted changes in

kinematics and behaviour when either sensory input was lost, but expected the greatest changes in locomotion to be observed when fish did not have access to either source of sensory information. We hypothesized that *Polypterus* has limited aerial vision capabilities, and that neuromasts are non-functional or poorly responsive in air. We therefore expected to observe little to no difference in terrestrial locomotion due to the loss of either or both sensory inputs.

1.2 METHODS

Animals

Polypterus senegalus were obtained from the pet trade (AQUAlity Tropical Fish Wholesale Inc., Mississauga, ON, Canada). They were kept at 25°C on a 12 h/12 h light/dark cycle, and were fed daily ad libitum. Each of four treatment groups included 6 fish, for a total of 24 individuals. Average total body length (BL) of the fish was 127.2 ± 5.9 mm (mean ± s.e.m.), while average mass was 63.23 ± 0.61 g, and neither varied significantly between treatment groups ($p \geq 0.6960$).

Inactivation of the lateral line system

Cobalt acts as a competitive antagonist at the calcium channels on neuromast hair cells, ultimately preventing signal transmission (Schwalbe et al., 2012). The lateral line is disabled without impairing the vestibular system (Schwalbe et al., 2012). *Polypterus* were exposed to 0.15 mmol/L (0.0357 g/L) cobalt (II) chloride hexahydrate (Sigma-Aldrich Corp., St Louis, MO, USA) for three hours, as in similar experiments by Liao (2006) and by Flammang and Lauder (2013). The cobalt was dissolved in calcium-free fresh water, prepared as outlined in Karlsen and Sand (1987). Control fish were placed in calcium-free fresh water containing no cobalt. Fish were placed in aquarium water for 5 minutes prior to trials to rinse off excess cobalt (II) chloride solution.

Although cobalt can be toxic to fishes, adverse behavioural and physiological effects have been observed only at much higher concentrations (1-2 mmol/L), and particularly when exposure time is increased (Janssen, 2000; Karlsen and Sand, 1987). We did not observe any of the outward signs of physiological and behavioural disturbances noted by these studies, nor did we observe any of the characteristic signs of heavy metal toxicity in fishes (Sorensen, 1991).

The loss of the escape response in response to a water jet from a syringe is often used to assess the inactivation of the lateral line (Flammang and Lauder, 2013; Liao, 2006); however, *Polypterus* do not reliably respond to this stimulus even when unimpaired. Instead, the fluorescent dye 4-(4-diethylaminostyryl)-1-methylpyridinium iodide (4-Di-2-ASP; Sigma-Aldrich Corp., St Louis, MO, USA), which is taken up by the ionic transduction channels of functioning hair cells, was used to verify that the neuromast block was successful (Nakae et al., 2012). Cobalt-exposed fish were placed in a solution of 50 mg/L 4-Di-2-ASP for at least 30 minutes, then compared to control fish under a fluorescence microscope. The superficial neuromasts and those of the lateral line canal were darkened following cobalt exposure, indicating that they were impaired. The cobalt exposure did not entirely disable the neuromasts of the head canals.

Filming set-up

Fish were filmed from above using a Photron Fastcam Mini UX 100 high-speed camera (1024 X 1024 pixel resolution). The filming area (24 cm X 28 cm) was lit from below, using a pair of either fluorescent lights, or infrared lights (850 nm, Smart Vision Lights, Muskegon, MI, USA), depending on whether the trial was to be performed using visible light or in the dark. The light was diffused through a sheet of white plexiglass. With the exception of the lights mentioned above, all trials were performed in a dark, windowless room. The aquarium was covered in black

paperboard, except where it would block the light source or the view of the camera. The laptop controlling the camera was concealed in a large box, the opening of which was blocked by a blackout curtain.

It is often assumed that most fishes cannot see infrared light, since these wavelengths are rapidly absorbed in clear water, while blue light penetrates deepest (Levine and MacNichol, 1982). However, in water containing more organic matter, red and infrared light predominate, and it has been shown that some fishes living in turbid environments are sensitive to infrared light (Matsumoto and Kawamura, 2005; Shcherbakov et al., 2012). *Polypterus* inhabit environments of varying water clarity, and the spectral sensitivity of their eyes is not known. Here we assumed, as in previous studies involving nocturnal fishes (e.g. Fitzpatrick et al., 2013), that infrared light is invisible to the fish, but this assumption remains to be tested.

Experimental protocol

Twelve fish were filmed using visible light, six of which were exposed to cobalt solution (abbreviated CC/LT) and six of which were exposed to a cobalt-free sham solution (SH/LT). Another 12 fish were filmed using infrared light. Again, six were exposed to cobalt (CC/DK) and six were exposed to the sham solution (SH/DK). Experiments were conducted over the course of six days, using one fish from each treatment group each day, and varying the order in which fish were used between days.

Fish were introduced to a uniform, enclosed aquarium area, with a water depth of approximately 5 cm, and their swimming behaviour was recorded for 10 minutes, at 0.5 frames s^{-1}. Immediately following this time period, short intervals of relatively steady swimming were captured at 250 frames s^{-1} for kinematic analysis. Efforts were made to capture swimming intervals in which the fish performed at least three tail oscillations, and in which the fish swam at

relatively constant speed with minimal change in direction. Walking trials were performed by repeatedly raising and submerging a textured platform in the aquarium, in order to minimize fish stress from handling. Once the platform was raised and the water drained, the movements of the fish were recorded at 500 frames s^{-1}. As with swimming, a trial was deemed successful if the fish demonstrated at least three tail oscillations.

Data processing, analysis and statistical tests

Videos were digitized using the MATLAB (Mathworks, Natick, MA, USA) program DLTdv5 (Hedrick, 2008). In all the 10-minute behaviour video segments, the nose of the fish was tracked. In the high-frame-rate swimming and walking kinematics videos, both the nose and the tip of the caudal fin were digitized, as well as the fin tips. A MATLAB script automatically traced the midline of the fish in each frame, and selected 15 points evenly spaced along this line for further analysis.

Kinematic and behavioural variables of interest were selected based on related lateral line system studies (Liao, 2006; Patton et al., 2010) and past analyses of *Polypterus* locomotion (Standen et al., 2016). From the 10-minute behaviour videos, differences in average distance travelled, variation in speed and average distance from walls of the filming area were assessed. The following kinematic variables were quantified in both walking and swimming locomotion: average speed of the midline point displaying the least lateral oscillation, maximum angle of fin abduction (Fig. S1.1), fin beat frequency, average minimum distance between the nose and tail as a proxy for maximum body curvature (Fig. S1.1), amplitude, frequency and wavelength of nose and tail oscillation (Fig. S1.2), amplitude of oscillation of body midline points, and body wave speed. The body wave speed, determined by tracking wave crests as they passed down the body of the fish (Fig. S1.1), was divided by the tail-beat frequency to obtain the body wavelength, as

in Liao (2006). Speed of forward travel was divided both by body wave speed and by fin beat frequency to provide estimates of relative propulsion effort.

All behaviour and magnitude variables were standardized relative to the total length of the fish, and the effect of average speed on all other variables was controlled for by taking the residuals. Equality of variances was assessed using Bartlett's test, and normality was assessed by Q-Q plot. For most variables, differences between treatment groups within each type of locomotion were evaluated by one-way ANOVA, followed by Tukey's HSD where applicable. If the assumptions for a parametric test were not met, a non-parametric Kruskal-Wallis test was performed, followed by Mann-Whitney U tests, Bonferroni-corrected for multiple comparisons, to determine pairwise significance. For the 15 body midline points, differences between treatments and between points were assessed by repeated-measures ANOVA. As the assumption of sphericity was violated in both swimming and walking (Mauchly's Test, $p<0.05$), Greenhouse-Geisser-corrected *P*-values were reported. T-tests were used to evaluate overall differences between swimming and walking for each magnitude variable.

In order to determine whether coordination of walking differed between treatments, timing of the maximum lateral displacement of nose, tail and body midline points, and timing of the maximum angle of fin abduction were standardized relative to the regular oscillation of the nose point. As in Standen et al. (2016), the maximum leftward displacement of the nose was defined as 0 degrees, while maximum rightward displacement of the nose was defined as 180 degrees. In order to assess timing differences in swimming, where nose oscillation is infrequent, the same analysis was performed twice and compared, once defining the locomotor cycle by the oscillation of the tail, and the second time defining it by the abduction of the left fin. In both swimming and walking, the assumption of equality of variances was not violated for any

variable. In walking, all variables were von Mises-distributed (Kuiper test, $p>0.05$), while in swimming, some body midline points closer to the nose were not von Mises-distributed ($p<0.05$), and were therefore excluded from further analyses. Directionality was assessed using Rayleigh's Test. No variables demonstrated predicable timing in swimming, regardless of whether the stroke cycle was defined by tail oscillation or by fin motion. Walking variables were directional; therefore possible timing differences between treatments were assessed via Watson-Williams multi-way test for circular data. When $p<0.05$, this was followed by pairwise Watson-Williams tests, adjusted for multiple comparisons using the Bonferroni correction. A Watson-Williams multi-way test was also used to evaluate differences in timing between timing variables irrespective of treatment.

Finally, as swimming fish demonstrated a variety of locomotor strategies across trials, Fisher's exact test was used to evaluate whether propulsion method preference differed between treatments.

1.3 RESULTS

Behaviour

Over a 10-minute swimming period, the average distance travelled by CC/DK fish was less than half that of the non-cobalt-exposed groups ($p=0.0147$, Fig. 1.1A & Table 1.1). Variance in speed over this period did not differ significantly between groups ($p=0.8125$, Fig. 1.1B). Fish remained relatively close to the walls of the set-up in all cases, although average distance of fish from wall was at least 30% greater in CC/DK than in all other groups ($p=0.1834$, Fig. 1.1C), and variance was significantly greater in CC/DK compared to SH/LT ($p=0.0081$).

Kinematics - Magnitude

All kinematics measurements were performed on short video segments representing the first periods of relatively steady, straight-ahead swimming observed in each fish. These segments of steady swimming captured at high frame rate for kinematic analysis did not show the same trend in speed as the longer videos used to assess overall behaviour. Average speeds of forward travel in these short clips, rather than declining in cobalt-exposed fish, were approximately three times higher than those of fish that had not been exposed to cobalt (p=0.0014, Fig. 1.2A & Table 1.2). Speed of terrestrial locomotion differed between treatment groups overall (p=0.0191). Although pairwise comparisons were not significant, speed was generally lower in cobalt-treated fish, particularly those filmed in the dark.

Walking *Polypterus* propel themselves forwards through alternating pectoral fin plants in combination with the curvature of the body into C-shapes on alternating sides, while swimming fish rely on smaller posterior undulations, fin movement, or a combination of the two (Table S1.1). As expected given past analyses of *Polypterus* kinematics (Standen et al., 2016), variables such as body curvature (as determined by minimum distance between nose and tail), maximum fin abduction angle, and nose oscillation differed between environments; these variables did not differ between treatments in either type of locomotion ($p\geq$0.1199, Table 1.2). The nose of the fish rarely oscillates in swimming, so variables associated with nose oscillation were only assessed in walking (Fig. 1.3ACE). Notably, variance in nose oscillation wavelength was at least five times greater in CC/LT than in all other groups (p<0.0001).

Several differences between treatments are only apparent before controlling for speed. In swimming, fin beat frequency was higher in the cobalt-exposed groups (p=0.0127, Fig. 1.2B), but was not longer significant after accounting for speed (p=0.8821, Table 1.2). Tail oscillation amplitude, frequency and wavelength also increased with speed (Fig. 1.3BDF), as did body wave

speed (Fig. 1.2C), although this did not always result in significant differences between treatment groups. Tail wavelength in swimming also demonstrated a unique pattern in variance, as variance of the CC/LT group was over three times larger than that of other treatment groups ($p<0.0001$). When fish speed was compared by ratio to body wave speed and fin beat frequency, fish speed/body wave speed was not significant ($p\geq0.0587$, Fig. 1.2E & Table 1.2), while fish speed/fin frequency was significantly higher in cobalt-exposed groups than in SH/DK ($p\leq0.0454$).

In addition to nose oscillation wavelength in walking, and tail oscillation wavelength in swimming, two other variables demonstrated increased variance in the CC/LT group. Body wavelength (body wave speed/tail oscillation frequency) variance was at least six times greater in CC/LT compared to other treatments, in both swimming and walking ($p<0.0001$, Fig. 1.2D & Table 1.2), making it one of the few kinematic variables to display similar trends under both modes of locomotion.

Similarly, in swimming fish, both mean and variance of body midline oscillation amplitude were greatest in CC/LT, but these differences were not significant ($p=0.4729$, Fig. 1.2F & Table 1.2). In walking fish, there were no apparent differences in amplitude of oscillation between treatments ($p=0.4939$). Overall, in swimming, amplitude increased slightly, although not significantly, towards the tail ($p=0.0698$, Table S1.4). In walking, amplitude differed significantly between many body midline points ($p=0.0002$), with maxima found at the nose and at approximately 60% BL along the body from the nose, and minima found at approximately 20% and 85% BL. At each point, amplitude was significantly greater in walking compared to swimming ($p<0.0001$).

Kinematics - Timing

Timing of body midline oscillation and fin movements showed no evidence of directionality in swimming, regardless of whether timing was determined relative to fin beats, or relative to tail oscillation, so no further analysis was performed ($p>0.05$). Past studies of *Polypterus* have also found limited directionality in kinematic variables associated with swimming (Standen et al., 2016).

In walking, body oscillation and fin abduction were directional relative to the left-right-left oscillation of the nose ($p<0.05$). There were no significant differences in timing between treatment groups ($p\geq0.1140$). Overall, for both oscillations to the left and to the right, the timing of maximum amplitude of oscillation at any given midline point was significantly different from that at all other points ($p<0.0001$, Fig. 1.4 & Table S1.5).

1.4 DISCUSSION

Exploratory behaviour

When introduced to a new aquarium, fish tend to spend more time swimming along the walls, exploring their new environment (Mikheev and Andreev, 1993). In some fishes, reliance on tactile feedback increases when other senses are impaired (Flammang and Lauder, 2013; Walton and Moller, 2010). On the other hand, in Mexican blind cavefish (*Astyanax mexicanus*), characteristic wall-following behaviour is partially impaired in lateral line inactivation (Patton et al., 2010). We were therefore interested in whether proximity to the enclosure walls changed in *Polypterus* in response to sensory deprivation. We did not observe any significant difference in mean distance to wall between groups, providing no evidence for a change in strategy for exploring a novel environment in impaired fish.

With regard to path length, which may be representative of the tendency to explore the environment (Gill and Andrews, 2001; Mikheev and Andreev, 1993), we observed a small

decrease when the lateral line was blocked, which became more pronounced when fish could neither see nor feel. In fish with functional lateral lines, darkness had no effect. That lateral line sense can compensate entirely for the loss of vision, while vision can only partially compensate for lateral line block, is expected, given that *Polypterus* is nocturnal. Similarly, in another nocturnal fish, the glass knifefish (*Eigenmannia virescens*), electrosensory input is favoured over visual input when attempting to maintain position beneath a moving refuge (Sutton et al., 2016). By contrast, the diurnal rainbow trout (*Oncorhynchus mykiss*) will exploit turbulent flows so long as it can see, regardless of lateral line status, but without vision, hydrodynamic exploration decreases (Liao, 2006). Generally, the sensory modality that predominates in each species appears suited to its ecology.

Speed and its effects on kinematics

When the lateral line was blocked, steady swimming bouts were faster, on average, as well as more variable. Mexican blind cavefish also demonstrate increased swimming speed due to lateral line block (Hassan et al., 1992), while sea lamprey (*Petromyzon marinus*) show increased variation in speed (Ayali et al., 2009). Lateral line feedback, or lack thereof, seems to affect the control of swim speed. Increases in speed might be an attempt to compensate for the loss of expected lateral line stimulation in response to swimming movement. Interestingly, speed of terrestrial locomotion decreased in response to lateral line block, particularly in the dark, although differences were not significant. Lateral line feedback might not be anticipated in response to forward movement on land in the same way as in water. In addition, fish might move with greater caution when missing sensory input in an novel environment.

Although strategies for increasing speed depend on the species, and on the style of locomotion, there are many kinematic variables that commonly vary with swim speed

(Bainbridge, 1957; Drucker and Jensen, 1996; Webb, 1973; Webb et al., 1984). Increases in the same variables might be expected to similarly affect speed on land. Several differences between treatments are therefore likely to be a natural consequence of change in speed, rather than indicating a change in movement coordination as a direct result of sensory impairment. In both swimming and walking, differences between groups in mean tail oscillation amplitude and frequency, as well as body wave speed, all reflected differences in speed. Fin beat frequency in swimming and tail oscillation wavelength in walking also increased with speed. For these variables, any significant differences between treatments disappeared after controlling for speed. Overall, kinematic differences between water and land were similar to those observed by Standen et al. (2016). Mean fin abduction angle, body curvature, and amplitude of tail oscillation were all greater in terrestrial locomotion in both studies.

Effect of lateral line block on kinematics

In *Polypterus* with lateral line block but no visual impairment, several variables showed a large increase in variability, and smaller, non-significant increase in mean. Although Dijkgraaf (1963) found that disabling the lateral line of cyprinids did not affect steady swimming performance, our findings are in line with more recent evidence demonstrating that lateral line disruption can indeed affect kinematics. We observed increased variation in amplitude of midline oscillation in swimming. Similarly, swimming against laminar flows, lateral displacement of the body midline increased both in yellowtail kingfish (*Seriola lalandi*) that had undergone unilateral ablation of superficial neuromasts (Yanase et al., 2014), and in golden shiner (*Notemigonus crysoleucas*) for which all lateral line input had been blocked (McHenry et al., 2010). Conversely, in trout swimming in structured turbulent flows, lateral movement of the centre of mass and tail decreased in absence of lateral line input (Liao, 2006). We also observed increased

variation in body wavelength in both swimming and walking. This was the only variable to display the same pattern of variation both in water and on land, making it particularly unusual, especially considering the drastic differences between the two types of locomotion. After pharmacological ablation, changes in body wavelength were also seen in tethered sea lamprey, where wavelength increased (Ayali et al., 2009), but no such changes were observed in free-swimming golden shiner (McHenry et al., 2010). In general, lateral line impairment may often alter displacement of the midline or body wavelength; however, variation in its effects between studies is expected, since the species studied use different movement patterns, and the experimental protocols differ.

Effect of lateral line block on efficiency

Significant perturbations in swimming kinematics may impact the efficiency of movement. For example, Yanase et al. (2012) found a decrease in critical swimming speed, and an increase in oxygen consumption in yellowtail kingfish with ablated neuromasts. Although we made no direct measures of efficiency, inferences might be made based on kinematics alone. Increased lateral displacement of the midline, even after controlling for swim speed, as observed here, suggests increased drag and less efficient locomotion (Yanase et al., 2014). Increased variation in body wavelength suggests inconsistent optimization of this parameter, and again, decreased efficiency. The comparison between the speed of the wave passing down the body of the fish and the speed of forward travel can also provide some insight into locomotor efficiency, with a lower ratio of fish speed to body wave speed suggesting decreased efficiency (Liao, 2006). The ratio of fish speed to fin beat frequency provides a similar estimate of propulsion efficiency for fin movement. On land, the relationship between body and fin movement and forward speed showed little variation within or between treatments. In water, fish speed/fin

frequency increased significantly when lateral line was blocked. This may be due to differences in relative contribution of the tail and fins as swim speed increases.

In one of the proposed mechanisms for lateral line influence on steady swimming, mechanosensory input from the head region is used to adjust the lateral movement of the head to reduce drag, and increase efficiency (Akanyeti et al., 2016; Lighthill, 1993). Though there is evidence for active drag reduction in herring (*Clupea harengus*; Lighthill, 1993; Rowe et al., 1993), none was found in golden shiner, despite changes in other kinematics due to lateral line ablation (McHenry et al., 2010). In *Polypterus*, head oscillation in swimming was rare except at very high speeds, regardless of whether the lateral line was blocked, but lateral line block caused changes in other kinematic variables. Lateral line feedback might be important in controlling head oscillation in highly active species, where head recoil as a result of tail oscillation is greater (Bainbridge, 1963); in less active species, it may affect other aspects of swimming, such as the lateral displacement of the body. The effects of lateral line block likely also depend on the speed of travel. In some species, lateral line block has a greater effect on kinematics at slow to intermediate swimming speeds compared to high swimming speeds (McHenry et al., 2010; Yanase et al., 2014). Lateral line feedback might be more important at lower speeds, at which maintaining stability is more difficult, and at which superficial neuromasts are more sensitive (Engelmann et al., 2000). One might hypothesize that control of movement on land, where lateral line input might be compromised, might bear similarity to rapid swimming in reduced reliance on sensory feedback. The consequent prediction would be little or no change in walking kinematics due to lateral line block. However, although differences in amplitude of oscillation of the midline were only observed in swimming fish, differences in body wavelength were observed in both environments, so there appears to be limited support for this hypothesis in *Polypterus*.

Inputs from multiple sensory modalities

Many fish behaviours are influenced by inputs from both visual and lateral line systems. For example, fish switch prey capture strategies when either or both senses are impaired (Gardiner and Motta, 2012; New et al., 2001; Schwalbe et al., 2012). To our knowledge, only one other study quantifying the effect of lateral line block on swimming kinematics also considered the effect of visual input. Liao (2006) found that in trout swimming in structured turbulence, visual impairment did not affect most kinematic variables, though it did result in increased variability in body wavelength. In *Polypterus*, vision loss by itself had little effect on kinematics, as expected in a nocturnal species, known to have poor visual acuity (see Chapter 2) and accustomed to relying on non-visual sources of sensory information.

Although sensory input from multiple modalities often improves performance on behavioural tasks (Sutton et al., 2016; Verhaal and Luksch, 2015; Walton and Moller, 2010), partial or full compensation for the impairment of one sense by reliance on others has been observed for various behaviours in fishes (Gardiner et al., 2014; Moller, 2002; Sutton et al., 2016; Von der Emde and Bleckmann, 1998). In some behaviors, vision can compensate for the loss of the lateral line (Liao, 2006; Sutterlin and Waddy, 1975); however, we found that lateral line block had an effect on kinematic variables such as body wavelength only when vision was not impaired. Some tasks require the integration of multiple sources of sensory information; for example, flies require visual input to locate an odor source (Frye et al., 2003). Similarly, the concordance of visual and lateral line input, or lack thereof, appears to affect locomotor control in *Polypterus*.

On land, visual acuity is poor, lateral line function is presumably reduced, electrosensation is non-functional, and lack of buoyancy likely impacts vestibular and tactile

input. Surprisingly, despite these presumably large changes in the available sensory information, the comparison of inputs from different modalities seems to remain important in terrestrial locomotion. Standen et al. (2016) proposed two mechanisms by which *Polypterus* might achieve its significantly altered locomotor pattern on land: Existing central pattern generators (CPGs) might be modified by local sensory feedback, or walking-specific CPGs might be configured through a combination of descending, afferent and neuromodulatory inputs. Lateral line and visual information appear to interactively influence descending control in both environments, but their loss did not severely impair motion in either environment. Greater disruption would likely be observed if proprioceptive or tactile feedback could be disabled.

Lateral line system and plasticity

Although some variation exists, likely due to differences in habitat and locomotion style between species (Webb, 1989), the general arrangement of the lateral line canals is well-conserved, and correlates with the areas where variation in pressure during swimming is greatest (Ristroph et al., 2015). The arrangement of superficial neuromasts is more variable, and differences have been observed between populations of the same species living in different environments (Trokovic et al., 2011; Wark and Peichel, 2010), and even between individuals of the same population raised under different conditions. Guppies (*Poecilia reticulata*) raised in presence of predator chemical cues have more facial neuromasts than those not exposed to these cues (Fischer et al., 2013). Western rainbowfish (*Melanotaenia australis*), which inhabit a variable-flow environment, have less superficial neuromasts on the nose and more on the tail when raised in high flows (Kelley et al., 2017).

Although the lateral line system is not present at all in terrestrial vertebrates, likely because desiccation, coupled with density differences, would result in impaired function

(Fritzsch, 1989), it appears to have some impact on the terrestrial locomotion of the amphibious *Polypterus*. The lateral line canals might be sufficiently fluid-filled during this brief emersion to function; however, *Polypterus* can be raised on land, surviving for months so long as the environment is moist, and it is not known how the lateral line or other sensory systems might change as a result. Plastic changes in pectoral anatomy are observed in land-raised fish (Standen et al., 2014), but potential changes in neuromast function or arrangement remain to be investigated, as does the effect of prolonged air exposure on the structure and function of the eye.

1.5 FIGURES

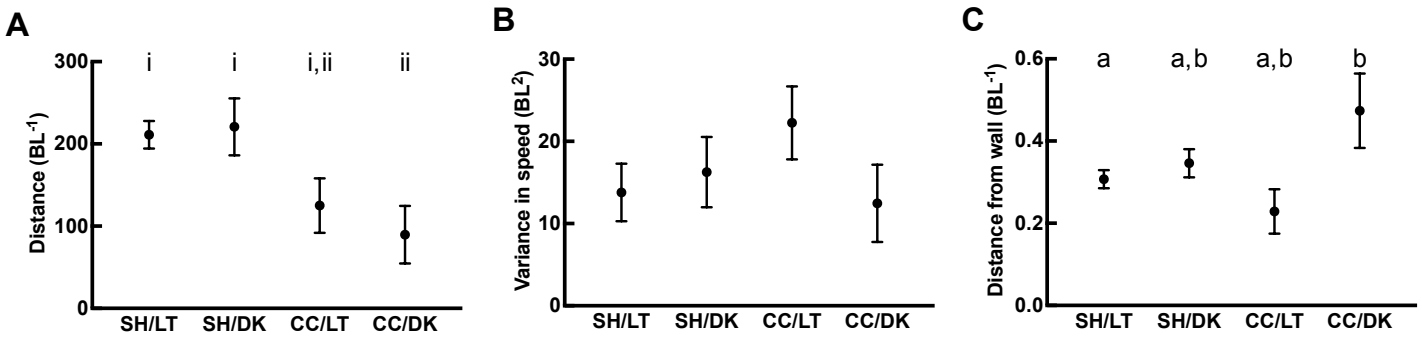

Figure 1.1. Behaviour variables, including distance travelled (A), variation in speed (B), and average distance from enclosure wall (C). CC = cobalt-treated, SH = sham, LT = filmed in light, DK = filmed in dark. Points represent means, and error bars are s.e.m. Shared Roman numerals represent means that do not differ significantly, while shared letters represent variances that do not differ significantly. If there is no pairwise significance between any groups, no numbering or lettering is used. Note that multiple comparison *P*-values are highly conservative (see Table 1.1).

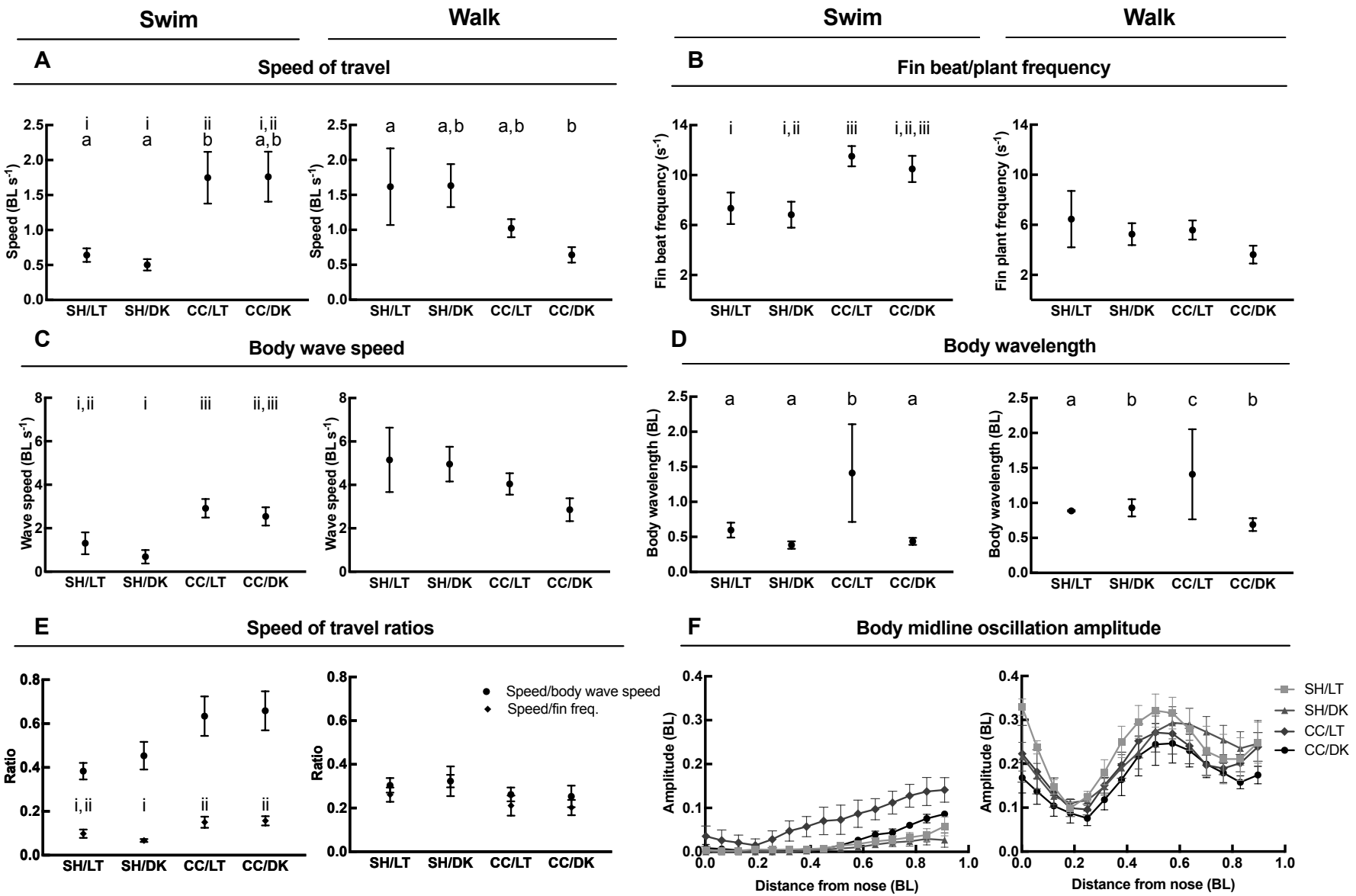
Swim
Walk
Swim
Walk
A
Speed of travel
B
Fin beat/plant frequency
Speed (BL s-1)
Fin beat frequency (s-1)
Fin plant frequency (s-1)
SH/LT
SH/DK
CC/LT
CC/DK
C
Body wave speed
D
Body wavelength
Wave speed (BL s-1)
Body wavelength (BL)
E
Speed of travel ratios
F
Body midline oscillation amplitude
Ratio
Speed/body wave speed
Speed/fin freq.
Amplitude (BL)
Distance from nose (BL)

Figure 1.2. Magnitude variables, including speed of travel (A), frequency of fin beats in swimming and fin plants in walking (B), body wave speed (C), body wavelength (D), ratios between speed of travel and body wave speed, and speed of travel and fin frequency (E), and maximum amplitude of oscillation for 15 points along the body midline (F). CC = cobalt-treated, SH = sham, LT = filmed in light, DK = filmed in dark. Points represent means, and error bars are s.e.m. For A-E, shared Roman numerals represent means that do not differ significantly, while shared letters represent variances that do not differ significantly. If there is no pairwise significance between any groups, no numbering or lettering is used. Note that multiple comparison *P*-values are highly conservative (see Tables S1.2 & S1.3).

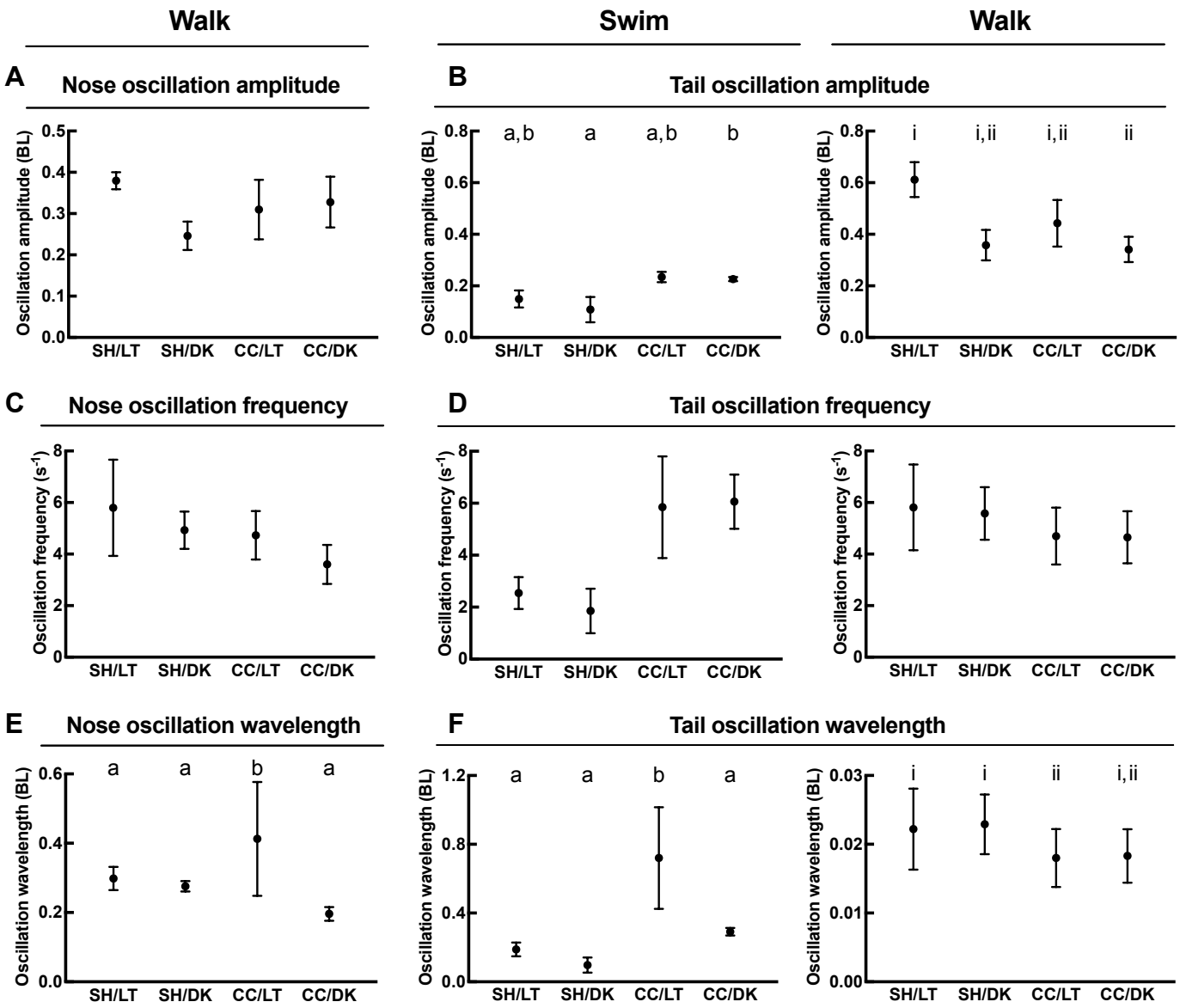
Walk
Swim
Walk
A
Nose oscillation amplitude
B
Tail oscillation amplitude
Oscillation amplitude (BL)
0.5
0.4
0.3
0.2
0.1
0.0
a,b
a
a,b
b
i
i,ii
i,ii
ii
0.8
0.6
0.4
0.2
0.0
SH/LT
SH/DK
CC/LT
CC/DK
C
Nose oscillation frequency
D
Tail oscillation frequency
Oscillation frequency (s-1)
8
6
4
2
0
E
Nose oscillation wavelength
F
Tail oscillation wavelength
Oscillation wavelength (BL)
a
a
b
a
i
i
ii
i,ii
0.6
0.4
0.2
0.0
1.2
0.8
0.4
0.0
0.03
0.02
0.01
0.00

Figure 1.3. Magnitude variables, including nose oscillation amplitude (A), frequency (C), and wavelength (E) in walking only, and tail oscillation amplitude (B), frequency (D), and wavelength (F) in both swimming and walking. CC = cobalt-treated, SH = sham, LT = filmed in light, DK = filmed in dark. Points represent means, and error bars are s.e.m. shared Roman numerals represent means that do not differ significantly, while shared letters represent variances that do not differ significantly. If there is no pairwise significance between any groups, no numbering or lettering is used. Note that multiple comparison *P*-values are highly conservative (see Tables S1.2 & S1.3).

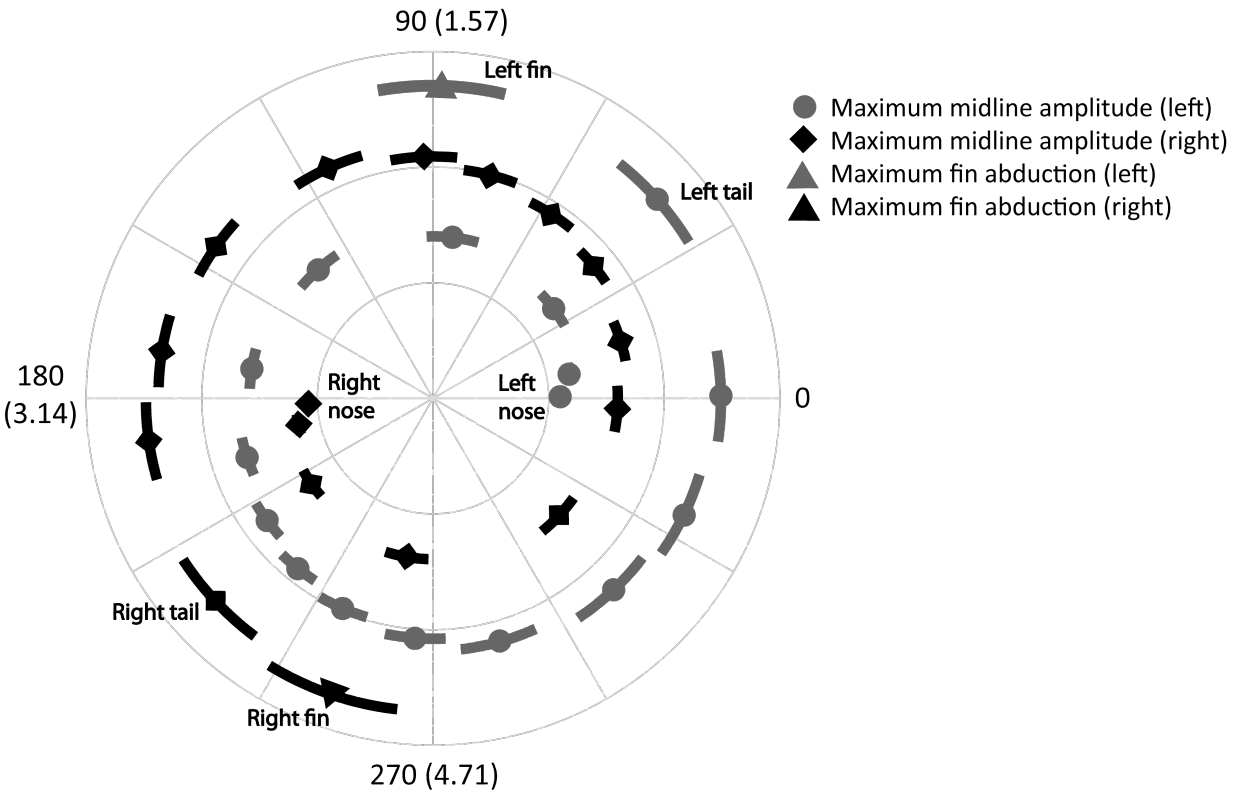

Figure 1.4. Timing variables in walking, pooled across all treatments. Start of cycle is defined by maximum leftwards displacement of nose (0°), mid-cycle by maximum rightwards displacement (180°), and end of cycle by return to maximum leftwards displacement (360°). Symbols represent means, and error bars are angular s.e.m. Innermost points on graph represent timing of maximum oscillation to the left and to the right of the body midline point closest to the nose. Each subsequent point along the body midline is plotted at a slightly larger radius, with the final two points representing the timing of tail oscillation and fin abduction. Timing is shown in degrees, with radian equivalent in brackets. Swim timing showed no directionality (Rayleigh's Test, $P>0.05$), and was not plotted.

1.6 TABLES

Table 1.1. Comparison of behaviour variables between treatment groups. Means are shown ± standard error of the mean. *P*-values for differences in mean and variance (var.) are accompanied by the appropriate test statistic (B = Bartlett's statistic) and degrees of freedom. Multiple comparisons were carried out for mean/variance where they differed significantly between groups (overall $P<0.05$). For pairwise comparisons, $P_{crit}=0.05$ for differences in mean path travelled, and $P_{crit}=0.0083$ for differences in variance in wall distance.

Variable	Treatment mean				*P* (mean)	*P* (var.)	Multiple comparison (*P*)						
	SH/LT	SH/DK	CC/LT	CC/DK			Type	SH/LT-SH/DK	SH/LT-CC/LT	SH/LT-CC/DK	SH/DK-CC/LT	SH/DK-CC/DK	CC/LT/-CC/DK
Total path travelled (BL)	211.1244± 16.6864	220.7998± 34.5947	125.0512± 33.0627	89.5703± 34.9522	0.0147 ($F_{3,22}=4.53$)	0.4247 ($B_3=2.79$)	**Mean**	0.9957	0.2481	0.0451	0.1735	0.0282	0.8550
Variance in speed (BL^2)	13.7787± 3.5031	16.2563± 4.2690	22.2573± 4.4354	12.4657± 4.6926	0.8125 ($\chi^2_{3,22}=0.41$)	0.8650 ($B_3=9.45$)	**None**	N/A	N/A	N/A	N/A	N/A	N/A
Distance from wall (BL)	0.3073± 0.0222	0.3460± 0.0342	0.2286± 0.0539	0.4738± 0.0903	0.1834 ($\chi^2_{3,22}=0.31$)	0.0239 ($B_3=0.73$)	**Var.**	0.3632	0.8724	0.0081	0.3234	0.0526	0.0124

Table 1.2. Comparison of magnitude variables between walking and swimming, and between treatment groups within each type of locomotion. Means are shown ± standard error of the mean. *P*-values for differences in mean and variance (var.) are accompanied by the appropriate test statistic (B = Bartlett's statistic) and degrees of freedom. Nose oscillation occurred too infrequently in swimming to statistically test related variables, and corresponding boxes have been crossed off. For multiple comparisons, see supplementary materials, Tables S1.2 & S1.3.

Variable		**Overall mean**	***P* (swim vs. walk)**	**Treatment mean**				**Between treatments**			
				SH/LT	**SH/DK**	**CC/LT**	**CC/DK**	**Not controlled for speed**		**Controlled for speed**	
								***P* (mean)**	***P* (var.)**	***P* (mean)**	***P* (var.)**
Speed (BL/s)	*Swim*	1.1274± 0.1732	0.7023 (t_{22}=0.54)	0.6420± 0.0966	0.5033± 0.0809	1.7480± 0.3703	1.7622± 0.3576	0.0014 ($\chi^2_{3,22}$=15.58)	0.0035 (B_3=13.59)	N/A	N/A
	Walk	1.2292± 0.1750		1.6182± 0.5476	1.6325± 0.3071	1.0233± 0.1292	0.6427± 0.1114	0.0191 ($\chi^2_{3,23}$=9.93)	0.0027 (B_3=14.14)	N/A	N/A
Fin abduction angle (rad)	*Swim*	1.1106± 0.0425	0.0008 (t_{22}=-3.59)	0.9622± 0.0622	1.1103± 0.0591	1.1454± 0.0787	1.2474± 0.1168	0.1250 ($F_{3,22}$=2.17)	0.5958 (B_3=1.89)	0.1984 ($F_{3,22}$=1.71)	0.4970 (B_3=2.38)
	Walk	1.3332± 0.0342		1.3136± 0.1056	1.3409± 0.0466	1.3153± 0.0768	1.3634± 0.0452	0.9797 ($\chi^2_{3,23}$=0.19)	0.1994 (B_3=4.65)	0.8238 ($\chi^2_{3,23}$=0.91)	0.7122 (B_3=1.37)
Fin beat frequency (s^{-1})	*Swim*	9.0807± 0.6581	0.0005 (t_{21}=-3.85)	7.3473± 1.2625	6.8304± 1.0372	11.5142± 0.8157	10.4908± 1.0485	0.0127 ($F_{3,21}$=4.79)	0.8149 (B_3=0.94)	0.8221 ($F_{3,21}$=0.30)	0.3394 (B_3=3.36)
	Walk	5.2315± 0.6503		6.4602± 2.2522	5.2573± 0.8776	5.5868± 0.7609	3.6217± 0.7162	0.2064 ($\chi^2_{3,23}$=4.57)	0.0236 (B_3=9.47)	0.7102 ($\chi^2_{3,23}$=1.38)	0.8720 (B_3=0.70)
Maximum body curvature (BL)	*Swim*	0.9617± 0.0138	<0.0001 (t_{22}= -12.00)	0.9899± 0.0068	0.9739± 0.0386	0.9113± 0.0276	0.9738± 0.0166	0.1199 ($\chi^2_{3,22}$=5.84)	0.0093 (B_3=11.50)	0.3137 ($\chi^2_{3,22}$=3.56)	0.0078 (B_3=11.87)
	Walk	0.5985± 0.0260		0.5724± 0.0563	0.6308± 0.0419	0.6351± 0.0266	0.5559± 0.0764	0.6432 ($F_{3,23}$=0.57)	0.1732 (B_3=4.98)	0.5766 ($F_{3,23}$=0.51)	0.1767 (B_3=4.93)
Nose amplitude (BL)	*Swim*										
	Walk	0.3159± 0.0259		0.3797± 0.0207	0.2461± 0.0344	0.3098± 0.0720	0.3279± 0.0614	0.3526 ($F_{3,23}$=1.15)	0.0622 (B_3=7.33)	0.3365 ($F_{3,23}$=1.20)	0.1190 (B_3=5.85)
Nose frequency (s^{-1})	*Swim*										

	Walk	4.7644± 0.5682		5.7972± 1.8643	4.9278± 0.7217	4.7317± 0.9400	3.6007± 0.7569	0.6231 ($F_{3,23}$=0.60)	0.1051 (B_3=6.14)	0.8763 ($\chi^2_{3,23}$=0.69)	0.6066 (B_3=1.84)
Nose wavelength (BL)	*Swim*										
	Walk	0.2956± 0.0427		0.2981± 0.0335	0.2757± 0.0149	0.4126± 0.1642	0.1960± 0.0197	0.1520 ($\chi^2_{3,23}$=5.29)	<0.0001 (B_3=31.96)	0.5151 ($\chi^2_{3,23}$=2.29)	<0.0001 (B_3=31.72)
Tail amplitude (BL)	*Swim*	0.1775± 0.0190	<0.0001 (t_{22}=-5.33)	0.1493 ± 0.0329	0.1081± 0.0488	0.2344± 0.0197	0.2266± 0.0080	0.0205 ($\chi^2_{3,22}$=4.18)	0.0109 (B_3=11.16)	0.9269 ($\chi^2_{3,22}$=0.46)	0.3562 (B_3=3.24)
	Walk	0.4384± 0.0389		0.6118± 0.0677	0.3579± 0.0590	0.4427± 0.0902	0.3413± 0.0489	0.0414 ($F_{3,23}$=3.30)	0.5987 (B_3=1.89)	0.0756 ($F_{3,23}$=2.86)	0.5625 (B_3=2.05)
Tail frequency (s^{-1})	*Swim*	3.990± 0.7059	0.2361 (t_{22}=-0.73)	2.5428± 0.6108	1.8543± 0.8568	5.8472± 1.9581	6.0619± 1.0445	0.0533 ($F_{3,22}$=3.06)	0.0686 (B_3=7.11)	0.8092 ($F_{3,22}$=0.32)	0.4193 (B_3=2.83)
	Walk	5.1850± 0.5832		5.8130± 1.6638	5.5767± 1.0187	4.6989± 1.1027	4.6514± 1.0106	0.8703 ($F_{3,23}$=0.24)	0.6264 (B_3=1.75)	0.7769 ($\chi^2_{3,23}$=0.47)	0.4178 (B_3=2.83)
Tail wavelength (BL)	*Swim*	0.3314± 0.0939	0.9784 (t_{22}=2.15)	0.1884± 0.0402	0.0976± 0.0441	0.7201± 0.2952	0.2915± 0.0222	0.0094 ($\chi^2_{3,22}$=11.48)	<0.0001 (B_3=32.92)	0.1208 ($\chi^2_{3,22}$=5.82)	<0.0001 (B_3=34.38)
	Walk	0.3357± 0.0381		0.0222± 0.0059	0.0229± 0.0043	0.0180± 0.0042	0.0183± 0.0039	0.5280 ($\chi^2_{3,23}$=2.22)	<0.0001 (B_3=16.83)	0.7323 ($\chi^2_{3,23}$=1.29)	<0.0001 (B_3=16.57)
Body wave speed (BL/s)	*Swim*	1.8343± 0.2764	0.0002 (t_{22}=-4.15)	1.3069± 0.5035	0.6881± 0.3110	2.9167± 0.4280	2.5438± 0.4222	0.0041 ($F_{3,22}$=6.18)	0.7829 (B_3=1.08)	0.6653 ($F_{3,22}$=0.53)	0.5917 (B_3=1.91)
	Walk	4.2524± 0.4678		5.1520± 1.4853	4.9589± 0.8002	4.0423± 0.4947	2.8562± 0.5254	0.1656 ($\chi^2_{3,23}$=5.09)	0.0535 (B_3=7.66)	0.7867 ($\chi^2_{3,23}$=1.06)	0.1923 (B_3=0.91)
Speed/body wave speed ratio	*Swim*	0.5553± 0.0473	0.0001 (t_{22}=-4.43)	0.3834± 0.0375	0.4540± 0.0632	0.6344± 0.0900	0.6588± 0.0892	0.0995 ($F_{3,17}$=2.53)	0.3097 (B_3=3.59)	N/A	N/A
	Walk	0.2817± 0.0165		0.3037± 0.0336	0.3230± 0.0286	0.2620± 0.0317	0.2529± 0.0492	0.3852 ($F_{3,23}$=1.07)	0.3932 (B_3=2.99)	N/A	N/A
Speed/fin beat freq. ratio (BL)	*Swim*	0.1186± 0.0124	0.0005 (t_{21}=-3.85)	0.0981± 0.0191	0.0668± 0.0067	0.1506± 0.0253	0.1568± 0.0210	0.0199 ($F_{3,21}$=4.23)	0.1105 (B_3=6.02)	N/A	N/A
	Walk	0.2506± 0.0244		0.2618± 0.0325	0.3230± 0.0678	0.2118± 0.0462	0.2027± 0.0348	0.0993 ($\chi^2_{3,23}$=6.27)	0.4762 (B_3=2.50)	N/A	N/A
Body wavelength (BL)	*Swim*	0.7467± 0.2354	0.0158 (t_{22}=-2.30)	0.5974± 0.1064	0.3835± 0.0532	1.4106± 0.6974	0.4366± 0.0505	0.4056 ($\chi^2_{3,18}$=1.53)	<0.0001 (B_3=27.28)	0.0482 ($\chi^2_{3,18}$=6.61)	<0.0001 (B_3=25.04)
	Walk	0.9795± 0.1638		0.8864± 0.0096	0.9306± 0.1227	1.4100± 0.6435	0.6910± 0.0910	0.2481 ($\chi^2_{3,23}$=4.13)	<0.0001 (B_3=47.56)	0.8638 ($\chi^2_{3,23}$=0.74)	<0.0001 (B_3=35.87)

1.7 SUPPLEMENTARY MATERIALS

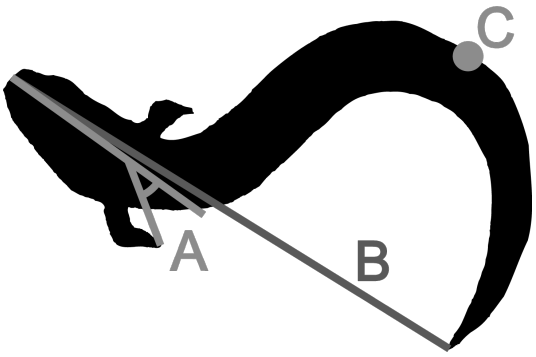

Figure S1.1. Walking fish (dorsal view) overlain with magnitude variable measurements. Fin abduction angle (A), is measured relative to a segment joining the nose and the midpoint between the pectoral fins. Minimum distance between nose and tail (B) is a proxy for body curvature. The wave crest (C) on the right side of the body is tracked as it passes from nose to tail to determine body wave speed.

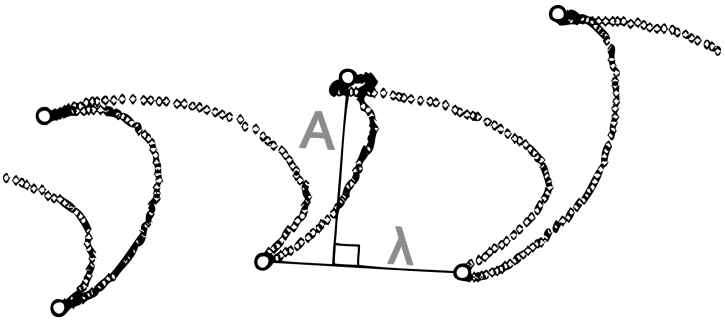

Figure S1.2. X-Y plot showing oscillatory movement of nose point in walking fish. Method of measurement for oscillation amplitude (A) and wavelength (λ) is consistent across all digitized points.

Table S1.1. Frequency of propulsion methods in swimming trials. Fisher's exact test revealed no significant differences between treatments (P=0.1713).

Treatment	Propulsion method		
	Fins only	Fins and tail	Tail only
SH/LT	1	5	0
SH/DK	3	2	1
CC/LT	1	5	0
CC/DK	0	5	0

Table S1.2. Multiple comparisons of treatment means, for magnitude variables where means differed significantly between groups (overall P<0.05, see Table 1.2). Threshold for pairwise significance depends on whether P-value was adjusted within the post-hoc test (parametric, P_{crit}=0.05) or not (non-parametric, P_{crit}=0.0083).

Variable		Control for speed?	P_{crit}	Multiple comparison (P)					
				SH/LT-SH/DK	SH/LT-CC/LT	SH/LT-CC/DK	SH/DK-CC/LT	SH/DK-CC/DK	CC/LT/-CC/DK
Speed (BL/s)	*Swim*	N/A	0.0083	0.7056	0.0107	0.0223	0.0048	0.0103	0.8026
Speed (BL/s)	*Walk*	N/A	0.0083	0.3367	0.1495	0.0250	0.0782	0.0250	0.0374
Fin beat frequency (s^{-1})	*Swim*	No	0.0500	0.9856	0.04293	0.1946	0.0281	0.1287	0.9035
Tail amplitude (BL)	*Swim*	No	0.0083	0.8068	0.0250	0.0176	0.0360	0.0654	0.2733
Tail amplitude (BL)	*Walk*	No	0.0500	0.0697	0.3236	0.0493	0.8151	0.9981	0.7215
Tail wavelength (BL)	*Swim*	No	0.0083	0.1215	0.1093	0.0176	0.0156	0.0099	0.8551
Body wave speed (BL/s)	*Swim*	No	0.0500	0.4064	0.0247	0.0996	0.0156	0.0057	0.4652
Speed/fin freq. ratio (BL)	*Swim*	N/A	0.0500	0.7101	0.2667	0.2191	0.0454	0.0385	0.9965
Body wavelength (BL)	*Swim*	Yes	0.0083	0.4561	0.2012	0.9168	0.0201	0.0526	0.1441

Table S1.3. Multiple comparisons of treatment variances, for magnitude variables where variance differed significantly between groups (overall $P<0.05$, see Table 1.2). Pairwise comparison is considered significant when $P<0.00833$.

Variable		Control for speed?	Multiple comparison (*P*)					
			SH/LT-SH/DK	SH/LT-CC/LT	SH/LT-CC/DK	SH/DK-CC/LT	SH/DK-CC/DK	CC/LT/-CC/DK
Speed (BL/s)	*Swim*	N/A	0.7056	0.0107	0.0223	0.0048	0.0103	0.8026
Speed (BL/s)	*Walk*	N/A	0.2298	0.0068	0.0035	0.0806	0.0444	0.7526
Fin beat frequency (s^{-1})	*Walk*	No	0.0595	0.0330	0.0255	0.7613	0.6658	0.8973
Maximum body curvature (BL)	*Swim*	No	0.0018	0.0083	0.1175	0.4807	0.0882	0.2524
Maximum body curvature (BL)	*Swim*	Yes	0.0013	0.0062	0.0796	0.4775	0.1004	0.2842
Nose wavelength (BL)	*Walk*	No	0.0991	0.0035	0.2699	<0.0001	0.5482	0.0003
Nose wavelength (BL)	*Walk*	Yes	0.0306	0.0069	0.1939	<0.0001	0.3200	0.0004
Tail amplitude (BL)	*Swim*	No	0.4064	0.2860	0.0117	0.0691	0.0027	0.0732
Tail wavelength (BL)	*Swim*	No	0.8443	0.0005	0.1977	0.0008	0.1485	0.0001
Tail wavelength (BL)	*Swim*	Yes	0.8514	0.0003	0.2919	0.0005	0.2263	0.0002
Tail wavelength (BL)	*Walk*	No	0.8654	0.0079	0.9298	0.0055	0.7969	0.0095
Tail wavelength (BL)	*Walk*	Yes	0.6080	0.0132	0.8317	0.0044	0.7625	0.0084
Body wavelength (BL)	*Swim*	No	0.1232	0.0054	0.0654	0.0050	0.7683	0.0001
Body wavelength (BL)	*Swim*	Yes	0.0800	0.0105	0.0985	0.0039	0.4453	0.0008
Body wavelength (BL)	*Walk*	No	<0.0001	<0.0001	0.0002	0.0026	0.5270	0.0006
Body wavelength (BL)	*Walk*	Yes	0.0214	<0.0001	0.0483	0.0019	0.6829	0.0008

Table S1.4. Maximum amplitude of oscillation to the left and to the right along the body midline. Means are shown ± standard error of the mean. In walking, amplitude varied significantly between points ($F_{14,259}$=7.12, *P*=0.0002), but variation between points did not depend on treatment ($F_{42,259}$=0.83, *P*=0.4939). In swimming, amplitude did not depend on either point position ($F_{14,259}$=0.74, *P*=0.4729) or the interaction between point and treatment ($F_{42,259}$=3.04, *P*=0.0698). At each point, amplitude was significantly greater in walking compared to swimming ($t_{21}\leq$-4.422, *P*<0.0001 at all points).

Variable	Distance from nose (BL)	Amplitude (BL)	
		Walk	Swim
Midline point 1	0.0026	0.2340±0.0228	0.0110±0.0061
Midline point 2	0.0626	0.1820±0.0173	0.0081±0.0056
Midline point 3	0.1264	0.1280±0.0118	0.0063±0.0042
Midline point 4	0.1903	0.0992±0.0079	0.0048±0.0034
Midline point 5	0.2547	0.1035±0.0074	0.0078±0.0048
Midline point 6	0.3197	0.1495±0.0115	0.0131±0.0064
Midline point 7	0.3845	0.2002±0.0168	0.0168±0.0070
Midline point 8	0.4496	0.2478±0.0196	0.0211±0.0090
Midline point 9	0.5150	0.2775±0.0203	0.0258±0.0092
Midline point 10	0.5805	0.2810±0.0200	0.0335±0.0094
Midline point 11	0.6458	0.2598±0.0197	0.0420±0.0090
Midline point 12	0.7112	0.2242±0.0191	0.0488±0.0102
Midline point 13	0.7763	0.2084±0.0176	0.0549±0.0108
Midline point 14	0.8415	0.2014±0.0165	0.0667±0.0131
Midline point 15	0.9092	0.2264±0.0201	0.0725±0.0141

Table S1.5. Timing variables in walking fish pooled across treatments, including maximum amplitude of oscillation to the left and to the right along the body midline, as well as fin abduction. Means are shown ± standard error of the mean. For each oscillation direction (left and right), timing of maximum oscillation at any given point was significantly different than at all other points ($F_{1,23} \geq 91.49$, $P < 0.0001$ for all pairwise comparisons). There was no significant difference in timing between treatments at any midline point ($F_{3,23} \leq 2.25$, $P \geq 0.1140$).

Variable	Distance from nose (BL)	Left oscillation timing (rad)	Right oscillation timing (rad)
Midline point 1	0.0026	0.0168±0.0395	3.2132±0.0418
Midline point 2	0.0626	0.1792±0.0656	3.3390±0.0667
Midline point 3	0.1264	0.6339±0.1232	3.7556±0.0932
Midline point 4	0.1903	1.4517±0.1535	4.5450±0.1305
Midline point 5	0.2547	2.3071±0.1359	5.5264±0.1250
Midline point 6	0.3197	2.9825±0.1039	6.2202±0.1215
Midline point 7	0.3845	3.4507±0.0966	0.2932±0.1001
Midline point 8	0.4496	3.7772±0.0942	0.6881±0.0939
Midline point 9	0.5150	4.0401±0.0929	0.9979±0.1070
Midline point 10	0.5805	4.3065±0.1092	1.3203±0.1160
Midline point 11	0.6458	4.6375±0.1236	1.6122±0.1344
Midline point 12	0.7112	4.9748±0.1497	2.0018±0.1399
Midline point 13	0.7763	5.4618±0.1633	2.5386±0.1191
Midline point 14	0.8415	5.8401±0.1549	2.9714±0.1295
Midline point 15	0.9092	0.0048±0.1586	3.2925±0.1365
Tail point	1	0.7254±0.1725	3.8928±0.1740
Fin abduction	N/A	1.5409±0.2016	4.3869±0.2122

CHAPTER 2.

Aquatic and aerial visual acuity of the amphibious fish *Polypterus senegalus*, as estimated by optokinetic response

ABSTRACT

Polypterus senegalus is an amphibious fish, capable of survival on land for extended periods. Previously, we had shown that removal of visual information affects both its aquatic locomotion and its terrestrial locomotion. Although thought to have poor vision, *Polypterus* visual acuity had not been quantified. We measured the optokinetic response of fish to stimuli of varying speed and spatial frequency, in both air and water. In water, fish tracked slow-moving (2 deg/s) stimuli moderately well, and tracked fast-moving stimuli very poorly. Spatial acuity was very low compared to many other species, and maximum response was observed at 0.05-0.075 stimulus cycles per degree of visual arc. In air, visual acuity was much poorer by every measure, but some visual ability persisted. Limited stimulus tracking ability might be expected in a nocturnal ambush predator such as *Polypterus*, where gaze stabilization may be less crucial, and other sensory inputs may have greater importance in perceiving the environment. Poor vision in air was also expected, as the eye shows no specialization for aerial vision; however, considering the plastic response of *Polypterus* to long-term emersion in other respects, eye anatomy and aerial acuity of land-raised fish should be examined.

2.1 INTRODUCTION

For most fishes, visual input is an important source of information about the environment. Animals with higher visual acuity can perceive their surroundings in greater detail, which may be important for foraging, navigation, predator avoidance, and other behaviours. However, the visual image must be in a fixed position on the retina, or vision will be blurred. When either the animal or elements of the surrounding environment are in motion, the visual image moves relative to the retina, reducing the quality of the information gathered. The oculomotor system works to maintain visual acuity when the fish is moving relative to its environment, through compensatory strategies such as the optokinetic reflex (OKR; Land, 1999). The OKR consists of two phases. During the slow phase, the eye rotates as it tracks an object moving relative to the animal, thus preventing optic blur. Once the maximum angle of rotation is reached, the eye is quickly rotated in the opposite direction, to reset it for the next tracking phase. During this saccade, vision is temporarily blurred (Land, 1999).

The OKR can be used to assess the visual capabilities of an organism. Various parameters of an experimental stimulus (usually a vertically-striped drum) can be varied to test contrast sensitivity, temporal acuity, and spatial acuity (Mueller and Neuhauss, 2010). Lack of OKR response to a given stimulus indicates that it cannot be perceived by the animal, providing information on the limits of visual perception. Although OKR tests are not the only method for determining visual acuity, they have several advantages over other techniques. Anatomical methods, such as estimates based on photoreceptor density, may over-estimate acuity compared to behavioural methods, as they do not account for the effects of higher-order neural processing on perception (Pettigrew et al., 1988). Other behavioural methods, such as the use of optomotor

response (OMR) or discrimination training, are more difficult to quantify, or less efficient (Mueller and Neuhauss, 2010).

Although the OKR is conserved across vertebrates, its characteristics depend on taxonomic group, on locomotion style, and on habitat. Fishes and mammals tend to track motion via eye movements alone, while birds, reptiles and amphibians rely to a greater extent on head and body movements (Mueller and Neuhauss, 2010). In teleosts, OKR ability has been found to be related to locomotion pattern, as species that move more slowly and intermittently do not track high-speed stimuli, and do not exhibit rhythmic OKR cycles (Dieringer et al., 1992). There is also a relationship between habitat and visual acuity, as actinopterygians in low-light or turbid environments have smaller eyes, and spatial acuity is positively correlated with eye size (Caves et al., 2017).

Owing to the difference in refractive indices between air and water, most fishes have myopic vision in an aerial environment. Some species which emerge onto land have eyes adapted to retain high visual acuity as they move between environments, with the extent of adaptation typically dependent on the relative amount of time spent in each medium (Sayer, 2005). Visual capabilities of amphibious fishes are often inferred from ability to capture prey (Sponder and Lauder, 1981) or avoid predators (Tytler and Vaughan, 1983) in the terrestrial environment. Differences in acuity between environments have not, to our knowledge, been assessed by OKR tests.

We tested the temporal and spatial acuity of *Polypterus senegalus*, an amphibious basal actinopterygian, using an OKR experiment. *Polypterus* are capable of terrestrial locomotion (Standen et al., 2016), although voluntary excursions onto land have yet to be observed in the natural environment (Du et al., 2016). Their visual abilities, in either air or water, were unknown;

however, absence of visual input has been shown to impact both terrestrial and aquatic locomotor behaviour (see Chapter 1). Although *Polypterus* are nocturnal and can live in turbid environments, their eyes are not noticeably reduced. Vision may therefore be a significant source of information for *Polypterus*, but visual acuity is expected to be poor in comparison to that of diurnal species inhabiting clearer waters. As *Polypterus* are not known to possess any adaptations for aerial vision (Kröger et al., 2014; Rochon-Duvingneaud, 1943, in Pfeiffer, 1968), they were expected to demonstrate decreased acuity in air relative to water, but not a complete loss of visual ability.

2.2 METHODS

Animals

Polypterus senegalus were obtained from the pet trade (AQUAlity Tropical Fish Wholesale Inc., Mississauga, ON, Canada). They were kept at 25°C on a 12 h/12 h light/dark cycle, and were fed daily ad libitum. Five fish were used. Average total body length was 67.0 ± 1.9 mm (mean ± s.e.m.), while average mass was 1.78 ± 0.20 g.

Filming set-up

Fish were embedded in agar to restrict their movement for the duration of the experiment. Each fish was anaesthetized in 125 mg/L MS-222 until it lost its ability to right itself when flipped upside down (1-3 minutes). The body of the fish was placed in a notch carved into agar in a petri dish. Agar cooled to gelling temperature (~37°C) was poured over top, immobilizing the body of the fish caudal to the gills. The head was gently secured by means of a thin strap caudal to the eyes but rostral to the gills. The secured fish was then placed in aquarium water and allowed at least two minutes to recover.

The optokinetic set-up (Fig. 2.1) was similar to that described by Mueller and Neuhauss (2010). The fish was placed in the centre of a white drum onto which a computer-generated stimulus video, of rotating, equally-spaced vertical bars, was projected from below. The movement of the eyes was filmed from above at 60 frames per second using a Nikon DSLR camera (AF-S DX 35mm f/1.8 lens) angled through the eyepiece of a Leica M60 microscope.

Experimental protocol

To test the temporal aspect of visual acuity, the speed of rotation was varied while the width of the projected bars was held constant, at 0.1 cycles per degree (cpd). This spatial frequency has been used as baseline in past acuity tests of teleosts (Mueller and Neuhauss, 2010; Ryan et al., 2016). Speeds selected for testing were 1, 2, 5, 10, 15, 20, 30, 40 deg/s, based on past OKR studies of other fishes (Mueller and Neuhauss, 2010; Ryan et al., 2016). Speed of rotation was changed every 10 s, first increasing from 1 deg/s to 40 deg/s, then decreasing back down to 1 deg/s. The sequential increase then decrease of the stimulus velocity allowed us to assess whether the residual anaesthesia had any effect on eye movement, through comparison of eye movements at the first and second occurrence of the same stimulus. A blank white screen was projected for 10 s at both the start and end of the trial as a control. In total, each trial lasted 3 minutes.

Preliminary analysis of temporal acuity data allowed selection of a stimulus rotation speed (20 deg/s) that produced a strong response at the baseline spatial frequency (0.1 cpd), to be used in all spatial frequency trials. Based on previous studies (Mueller and Neuhauss, 2010; Ryan et al., 2016), spatial frequency was varied (0.025, 0.05. 0.075, 0.1, 0.125, 0.15, and 0.2 cpd) every 10 s, first increasing and then decreasing the number of bars, with a blank-screened

control interval at both the beginning and end of the trial, for a total trial time of 2 minutes and 40 seconds.

Both eyes were stimulated, as binocular stimulation generally produces a stronger response (Beck et al., 2004; Dieringer et al., 1992). In other animals, the temporal-to-nasal slow phase tracking response is known to be stronger than the nasal-to-temporal response, particularly when eyes are positioned laterally (Masseck and Hoffmann, 2009). Therefore, as the stimulus video was rotated counter-clockwise, we decided to analyze the movement of the right eye only, since it was likely to demonstrate a stronger response. Both temporal and spatial acuity of each fish were tested both in air and in water. Two replicates of each trial were run, and the one that showed the strongest response, as defined by the total number of right-eye saccades, was selected for further analysis. To minimize stress on the fish, they were not subjected to more than one trial every three days.

Data processing, analysis and statistical tests

Videos were analyzed in ImageJ 1.50i (NIH, Maryland, USA). The coordinates of both corners of the pupil were recorded at the beginning and end of each saccade. The changes in angle both over the saccade itself and over the interval between saccades (the slow, tracking phase) were calculated, and divided by the duration to obtain average angular velocities. The gain was calculated by dividing the angular velocity of the eye during the slow phase by the angular velocity of the drum rotation during that interval.

For these calculations, saccade start and end was determined by a human observer, rather than from frame-by-frame digitization of pupil corners, as noise from fish opercular movement and user digitization error made it difficult to reliably separate saccadic and tracking intervals. Later, for the aquatic temporal acuity trials, pupil corners were digitized at 15 frames/s using the

MATLAB (Mathworks, Natick, MA, USA) program DLTdv5 (Hedrick, 2008; Fig. 2.2). Since slow phase start and end had already been determined by the previous method, this digitization allowed us to calculate instantaneous slow phase angular velocity, which was smoothed by running average of three frames. Gain calculated using instantaneous angular velocity could then be compared to that calculated using average angular velocity.

Linear mixed-effect analyses were performed in MATLAB to assess relationships between stimulus frequency and the displacement, duration and velocity of both saccade and tracking movements. Relationships between stimulus frequency and the number of saccades observed were analyzed using Poisson mixed-model regression. In all models, fixed effects included stimulus temporal or spatial frequency, trial environment (air vs. water), the interaction between these two terms, and whether the stimulus frequency was being increased or decreased. Differences between individual fish were accounted for by random intercept. In Poisson regression, a random intercept for observation number was also included to account for overdispersion (Elston et al., 2001). When visual inspection of Q-Q and residual plots revealed substantial heteroscedasticity or non-normality, a log or square root transformation was applied as a correction. Terms deemed non-significant ($p>0.05$) by likelihood ratio test were removed from final models.

2.3 RESULTS

Saccade rate

When stimulus angular velocity was varied, mean number of saccades per second was interactively dependent on stimulus velocity and medium ($p=0.0170$, Table 2.1). In water, saccade rate was greatest at intermediate stimulus velocities. At stimulus velocities ranging from 5-30 deg/s, mean saccade rate was more than two times greater than it was at more extreme

velocities (Fig. 2.3A). In air, mean saccade rate remained relatively low regardless of stimulus velocity, although saccade rate did decrease further at the highest stimulus velocities (30-40 deg/s). The highest saccade rate in water was 3.5 times greater than the highest saccade rate in air. Variation in stimulus spatial frequency had no significant effect on saccade rate (p=0.8097, Table 2.2). However, mean saccade rate in water was greatest at 0.05-0.075 cpd, dropping sharply when spatial frequency was either increased or decreased (Fig. 2.3B). Again, the number of saccades in air was consistently lower (p<0.0001). With the exception of the control condition, mean saccade rate was at least 1.8 times greater in water than in air at every spatial frequency tested.

Saccade angular velocity

In temporal frequency trials, changes in the angular velocity of the saccade depended on the interaction between medium and stimulus velocity (p=0.0465). However, stimulus velocity had a relatively small effect on saccade velocity in either medium, except at very low stimulus velocities (Fig. 2.3C). The effect of medium was much larger and more consistent. At stimulus velocities of 2 deg/s or greater, the saccade velocity in water was at least 2.5 times greater than that in air. Differences in saccade velocity were driven by changes in the total angular displacement of the eye over the course of the saccade (p=0.0446 for interaction term), rather than by any changes in saccade duration ($p\geq$0.2221).

Saccade angular velocity was not significantly affected by spatial frequency (p=0.1506). In water, mean saccade velocity peaked at stimulus frequencies of 0.025-0.05 cpd and then declined, but within-group variation was too large to draw any firm conclusions (Fig. 2.3D). Once again, medium had an important effect on saccade velocity, as mean aquatic saccade velocities were at least 1.5 times greater than mean aerial velocities in all cases except the

control condition ($p<0.0001$). Lower saccade velocities in air were a result of both increased saccade duration ($p=0.0152$) and decreased angular displacement ($p=0.0002$).

Slow phase angular velocity

As expected, all mean slow phase velocities were of much lower magnitude than saccade velocities. They were not significantly affected by variation of stimulus velocity ($p=0.0758$), but depended strongly on the medium in which the trial was conducted ($p<0.0001$, Fig. 2.3E). Except at stimulus velocities lower than 5 deg/s, slow phase velocity in water was more than five times greater than in air. The difference between media was largely due to longer slow phase duration in air ($p=0.0053$ for interaction term), as well as differences in angular displacement ($p=0.0926$). In air, there were longer time intervals between saccades, in which the eye would demonstrate short, small temporal-to-nasal tracking movements, interrupted by long pauses, until an eventual saccade reset occurred.

In spatial frequency trials, both stimulus frequency ($p=0.0022$) and medium ($p<0.0001$) impacted slow phase velocity (Fig. 2.3F). As in temporal trials, slow phase velocity in air was often near-zero, and demonstrated no overall trend across stimulus frequencies. In water, mean slow phase velocity peaked at 1.99 ± 0.64 deg/s, at a stimulus frequency of 0.05 cpd. Significant changes in slow phase velocity were a result both of changes in total angular displacement of the eye ($p=0.0006$ for interaction term) and of changes in duration of the slow phase ($p\leq0.0022$) between different trial conditions.

Gain

With the exception of the lowest stimulus velocity (1 deg/s), gain improved as stimulus velocity decreased ($p=0.0136$, Fig. 2.3G). Mean gain was also generally greater in magnitude in aquatic trials, although this difference was not significant ($p=0.1176$). Maximum mean gain

(0.48 ± 0.14) was observed at a stimulus velocity of 2 deg/s, in water. Instantaneous gain, calculated using slow phase instantaneous angular velocity obtained through frame-by-frame digitization, did not closely resemble gain calculated based on only on eye position at the start and end of the slow phase. Across all aquatic temporal trials, when stimulus velocity was 2 deg/s, instantaneous gain during the inter-saccade interval was often far less than 0.48 ± 0.14 (Fig. 2.4). In addition, sometimes instantaneous eye velocity greatly exceeded stimulus velocity. The two were not matched particularly often.

In spatial frequency trials, stimulus velocity was held constant, so gain varied in the same way as slow phase velocity, affected by both stimulus frequency ($p=0.0022$) and medium ($p<0.0001$, Fig. 2.3H). Here maximum mean gain (0.10 ± 0.03) was observed at a stimulus frequency of 5 cpd, again in water. Under no set of stimulus parameters did the mean tracking velocity of the eye approach the velocity of the stimulus.

Increasing vs. decreasing stimuli

In all trials, stimulus frequencies were sequentially increased, then decreased, allowing fish two opportunities to respond to each stimulus level. For most response variables, there was no significant difference between the "stepping up" and "stepping down" occurrences of the same stimuli. However, in spatial trials, both saccade and slow phase duration were significantly longer in the first half of the trial ($p\leq0.0216$), and as a result, slow phase angular velocity and gain also demonstrated the same pattern ($p=0.0180$). These differences, which were accounted for in the models, may have been due to residual effects of anaesthesia.

2.4 DISCUSSION

In water, slow phase velocity did not increase in proportion to stimulus velocity, so one might infer that at higher stimulus velocities, fewer stimulus bars passing the eye were tracked

by the fish. As a result, gain, the ratio of slow phase velocity to stimulus velocity, peaked (0.48 ± 0.14) at 2 deg/s, and declined at higher velocities. It should be noted that instantaneous gain at stimulus velocity of 2 deg/s was quite variable. From this measure, it appeared that the eyes of the fish spent most of the inter-saccade interval, ostensibly the tracking phase, moving very slowly (≤0.4 deg/s), with fewer, momentary instances of more rapid movement, and these not all in the same direction as stimulus movement. Although overall temporal-to-nasal eye motion was stimulated by stimulus motion, the instantaneous gain distribution did not suggest smooth, consistent tracking of individual stimulus bars. However, the instantaneous measure is likely to be more strongly influenced by opercular movements, and is also more affected by digitization error, both which might increase its variability. Overall, gain from average velocity did not capture full variation in inter-saccadic eye movement, but it was used as a general comparison between the motion of the eye and the motion of the stimulus.

Polypterus tracking ability, even at maximum performance, appears poor relative to that of zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), and goldfish (*Carassius auratus*), all for which gain can approach 1 (Beck et al., 2004; Dieringer et al., 1992; Mueller and Neuhauss, 2010; Rinner et al., 2005). However, other species demonstrate much lower maximum gain (e.g. pond loach *Misgurnus fassilis*, max. gain <0.1, Dieringer et al., 1992; benthic sharks *Heterodontus portusjacksoni* and *Chiloscyllium punctatum*, max. gain ~ 0.21 and 0.06 respectively, Ryan et al., 2016). Differences in tracking ability may be related to the habitat and locomotor style of the species. For example, benthic fishes are less likely to show a consistent response to an optomotor stimulus (Jones, 1963), and their maximum gain is often, although not always, lower compared to pelagic fishes (Dieringer et al., 1992; Ryan et al., 2016). Gain generally peaks at the lowest stimulus velocities, but the extent of decline in gain as stimulus

velocity increases varies between species. Bottom-dwellers or ambush predators tend to track high-velocity stimuli much more poorly (Dieringer et al., 1992). At a stimulus velocity of 10 deg/s, gain in the brown bullhead (*Ictalurus nebulosus*) has declined to less than a quarter of the maximum, while in piranha (*Serrasalmus nattereri*), it has declined by only ~25% (Dieringer et al., 1992). In *Polypterus*, gain fell by more than half as stimulus velocity increased from 2 deg/s to 10 deg/s. Overall, *Polypterus* have a moderate response to stimuli at low velocities, but respond very poorly at high velocities, suggesting some gaze stabilization ability at slow swimming speeds, as well as possible tracking of some slow-moving objects in the surroundings. This might be expected given that *Polypterus* is relatively sedentary, avoids high-flow environments, and is an ambush predator.

In water, saccade velocity did not vary substantially at stimulus speeds of 5 deg/s or higher, nor did it vary greatly at stimulus spatial frequencies of 0.025 cpd or greater. Mean saccade speed in *Polypterus* (14.01 ± 0.76 deg/s) was somewhat lower than in many other fishes, as saccade speeds often range from 20-100 deg/s (Montgomery and Macdonald, 1984; Montgomery et al., 1983; Segev et al., 2007) and instantaneous saccade speeds can exceed 200 deg/s (Beck et al., 2006; Easter Jr., 1975; Mueller and Neuhauss, 2010). Under the aquatic control condition, across all fish and all trials, a single nasal-to-temporal eye movement, followed by a single saccade, was observed. Saccade rate was therefore lower under the control condition than under any stimulus condition, even those with a very low saccade rate, which might imply that *Polypterus* have some ability to track all temporal and spatial stimuli presented, albeit sporadically. However, at the slowest temporal frequency (1 deg/s), slow phase velocity and gain are highly variable, and the eye is often moving in the opposite direction to the stimulus. In addition, at spatial frequencies exceeding 0.125 cpd, saccade rate may be greater

than in the control, but slow phase velocity and gain are very low. At these extremes, tracking ability is exceedingly poor.

Strongest OKR is observed at 0.05-0.075 cpd, as saccade rate, slow phase velocity, and gain all reach their peak in this range of stimulus frequencies. This spatial frequency is lower than that which produces the maximum response in zebrafish and medaka (0.12 cpd, Mueller and Neuhauss, 2010), but comparable to larval zebrafish (0.025-0.075 cpd; Qian et al., 2005; Rinner et al., 2005; Schoonheim et al., 2010). Zebrafish also exhibit maximum contrast sensitivity at 0.05-0.12 cpd (Hollbach et al., 2015; Tappeiner et al., 2012). Both in *Polypterus* and in other fishes, OKR appears to perform optimally when tracking relatively large stimuli.

Spatial acuity is estimated through OKR by determining the finest stimulus to elicit a tracking response. For example, although their maximum response is observed at 0.12 cpd, zebrafish can detect optokinetic stimuli at spatial frequencies up to 0.59 cpd (Tappeiner et al., 2012). A review of spatial acuity in actinopterygians by Caves et al. (2017) found great variation between species, ranging from 40 cpd in rock bass (*Ambloplites rupestris*, Williamson and Keast, 1988) to 0.56 cpd in medaka (Tappeiner et al., 2012). In other groups, such as benthic sharks, acuity can be even lower (0.38 cpd; Ryan et al., 2016). As with temporal acuity, spatial acuity may be related to the habitat of the organism. Within actinopterygians, species living in poorly-lit or turbid waters tend to have smaller eyes, and thus poorer spatial acuity, and those living in complex environments have better spatial acuity, even controlling for eye size (Caves et al., 2017). *Polypterus* are nocturnal and may live in turbid waters. Based on the relationship between *Polypterus* body length (BL) and lens radius, as measured from past micro-CT scans (for scanning procedure, see Standen et al., 2014), fish in this study (BL 67.0 ± 1.9 mm) might have a mean lens diameter of 1.07 ± 0.01 mm. As the lens diameter vs. BL regression by Caves et al.

(2017) has an ordinal intercept of about 2.5 mm, *Polypterus* seem to have comparatively low eye investment. Additionally, spatial acuity of less than 1 cpd is low even for the smallest of fish eyes (Caves et al., 2017), so *Polypterus* spatial acuity is very poor in comparison to other fishes.

Perhaps other elements of vision or other sensory modalities are more important in the perception of the environment by *Polypterus*. In well-lit, open waters, high acuity can be critical to predator avoidance (Dobberfuhl et al., 2005); however, when visibility is poor, sensitivity to contrast or to light might be maximized at the expense of spatial and/or temporal acuity (Brokovich et al., 2010; Ryan et al., 2016). In addition, the mechanosensory lateral line and the electroreceptive ampullae of *Polypterus* provide further information about the surroundings, although they only function at fairly close range.

Evidently, temporal acuity cannot be assessed if the stimulus surpasses the limits of spatial acuity, and vice versa (Carvalho et al., 2002), but even within these limits, optokinetic performance is impacted by the interaction between the spatial and temporal aspects of the stimulus (Bilotta and Powers, 1991; Rinner et al., 2005). We did not examine this interactive effect. For temporal frequency trials, a constant spatial frequency of 0.1 cpd was selected based on previous OKR studies (Mueller and Neuhauss, 2010; Ryan et al., 2016). Preliminary analysis revealed that the saccade rate was highest at 20 deg/s during the temporal frequency trials, so this stimulus speed was selected for spatial frequency trials. Ultimately, the maximal response was observed at less than 0.1 cpd, and although saccade rate was highest at 20 deg/s, gain peaked at a much lower velocity. We may have observed stronger responses if we had used wider stimuli for temporal frequency trials and slower stimuli for spatial frequency trials.

Polypterus performed more poorly in air than in water across all measures. Saccade rate, saccade velocity, slow phase velocity and gain were all lower in air. Lower slow phase velocity

was due both to longer slow phases and to smaller angular displacement, while lower saccade velocity was mostly due to smaller angular displacement. Across vertebrate taxa, saccade velocity is well known to decrease when angular displacement decreases (Easter and Nicola, 1997; Garbutt et al., 2001; Huang and Neuhauss, 2008); however, the cause of decreased displacement in air is not clear. It may be that not all observed nasal-to-temporal movements in air are true saccades, but merely the slower, drifting return of the eye to a neutral position. It is possible that the difference is not caused by reduced acuity, rather, the neuromuscular machinery underlying the saccade reflex itself may be somehow compromised in air. Alternatively, if the irregular saccades of *Polypterus* are initiated by some perception of the approaching stimulus in the visual periphery, in air, where acuity is lower, poorly-functional peripheral vision might result in smaller saccades.

In air, the responses to stimuli and control were often similar. However, gain in both spatial and temporal trials, and slow phase velocity in temporal trials, all depended significantly on the non-interactive effect of stimulus frequency. This suggested some limited stimulus tracking ability in air, as the effect of stimulus did not depend on medium. *Polypterus* was expected to be able to see in air, as visual input may influence the kinematics of terrestrial locomotion, particularly when other senses are inhibited (see Chapter 1). However, poor acuity was expected, since the *Polypterus* eye is anatomically similar to that of typical teleosts (Kröger et al., 2014; Rochon-Duvingneaud, 1943, in Pfeiffer, 1968), and does not possess common adaptations for improving aerial acuity in amphibious fishes, like flattened lenses or corneas (Sayer, 2005).

In amphibious fishes, visual ability in air is often inferred through observation of behaviours such as feeding, predator avoidance, or orientation towards water (Bressman et al.,

2016; Sponder and Lauder, 1981; Tytler and Vaughan, 1983). In *Polypterus*, although vision is known to increase feeding success in water (Pfeiffer, 1968), whether feeding occurs on land is not known, nor has the importance of vision in other directed terrestrial behaviors been studied. To our knowledge, no OKR or OMR tests have performed in other fishes capable of terrestrial locomotion. Interestingly, the four-eyed fish, *Anableps anableps*, whose eyes are adapted for simultaneous aerial and aquatic vision at the water's surface, exhibits OMR only when the aerial field of view is stimulated, and OKR only when the aquatic field of view is stimulated (Saidel and Fabiane, 1998). Compared to aerial OKR gain in *Polypterus*, aerial OMR gain is much higher in *A. anableps*, and peaks at higher temporal frequencies (gain~1 for 50-100 deg/s; Saidel and Fabiane, 1998), indicating far superior tracking ability in air. Although the aerial vision of *Polypterus* raised in water is poor, the effects of long-term emersion on visual acuity and eye anatomy are not known. The fish is known to demonstrate plastic change in pectoral anatomy when raised on land (Standen et al., 2014), but sensory changes remain to be investigated.

Pfeiffer (1968) estimated that the *Polypterus* retina contained approximately $2.0X10^4$ photoreceptors per mm^2, and $3.0X10^3$ ganglion cells per mm^2. Since these densities were relatively low compared to past estimates in many teleosts (Wunder, 1936, in Pfeiffer, 1968), he determined that vision in *Polypterus* was poor. Lens measurements which would allow calculation of spatial acuity were not recorded. However, based on estimates of lens diameter for our fish, using formulas in Collin and Pettigrew (1989), and estimations of tissue shrinkage for similar preparations by Shand (1994), spatial acuity as estimated by retinal ganglion cell density is ~0.67 cpd. (This is a very rough estimate which does not account for variation in ganglion density over the retina, nor decrease in ganglion density with eye growth (Johns and Easter, 1977; Kock and Reuter, 1978)). Although still low relative to other species, this anatomical

estimate of spatial acuity is higher than the behavioural estimate, as is often the case (Caves et al., 2017). Anatomical acuity measurements do not account for the effects of higher neural processing (Pettigrew et al., 1988), so behavioural measures are generally a better assessment of the functional visual capabilities of the organism (Browman et al., 1990).

Comparison of OKR studies is complicated by variation in experimental protocol. Two studies designed to test spatial acuity by OKR may use different stimulus paradigms, or different set values of temporal acuity and contrast (Dieringer et al., 1992). Furthermore, OKR can depend on attention paid by the subject (Wyatt and Pola, 1987), as well as on the size of the animal, as visual acuity tends to improve as fish grow and their eyes increase in size, particularly in larvae and juveniles (Beck et al., 2004; Carvalho et al., 2002; Dobberfuhl et al., 2005; Easter and Nicola, 1997). Our trials were short, and stimulus parameters were varied frequently in an effort to retain the attention of the fish. The individuals selected for the study were relatively small, as smaller fish were easier to restrain. It is likely that larger *Polypterus* have improved visual acuity.

2.5 FIGURES

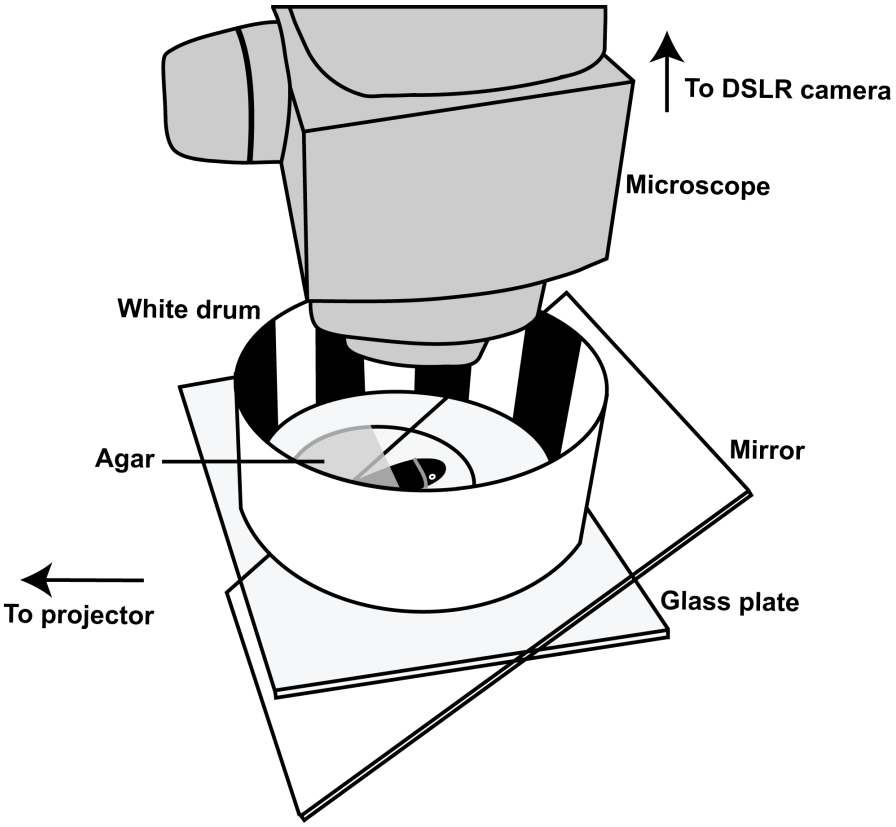

Figure 2.1. Schematic drawing of set-up used to measure optokinetic response. Rotating bars were projected onto a white drum using an angled mirror. The drum was filled with water during the aquatic trials, and empty during the aerial trials. Fish were immobilized in agar caudal to the gills, and a small strap behind the eyes secured the head. A DLSR camera angled through an eyepiece of the microscope captured eye movements.

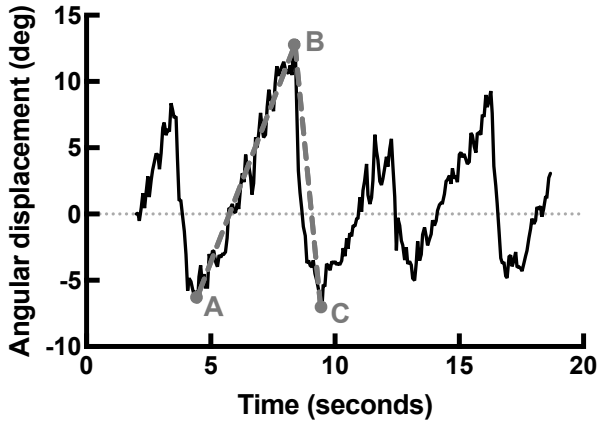

Figure 2.2. Angular displacement of the eye, relative to initial angle, over time, as calculated from digitized coordinates. Slope of the line AB is the angular velocity of the slow, tracking phase. Slope of the line BC is the angular velocity of the saccade. Temporal-to-nasal displacement is positive and nasal-to-temporal displacement is negative.

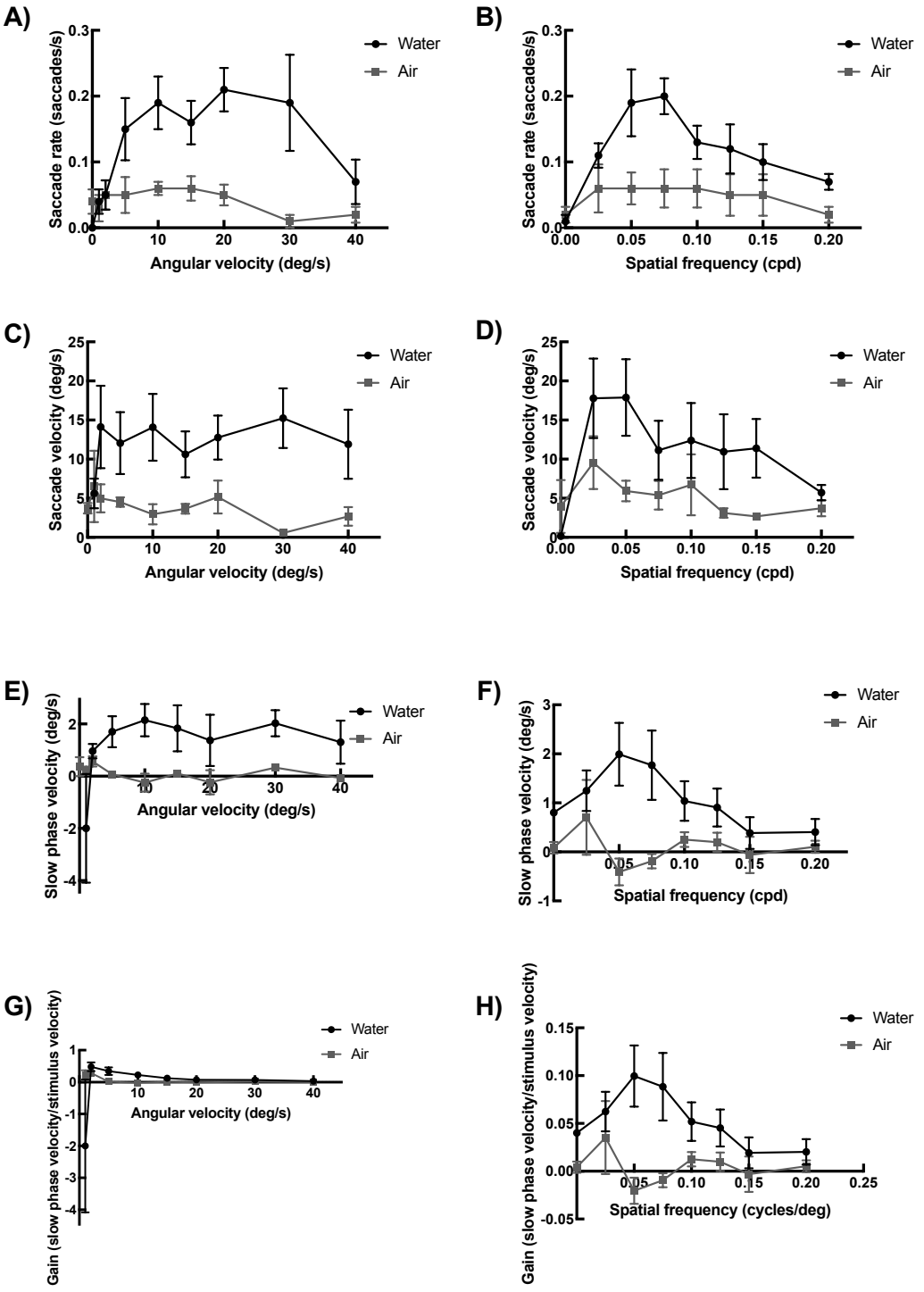

Figure 2.3. Effects of stimulus frequency and medium (water vs. air) on optokinetic response measures, including saccade rate (A, B) and velocity (C, D), slow phase velocity (E, F), and gain (G, H). A, C, E, and G correspond to temporal acuity trials, while B, D, F and H correspond to spatial acuity trials. Points represent means, and error bars are s.e.m.

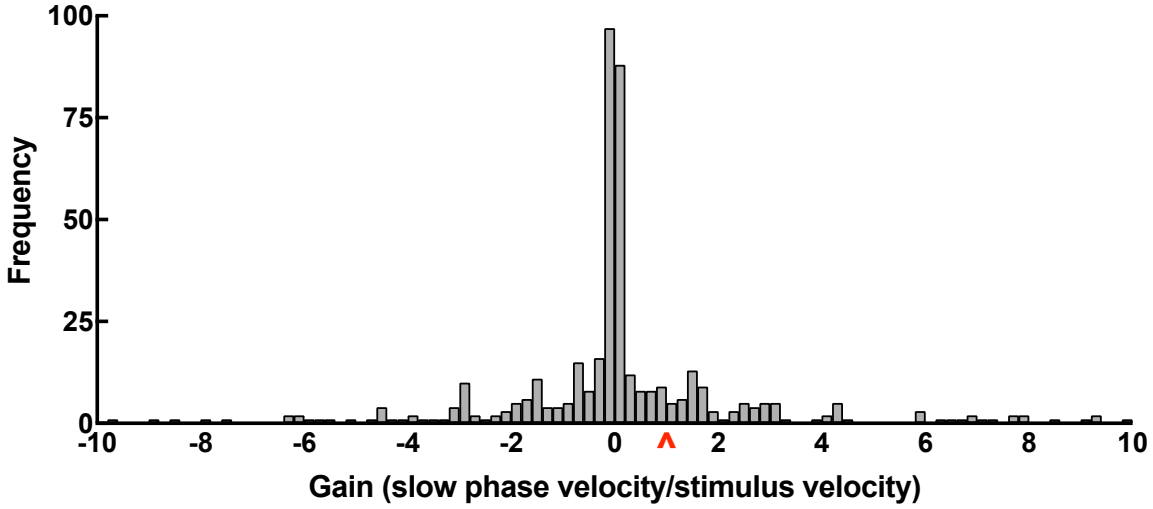

Figure 2.4. Distribution of instantaneous gain values for the stimulus velocity (2 deg/s) demonstrating the highest average gain (Fig. 2.3G), for all aquatic temporal acuity trials. Gain bin size is 0.2. Gain = 1 at red chevron, indicating a match between eye velocity and stimulus velocity. Nine values were outside of range of this histogram, five lower and four higher.

2.6 TABLES

Table 2.1. Effects of stimulus temporal frequency and medium (water vs. air) on various measures of the optokinetic response. Mixed models were used, with fish as random factor to account for inter-individual variation. Dependent variable was transformed to meet assumptions where necessary. Up/down refers to whether stimulus frequency was being increased or decreased. Significance of terms determined by maximum likelihood ratio test comparing models with and without each variable. When $P>0.05$, term was not included in final model. Effect sizes are shown ± standard error of the mean.

Dependent variable	Model (transform)	Significance of term (*P*)					Final model	Interaction		Step		Medium	
		Up/down	Fish	S*M	Step	Medium		Effect	*P*	Effect	*P*	Effect	*P*
Saccade number	Poisson (none)	0.4512 (χ^2_1=0.57)	0.0923 (χ^2_1=2.83)	0.0170 (χ^2_1=5.70)	N/A	N/A	Dependent ~ Step*Medium + (1|Obs_effect)	-0.0368± 0.0167	0.0288 (t_{176}=-2.20)	0.0548± 0.0215	0.0116 (t_{176}=2.55)	-0.5485± 0.2899	0.0601 (t_{176}=-1.89)
Saccade ang. displacement	Linear (log(y))	0.4041 (χ^2_1=0.70)	<0.0001 (χ^2_1=28.70)	0.0446 (χ^2_1=4.04)	N/A	N/A	Dependent ~ Step*Medium + (1|Fish)	-0.0270± 0.0133	0.0449 (t_{139}=-2.02)	0.0226± 0.0176	-0.0123 (t_{139}=1.28)	-0.6361± 0.2281	0.0060 (t_{139}=-2.79)
Saccade duration	Linear (none)	0.0916 (χ^2_1=2.85)	0.0002 (χ^2_1=13.45)	0.7091 (χ^2_1=0.14)	0.2399 (χ^2_1=1.38)	0.2221 (χ^2_1=1.49)	Dependent ~ (1|Fish)	N/A	N/A	N/A	N/A	N/A	N/A
Saccade ang. velocity	Linear log(y)	0.6673 (χ^2_1=0.18)	<0.0001 (χ^2_1=29.31)	0.0465 (χ^2_1=3.96)	N/A	N/A	Dependent ~ Step*Medium + (1|Fish)	-0.0285± 0.0142	0.0467 (t_{139}=-2.01)	0.0300± 0.0188	0.1122 (t_{139}=1.60)	-0.6882± 0.2428	0.0053 (t_{139}=-2.83)
Slow phase ang. displacement	Linear (log(y+1.1)	0.3356 (χ^2_1=0.93)	1 (χ^2_1=0)	0.6439 (χ^2_1=0.21)	0.2302 (χ^2_1=1.44)	0.0926 (χ^2_1=2.83)	No sig. effects	N/A	N/A	N/A	N/A	N/A	N/A
Slow phase duration	Linear log(y)	0.3627 (χ^2_1=0.83)	1 (χ^2_1=0)	0.0053 (χ^2_1=7.78)	N/A	N/A	Dependent ~ Step*Medium	0.0398± 0.0141	0.0054 (t_{135}=2.83)	-0.0459± 0.0184	0.3235 (t_{135}=-2.50)	0.2261± 0.2282	0.0136 (t_{135}=0.99)
Slow phase ang. velocity	Linear (none)	0.2662 (χ^2_1=1.24)	<0.0001 (χ^2_1=30.25)	0.0758 (χ^2_1=3.15)	0.6290 (χ^2_1=0.20)	<0.0001 (χ^2_1=18.14)	Dependent ~ Medium + (1|Fish)	N/A	N/A	N/A	N/A	0.0248± 0.0054	<0.0001 (t_{137}=4.64)
Slow phase gain	Linear (log(y+1.2)	0.1479 (χ^2_1=2.09)	1 (χ^2_1=0)	0.4403 (χ^2_1=0.60)	0.0136 (χ^2_1=6.09)	0.1176 (χ^2_1=2.45)	Dependent ~ Step	N/A	N/A	0.0147± 0.0066	0.0276 (t_{133}=2.23)	N/A	N/A

Table 2.2. Effects of stimulus spatial frequency and medium (water vs. air) on various measures of the optokinetic response. Mixed models were used, with fish as random factor to account for inter-individual variation. Dependent variable was transformed to meet assumptions where necessary. Up/down refers to whether stimulus frequency was being increased or decreased. Significance of terms determined by maximum likelihood ratio test comparing models with and without each variable. When $P>0.05$, term was not included in final model. Effect sizes are shown ± standard error of the mean.

Dependent variable	**Model (transform)**	**Significance of term (*P*)**					**Final model**	**Up/down**		**Interaction**		**Step**		**Medium**	
		Up/down	**Fish**	**S*M**	**Step**	**Medium**		**Effect**	***P***	**Effect**	***P***	**Effect**	***P***	**Effect**	***P***
Saccade number	Poisson (none)	0.6622 (χ^2_1=0.19)	0.0030 (χ^2_1=8.84)	0.7127 (χ^2_1=0.14)	0.8097 (χ^2_1=0.06)	<0.0001 (χ^2_1=23.82)	Dependent ~ Medium + (1|Fish) + (1|Obs_effect)	N/A	N/A	N/A	N/A	N/A	N/A	-0.8950± 0.1929	<0.0001 (t_{158}=-4.64)
Saccade ang. displacement	Linear (log(y))	0.8488 (χ^2_1=0.04)	<0.0001 (χ^2_1=62.45)	0.7177 (χ^2_1=0.13)	0.1503 (χ^2_1=2.07)	0.0002 (χ^2_1=13.94)	Dependent ~ Medium + (1|Fish)	N/A	N/A	N/A	N/A	N/A	N/A	-0.6000± 0.1607	0.0003 (t_{129}=3.73)
Saccade duration	Linear (log(y))	0.0216 (χ^2_1=5.28)	0.0092 (χ^2_1= 6.78)	0.3330 (χ^2_1= 0.94)	0.8989 (χ^2_1=0.02)	0.0152 (χ^2_1=5.89)	Dependent ~ Up/down + Medium + (1|Fish)	-0.2034± 0.0787	0.0108 (t_{128}=-2.59)	N/A	N/A	N/A	N/A	0.2145± 0.0869	0.0149 (t_{128}=2.47)
Saccade ang. velocity	Linear (log(y))	0.1733 (χ^2_1=1.85)	<0.0001 (χ^2_1=41.04)	0.2544 (χ^2_1=1.30)	0.1506 (χ^2_1=2.07)	<0.0001 (χ^2_1=21.93)	Dependent ~ Medium + (1|Fish)	N/A	N/A	N/A	N/A	N/A	N/A	-0.8162± 0.1708	<0.0001 (t_{129}=4.78)
Slow phase ang. displacement	Linear (none)	0.6838 (χ^2_1= 0.17)	<0.0001 (χ^2_1=36.34)	0.0006 (χ^2_1=11.66)	N/A	N/A	Dependent ~ Step *Medium + (1|Fish)	N/A	N/A	-1.0629± 0.3034	0.0006 (t_{126}=-3.50)	1.7303± 0.4180	<0.0001 (t_{126}=4.14)	0.1964± 0.0308	<0.0001 (t_{126}=6.37)
Slow phase duration	Linear (log(y))	<0.0001 (χ^2_1= 22.61)	0.0095 (χ^2_1=6.73)	0.1748 (χ^2_1=1.84)	0.0856 (χ^2_1=2.96)	0.0002 (χ^2_1=14.08)	Dependent ~ Up/down + Medium + (1|Fish)	-0.6410± 0.1449	<0.0001 (t_{126}=-4.42)	N/A	N/A	N/A	N/A	0.4882± 0.1607	0.0029 (t_{126}=3.04)
Slow phase ang. velocity	Linear (none)	0.0180 (χ^2_1=5.60)	<0.0001 (χ^2_1=20.06)	0.3462 (χ^2_1= 0.89)	0.0022 (χ^2_1=9.37)	<0.0001 (χ^2_1=20.77)	Dependent ~ Up/down + Step + Medium + (1|Fish)	-0.0111± 0.0042	0.0087 (t_{126}=-2.67)	N/A	N/A	0.1300± 0.0417	0.0023 (t_{126}= 3.12)	0.0218± 0.0046	<0.0001 (t_{126}=4.75)
Slow phase gain	Linear (none)	0.0180 (χ^2_1=5.60)	<0.0001 (χ^2_1=20.06)	0.3462 (χ^2_1= 0.89)	0.0022 (χ^2_1=9.37)	<0.0001 (χ^2_1=20.77)	Dependent ~ Up/down + Step + Medium + (1|Fish)	-0.0006± 0.0002	0.0087 (t_{126}=-2.67)	N/A	N/A	0.0065± 0.0021	0.0023 (t_{126}= 3.12)	0.0011± 0.0002	<0.0001 (t_{126}=4.75)

CHAPTER 3.

An attempt to observe electrogenesis in *Polypterus senegalus*

ABSTRACT

Many fishes have electroreceptive abilities, and a subset of these can also generate electric pulses. The number of species known to produce weak electric discharges has been expanding. These pulses may be used in communication, navigation, and more. A previous study by Baron and Pavlov (2003) found that *Polypterus senegalus* could produce electric discharges, but did not determine their purpose. Interested in the relative importance, and integration, of the many sensory inputs in this species, we sought to record these discharges in *Polypterus*, and to attempt to link them to fish behaviour. However, in the four individuals used in this experiment, we did not observe electrical disturbances closely resembling those reported by Baron and Pavlov (2003). It was unclear whether our fish produced electric discharges at all, highlighting the difficulty in, and importance of, repeating such experiments. We compare the results of the two studies, and suggest additions and modifications to our experiments which might produce more definitive results.

3.1 INTRODUCTION

The ability to perceive electrical stimuli in the environment is likely to be an ancestral characteristic of vertebrates (Zupanc and Bullock, 2005). It is represented in all major anamniotic taxa except the hagfishes, and is particularly common in non-teleost fishes (Jørgensen, 2005). Conversely, the ability to generate electric stimuli is much less widespread, and probably evolved independently in several taxa (Bass, 1986; Bennett, 1971).

Polypterus senegalus is a freshwater basal actinopterygian possessing electrosensory abilities. As in all non-teleost electroreceptive fish, its receptors take the form of ampullae (Jørgensen, 1982; Roth, 1973). These tubes in the epidermis, which are filled with mucous, are open to environment at one end and contain the sensory epithelium at the other (Jørgensen, 2005). In *Polypterus*, ampullae are found on the head, with the highest density on the snout (Northcutt, 1986 in Jørgensen, 2005). Electroreception by ampullary receptors is involved in behaviours such as prey detection and navigation in elasmobranchs (Kalmijn, 1982), and communication in gymnotids (Metzner and Heiligenberg, 1991). In a close relative of *Polypterus*, the reedfish *Calamoichthys calabaricus*, electroreception is estimated to be sensitive enough to detect the electric field generated by the respiration of another fish from a distance of about 5 cm (Roth, 1973). This suggests that electrosensation might aid in prey detection in *Polypterus*.

Some fish that are electroreceptive can also generate electric discharges through specialized electric organs, composed of modified, synchronously-active muscle or nerve cells (Zupanc and Bullock, 2005). In general, weakly-electric fish produce electric organ discharges (EODs) ranging from hundreds of millivolts to a few volts, while strongly-electric fish produce EODs of several hundred volts (Zupanc and Bullock, 2005). Weak electrogenesis was first

discovered in mormyrids (elephant nose fishes) and gymnotids (knifefishes; Lissmann, 1958). These fish produce EODs near-constantly (Coates et al., 1954), and use them in a range of behaviours, including electrolocation (Lissmann and Machin, 1958), species recognition (Hopkins, 1976), and communication (Hopkins, 1986).

More recently, weak electric discharges have been recorded in fishes from a range of other taxa, including four families of catfishes (Baron and Olshansky, 2009; Baron et al., 1994; Olshansky, 2010; Orlov et al., 2015a), three arowanas (*Heterotis niloticus*, *Osteoglossum bicirrhosum, Scleropages* sp.; Olshansky 2014, in Orlov et al., 2015b), a lungfish (*Protopterus aethuipicus*; Orlov et al., 2015b), and two Polypteriformes (Baron and Pavlov, 2003). Compared to those of mormyrids and gymnotids, the electric discharges of these fishes are far less frequent, and are often weaker. Some discharges have been found to be related to intraspecific interactions, such as reproduction or displays of aggression, while others might occur spontaneously, or in response to tactile stimulation.

Baron and Pavlov (2003) reported that *Polypterus senegalus* produce occasional electric discharges, at a rate of about once an hour during the day, and 2-3 times per hour at night. Discharge frequency did not change when fish were tested in pairs, nor was it noted to be related to any other factor than time of day. As we are interested in the sensory environment of *Polypterus*, we intended to record the electric discharges of the fish ourselves, and to compare them to fish behaviour in an attempt to determine their function(s). However, we did not find clear evidence that our fish were producing electric discharges.

Here we summarize the types of electrical disturbances observed in our trials, and the accompanying fish behaviours. We then compare our observations to the discharges recorded by Baron and Pavlov (2003), and discuss the likelihood of electrogenic ability in the species.

3.2 METHODS

Polypterus senegalus were obtained from the pet trade (AQUAlity Tropical Fish Wholesale Inc., Mississauga, ON, Canada). They were kept at 25°C on a 12 h/12 h light/dark cycle, and were fed daily ad libitum. Four fish were used, with an average total body length of 101.0 ± 4.7 mm (mean ± s.e.m.), and an average mass of 6.52 ± 0.88 g. Trials were performed in an enclosed space in an aquarium, 18 cm in length by 19 cm wide. The water was 11 cm deep, and was kept heated to 25°C. For periods of approximately 24 h each, single fish were placed in the enclosure, and behaviour and electrical output were recorded. The experiment was also repeated with two fish in the enclosure. To serve as a control, electrical output of the experimental set-up was recorded in absence of fish for three days.

A pair of short (30 cm), PFTE-wrapped, solid silver wires (AG-18), stripped bare about 5 mm from the end, served as electrodes. Silver, as a noble metal, was chosen to minimize instability of the electrode potential (Olshansky, 2010). Electrodes were secured at either end of the enclosure. The exposed ends were planted in small agar blocks, prepared using aquarium water, to minimize the effects of movement on electrical recording without altering conductivity. The aquarium and recording equipment were surrounded by grounded Faraday cages, and a ground wire was also placed in the water.

Signals were amplified 5000X using an AC amplifier (Grass P511, Natus Neurology Inc., Oakville, ON, Canada), band-pass-filtered from 0.1 Hz to 10 KHz, and sampled at 1 k/s with a DAQ system (PowerLab 16/35, ADInstruments Inc., Colorado Springs, CO, USA) connected to a computer running LabChart Pro (v.8.1.1, ADInstruments Inc., Colorado Springs, CO, USA). Fish behaviour was recorded at 30 frames/s, at 640 X 480 pixel resolution, using a sports camera (Shenzhen Weitong Technology Co. Ltd, Xixi, China). At night, the filming area was illuminated

with infrared lights (850 nm, Smart Vision Lights, Muskegon, MI, USA). Most fishes cannot see infrared light (Levine and MacNichol, 1982), and we assumed, as in previous studies of nocturnal fishes (e.g. Fitzpatrick et al., 2013), that infrared light is invisible to *Polypterus*, although the spectral sensitivity of the eye has not been measured.

The baseline electrical noise (flat trace) ranged ±0.004 mV. Based on this, only disturbances of 0.04 mV or greater (≥10X baseline) were considered notable. Segments of electrical recording were classed alphabetically according to the characteristics described in Table 3.1 and shown in Fig. 3.1. Video segments were classed numerically according to fish behaviour, as described in Table 3.2. Mean rate, amplitude and duration of each class of disturbances were calculated for each trial individually, then for all single-fish trials overall.

Linear mixed-effect analyses were performed in MATLAB (Mathworks, Natick, MA, USA) to assess effects of disturbance class, trial (four single-fish trials, and one paired-fish trial), and time of day (day vs. night) on both the rate and the amplitude of disturbances. When visual inspection of Q-Q and residual plots revealed substantial heteroscedasticity or non-normality, a transformation of the dependent variable was applied as a correction. Multinomial regression using the "nnet" package (Venables and Ripley, 2002) in R 3.3.1 (R Core Team, 2016) was used to assess the effects of behaviour class, trial and time of day on the observed disturbance class. For all models, independent variables deemed non-significant ($p>0.05$) by likelihood ratio test were removed from the final model. Within significant independent categorical variables, significance of pairwise differences between categories was determined by changing the reference category and re-running model. Resultant *P*-values were Bonferroni-corrected for multiple comparisons.

3.3 RESULTS

Controls

By comparison of controls run both with and without power to water heater, we determined that large changes in amplitude with consistent shape (class D) were likely the result of the heater turning on and off (Table 3.3); therefore these disturbances were ignored in further analysis. Single spikes (class E) also occurred at a moderate rate when the heater was on (4.62 obs./d), but not when it was off. Mean single spike rate was much higher when fish were present (18.91 ± 5.13 obs./d) than in any controls, so it is likely that most single spikes observed in fish trials were related to the presence of fish; however, as they also occur in controls, some may have been heater artefacts. Over 95% of disturbances recorded during the controls fell into classes D or E.

Initial analysis

We compared fish behaviour with the characteristics of the electrical recording for one 24 h trial, with the goal of determining whether particular behaviours reliably produced particular electrical trace types. As might be expected, a flat trace (class A) was common for behaviours in which the fish was relatively still. When the fish was sitting on the bottom, the trace was at baseline 42.38% of the time, and when the fish was hanging near the surface of the water, the trace was at baseline 98.36% of the time. Small irregular disturbances (class B) accounted for most of the remaining time spent in these behavioural states (57.44% and 1.64%, for sitting and hanging, respectively). During movement behaviours, including swimming and swimming vigorously, or behaviours that often co-occur with movement, such as when the mouth or body breaks the surface of the water, small irregular deviations (class B) are most common, accounting for upwards of 96.52% of total time spent in each of these behaviours. Extended

irregular disturbances were therefore determined to likely be a result of movement, and only disturbances of classes E-I were noted in all further analyses.

All of the shorter electrical disturbances (classes E-I) in combination did not represent more than 1% of time spent in any single behaviour. These disturbances, which are more likely to indicate an electric discharge than those of classes A-D, were not reliably produced by any particular fish behaviour. For example, taking a breath did not consistently generate a single spike in the electrical trace. For this reason, in further analyses, we classified behaviour of the fish only during electrical disturbances, rather than over the full 24 h period.

Single fish trials

Over four single-fish trials, 184 electrical disturbances were recorded which might correspond to electrogenic pulses. In single-fish trials, the single spike was the most common disturbance type, observed approximately 19 times per day on average (Table 3.4), and accounting for 36.41% of all disturbances (Fig. 3.2A). Prolonged spikes (class G), were next-most common (28.80%), although their rate of occurrence varied greatly between trials (15.91 ± 15.56 obs./d). Third-most common were sets of several spikes (class H, 25.54%, 13.55 ± 4.22 obs./d).

Mean amplitude was less than 0.2 mV for all disturbance classes, and varied significantly between classes ($p<0.0001$, Table 3.6). Sets of spikes demonstrated the largest amplitudes (0.18 ± 0.04 mV), while prolonged spikes had a mean amplitude less than half that of any other defined class (0.04 ± 0.01 mV, $p<0.0001$ for all pairwise comparisons except class I). Duration also differed between classes, because it was constrained by the class definitions themselves (Table 3.1). Single spikes and bipolar spikes (class F) were notably brief, lasting less than 5 ms

on average (Table 3.4). Sets of several spikes lasted about 70 ms on average, while prolonged spikes had longer durations.

The likelihood that a given disturbance belonged to a particular disturbance class depended on the time of day, the trial, and the behaviour of the fish at the moment of the disturbance ($p<0.0001$ for all terms, Table 3.7). In the daytime, disturbances were more likely to be prolonged spikes, while night-time disturbances were more likely to be single spikes or spike sets ($p=0.0002$, Fig. 3.4). Some fish were more likely to be associated with a particular type of disturbance than others; for example, single spikes were significantly more likely than prolonged disturbances to be observed when the smallest fish was in the enclosure ($p<0.0001$).

When disturbances occurred, the fish were most often swimming (52.72%), sitting on the bottom (27.17%), or swimming vigorously (11.41%, Fig. 3.2F). Less than 10% of disturbances occurred during all other behaviours. Most single spikes were observed when fish were sitting on the bottom (50.75%), or swimming (44.78%, Fig. 3.2B). Furthermore, approaching the relationship from the opposite perspective, most disturbances that occurred when fish were sitting on the bottom were single spikes (68.00%, Fig. 3.2G), which were more likely to be associated with this behaviour than was any other disturbance type ($p\leq0.0068$). By contrast, most prolonged spikes (84.91%, class G, Fig. 3.2D), and bipolar spikes (60.00%, Fig. 3.2C) occurred while the fish were swimming, not sitting. Nearly half (46.39%, Fig. 3.2H) of all disturbances during swimming were prolonged spikes, and they were significantly more likely to occur during this behaviour than were all other disturbance classes except bipolar spikes ($p\leq0.0009$).

In comparison to other disturbance types, sets of several spikes were more widely distributed amongst behaviour classes (Fig. 3.2E). Vigorous swimming and coughing behaviours were more likely to be associated with spike sets than with other disturbance classes, especially

single spikes ($p<0.0001$), occurring in this class at approximately three times their overall rates. Sets of several spikes accounted for 76.19% of disturbances when fish were swimming vigorously (Fig. 3.2I), and 85.71% of disturbances during coughing behaviours.

Paired fish trial

Over all trials, the rate of disturbance depended on the interactive effect between trial and time of day ($p=0.0043$, Tables 3.5&6). In general, the greatest number of disturbances were observed in the first two hours of a trial (Fig. 3.4A), and this trend was exaggerated in the paired-fish trial (Fig. 3.4B) Regardless of disturbance type, amplitude was significantly higher in the paired fish trial compared to all others ($p\leq0.0064$, Tables 3.5&6).

The rate of occurrence of a given disturbance class varied significantly between trials ($p=0.0002$, Table 3.6). In the paired-fish trial, the frequency of sets of several spikes was nearly eight-fold greater, while most other disturbance rates decreased somewhat (Table 3.5). Relative to single-fish trials, when two fish were placed in the enclosure together, a greater proportion of disturbances occurred when at least one fish was swimming vigorously (49.12% vs. 11.41%, Fig. 3.3A). Spike sets accounted for 78.07% of all disturbances in the paired-fish trial (Fig. 3.3B), compared to 25.54% of disturbances in the single-fish trials.

Polypterus in close proximity will interact, often with displays of aggression. Most rapid movement (89.29%), and spike sets (78.65%) occurred during these interactions. About half (56.18%) of spike sets occurred during vigorous swimming, and most of these (88.00%) also involved fish interaction (Fig. 3.3C). Less than 10% of spike sets occurred when both fish were sitting relatively still on the bottom.

3.4 DISCUSSION

Overall comparison of disturbance rate and amplitude with previous studies

We observed a variety of electric disturbances, some of which bore some similarity to the observations in the previous study of *Polypterus* electrogenesis (Baron and Pavlov, 2003). The electric discharges observed by Baron and Pavlov (2003) included solitary discharges and sets of discharges. Sets of spikes lasted 80-100 ms, and the largest spikes attained amplitudes of 6-8 mV. From the examples provided, there appeared to be substantial variation in discharge shape and pattern. In both our study and the previous, recordings were performed on free-swimming fish, so some variation likely resulted from the changing orientation of the fish relative to the electrodes.

When recording a single *Polypterus*, Baron and Pavlov (2003) noted nine discharges in eight hours of daytime recording, and 24 discharges in eight hours of nighttime recording, which might be extrapolated to approximately 50 discharges over a 24 h period, assuming 12 hours at daytime discharge rate, and 12 hours at nighttime rate. In their study, recording of a single fish appears to have been performed only once. We observed an average of 53.15 ± 25.57 obs./d in our single-fish trials. Although the mean rate was similar, inter-individual variation was high. Furthermore, as discussed below, it is unlikely that all observed disturbances represent electrogenic discharges.

In our single-fish trials, mean amplitude never exceeded 0.2 mV for any disturbance type. In contrast, Baron and Pavlov (2003) observed maximum amplitudes of 6-8 mV. Although amplitude can be influenced by numerous features of the recording set-up, and should not be attributed outsize importance, this discrepancy is quite large. However, some other studies of sporadically-electric fishes have reported discharge amplitudes in a similar range to the amplitude of our disturbances. Orlov et al. (2017) recorded discharges ranging 0.4-1 mV in the

catfish *Synodontis caudovittatus*, and Olshansky (2014, in Orlov et al., 2015b) recorded discharges no greater than 0.15 mV in the Asian arowana, *Scleropages* sp.

Possible causes of single spikes

At 18.91 ± 5.13 obs./d, single spikes were the most common disturbances in single-fish trials, and among the least variable between trials. Unlike other disturbance types, they were most often observed when the fish was sitting, relatively motionless, on the bottom of the enclosure. They are therefore unlikely to be movement-related artefacts. Although a few single spikes occurred in the control recordings, the rate was over four times greater when fish were present, so most were likely biological in origin, if not electrogenic. These single spikes may be equivalent to the "solitary discharges" observed by Baron and Pavlov (2003), although those appeared to be somewhat biphasic in form, with a larger initial phase. Biphasic discharges are common in sporadically-electric fishes (e.g. Orlov et al., 2015a; Orlov et al., 2015b; Orlov et al., 2017), but we observed relatively few (4.27 ± 1.61 obs./d). From their figure, the initial phase of solitary discharges observed by Baron and Pavlov (2003) appears to be similar in duration to single spikes observed here, usually lasting 5 ms or less.

Possible causes of prolonged spikes

Extended monopolar disturbances, typically lasting upwards of 100 ms, are likely too long to be electrogenic in origin. Production of prolonged monopolar discharges by an electric organ is somewhat uncommon in freshwater weakly-electric fishes (Bass, 1986; Hopkins, 1980), and is thought to be the result of more sophisticated electrogenic machinery (Baron et al., 1994; Orlov et al., 2015a). Although longer monopolar disturbances (50-300 ms) have been observed in other sporadically-electric fishes, such as the catfish *Parasilurus asotus* (Baron and Olshansky, 2009), Baron and Pavlov (2003) did not report extended monopolar discharges in

Polypterus. The prolonged disturbances we observed were also of much lower amplitude than disturbances of all other classes, except class I (other disturbances). Furthermore, prolonged disturbances were highly correlated with movement, nearly always (84.91% of the time) occurring during swimming. These disturbances lasted far too long to be myogenic in origin, but could be caused by the action of moving water on the electrodes, despite the agar encasement. In support of this hypothesis, they were also most common in the trial using the biggest fish, which would cause greater water disturbance when swimming.

Possible causes of spike sets

Sets of several spikes were observed when the fish were exhibiting a broad range of behaviours. About 20% of the spike sets occurred while fish were sitting on the bottom, and 30% occurred during normal swimming. Coughing and vigorous swimming were more often associated with sets of several spikes than they were with other disturbances. Increase in electric discharges during motion might be expected if the fish were using the pulses to explore their surroundings; however, thus far, use of pulses in electrolocation is only known to exist in the continuously-discharging weakly-electric fishes (Lissmann and Machin, 1958). Furthermore, if discharges functioned in active electrolocation, one would expect increased discharge rate during normal as well as vigorous swimming, and one would not expect an increased association with coughing behaviour. As another possible explanation, this disturbance type might be an artefact of particularly large or rapid water disturbances, generated by rapid jaw or body movement.

Sets of several spikes were similar in duration (72.83 ± 8.23 s) to those observed by Baron and Pavlov (2003), which lasted 80-100 ms. Although, as with all disturbances, they were lower in amplitude than the discharges recorded in the previous study, they had the highest mean amplitude (0.18 ± 0.04 mV) of all disturbance classes. The pulse sets observed by Baron and

Pavlov (2003) showed substantial variation in pattern, but none of the examples presented closely resembled the spike sets observed here. Baron and Pavlov (2003) observed spike sets in which each spike was quite similar to the one which came before, while we observed spike sets containing spikes of widely-varying amplitude and shape. They more closely resembled the spike sets recorded in the catfish *P. asotus* (Baron and Olshansky, 2009), which were similar in irregularity of shape, and in amplitude (0.4-0.6 mV), although shorter in duration (<25 ms). Our spike sets also bore similarity to the irregular discharges produced by juvenile *Polypterus delhezi*, (Baron and Pavlov, 2003) as is discussed in more detail below.

Effect of time of day

When recording a single fish, the previous study found discharge rates to be three times higher at night compared to during the day (Baron and Pavlov, 2003). We found that the greatest number of disturbances occurred during the first couple hours of the experiment, in the morning, when the fish were first introduced to the enclosure. Prolonged spikes occurred more often during the day, while single spikes and spike sets were more likely to be observed at night, so if *Polypterus* do indeed emit more discharges at night, our single spike and spike set classes might be more likely to represent electrogenic discharges. However, activity levels are also likely to vary between night and day, as *Polypterus* are nocturnal, so the rate of movement-related artefacts might be altered along with the rate of electric discharges.

Changes in paired-fish trials

In comparison to the single-fish trials, in the paired-fish trial, the rate of spike sets increased nearly eightfold, from 0.56 ± 0.18 obs./h to 4.46 obs./h, and mean amplitude doubled. For all other disturbance classes, rate remained similar, or decreased slightly, relative to single-fish trials, and changes in amplitude were less pronounced. Baron and Pavlov (2003) did not

observe a change in discharge rate when fish were in pairs, as the single fish emitted three discharges per hour at night, while the pairs emitted 6.04 ± 0.48 discharges per hour, between the two fish. When placed together in close quarters, *Polypterus* behave aggressively towards each other. As they chase and bite the other fish, the result is vigorous swimming of an intensity rarely observed in other trials. If spike sets are caused by water displacement, increase in the frequency and vigour of fish movement could cause an increase in the number and amplitude of spike sets. However, in some other sporadically-electric fishes, discharge rate and amplitude increase substantially during fish interaction (Baron et al., 1994; Orlov et al., 2017). As relatively few spike sets occurred when *Polypterus* were interacting, but not swimming vigorously, it is unclear whether changes in rate and amplitude are due to changes in electric discharge characteristics during interaction, or are a by-product of increased water movement.

Myogenic vs. electrogenic disturbances

In studies of sporadic electrogenesis, one of the challenges is in distinguishing electric discharges from myogenic disturbances. Although dependent on the organism and the experimental set-up, the amplitude of fish myograms is generally on the order of microvolts, even during rapid movement, and even in fish larger than those tested here (Barham et al., 1969; Baron and Olshansky, 2009). However, there are sporadically-electric fishes which produce pulses within this amplitude range. For example, pulses from smaller (6-8 cm) catfish *Synodontis nigrita* will range 0.05-0.1 mV, even when the fish is close to the recording electrodes (Baron et al., 1994). Electrogenic and myogenic disturbances therefore cannot always be reliably differentiated by amplitude alone. Generally, myograms are more irregular in shape, and myogenic disturbances also tend to be shorter than electrogenic pulses (Baron and Olshansky, 2009).

Electric discharges are produced by action of modified muscle cells. Amongst sporadically-electric fishes, the electric organ has been located only in catfishes, where it was found to originate from sonic muscle (Boyle et al., 2014). Variation in discharge shape likely results from variation in electric organ characteristics. Discharges which are irregular or short (<8 ms) are thought to result from the asynchronous activity of more primitive electric organs, which bear greater similarity to the original muscle tissue (Orlov et al., 2017). Pulses with more regular shape or longer duration might result from improved neural control, and in the case of extended monopolar disturbances, tetanic summation (Orlov et al., 2017). Often, recorded discharges are corrected for polarity, standardized by amplitude and aligned by anterior front to determine the average shape of the discharge (Olshansky, 2010). We found the spike sets observed in our trials too variable to be matched to one another in this way. Similarly, Baron and Pavlov (2003) opted instead to provide examples of several discharge types. If they are indeed electrogenic in origin, the irregular characteristics of the spike sets observed here, coupled with the short duration and relatively low amplitude of the individual spikes, suggest a relatively primitive, unspecialized electric organ in *Polypterus*.

Variation in study parameters

Differences in experimental design between our study and that of Baron and Pavlov (2003) included use of silver wire electrodes instead of stainless steel plates. Although both are appropriate materials for electrodes, larger electrodes might result in decreased electrical noise, as well as decreased fluctuation of electrode potential (Olshansky, 2010). Despite this, baseline noise in our set-up was low (±0.004 mV). When electrodes are smaller in size, amplitude of recorded discharges becomes more strongly dependent on the location of the fish in the recording area; however, our recording area was substantially smaller than that of Baron and Pavlov (2003)

in order to somewhat minimize this effect (18 X 19 X 11 cm^3 vs. 70 X 20 X 40 cm^3). Future experiments might decrease recording area size even more, to further minimize variation. Multiple pairs of recording electrodes might also be used to reduce the confounding effect of fish orientation on results (Olshansky, 2010).

Results of electrical recordings might also depend on the animals tested. Baron and Pavlov (2003) tested *Polypterus senegalus* which were larger (15-18 cm) than our fish (10.10 ± 0.47 cm), and *Polypterus delhezi* (4-6 cm) which were smaller than our fish, and found different discharges in the two groups. Discharges of *P. delhezi* were highly irregular in shape, bearing somewhat greater similarity to our observations than the more regular discharges of the larger *P. senegalus*. They were also of smaller amplitude (2 mV), despite use of a smaller recording area (15 X 10 X 10 cm^3). Although there may be interspecific differences, it is likely that smaller fish simply produce smaller, less regular discharges. Future experiments could compare disturbances observed when recording smaller compared to larger *Polypterus senegalus*, to investigate the effect of ontogeny.

In addition, since sample sizes tend to be small for long-term recording of sporadically-electric fishes, inter-individual variation, independent of size, might strongly influence results. Inter-individual variation in electrogenesis has been noted in several catfishes. For example, in *Auchenoglanis occidentalis*, some individuals produce long biphasic pulses and others produce bursts of pulses (Orlov et al., 2015a), while in clariid catfish, a series of low-amplitude (0.25-0.90 mV) discharges are produced during spawning by the female only (Olshansky, 2010). Some *Polypterus* might produce more discharges than others, or might produce discharges with different characteristics, depending on sex or other factors. A larger sample size would be necessary to determine the extent of inter-individual variation in the species.

Functional and evolutionary implications

If *Polypterus* do produce sporadic electric discharges, their function remains to be determined. In mormyrids and gymnotids, near-continuous discharges are used in electrolocation (Lissmann and Machin, 1958), species recognition (Hopkins, 1976), and communication (Hopkins, 1986). However, sporadic discharges are unlikely to be of use in electrolocation, as they would provide little information. Most sporadically-electric catfishes produce discharges during aggressive interactions, mating interactions, or both (eg.: Baron et al., 1994; Olshansky, 2010; Orlov et al., 2017). Though they may also produce spontaneous discharges, interaction-associated discharges often differ in rate or character, and likely have some function in communication. Interestingly, catfish electroreceptors display maximal sensitivity to pulses exceeding approximately 30 ms in duration (Peters and Buwalda, 1972), so effective communication required evolution of sustained pulses (Orlov et al., 2017). To our knowledge, the frequency sensitivity of *Polypterus* electroreceptors has not been assessed. Since it is not known whether the fish can detect discharges that might be produced by nearby conspecifics, it remains unclear whether any discharges produced might have communicative function.

Previously, electrogenesis was thought to have evolved independently at least six times, in taxa which were already electroreceptive: twice in chondrichthyans, and four times in actinopterygians (mormyrids, gymnotids, silurids, and the stargazer, *Astroscopus* sp.; Bass, 1986; Bennett, 1971). Recently, the list of sporadically weakly-electric species has expanded to include such groups as lungfish, arowana (Orlov et al., 2015b) and even Chinese giant salamander (*Andrias davidianus*; Olshanskii et al., 2016). There is also some evidence of synchronized potentials in the lamprey (Kleerekoper and Sibakin, 1956), which might be precursors to electric discharges (Zupanc and Bullock, 2005). Taken together, these findings raise the possibility that

electrogenic ability might have more basal origins than previously suspected, and electrogenesis in *Polypterus* would further support this idea.

Conclusions

Most studies of sporadically-electric fishes have been the work of a single team of researchers, and although they have provided details of their protocol for this type of electrical recording (Olshansky, 2010), the differences between our results and theirs might suggest some value in reproducing such studies. Overall, it is unclear whether we could not distinguish *Polypterus* discharges from movement-related artefacts, whether our fish were not producing discharges, or whether *Polypterus* are simply not electrogenic. If they are not, passive electroreception is likely to be important nonetheless, given that *Polypterus* are nocturnal and have poor vision. Passive electroreception can be used in prey detection (Kalmijn, 1982), and is likely sufficiently sensitive in *Polypterus* to detect nearby fish (Roth, 1973). In future studies, one could investigate whether *Polypterus* are drawn to electrical stimuli mimicking fish respiration, in the absence of other sensory information, to gain a clearer understanding of the role passive electroreception might play in predation.

3.5 FIGURES

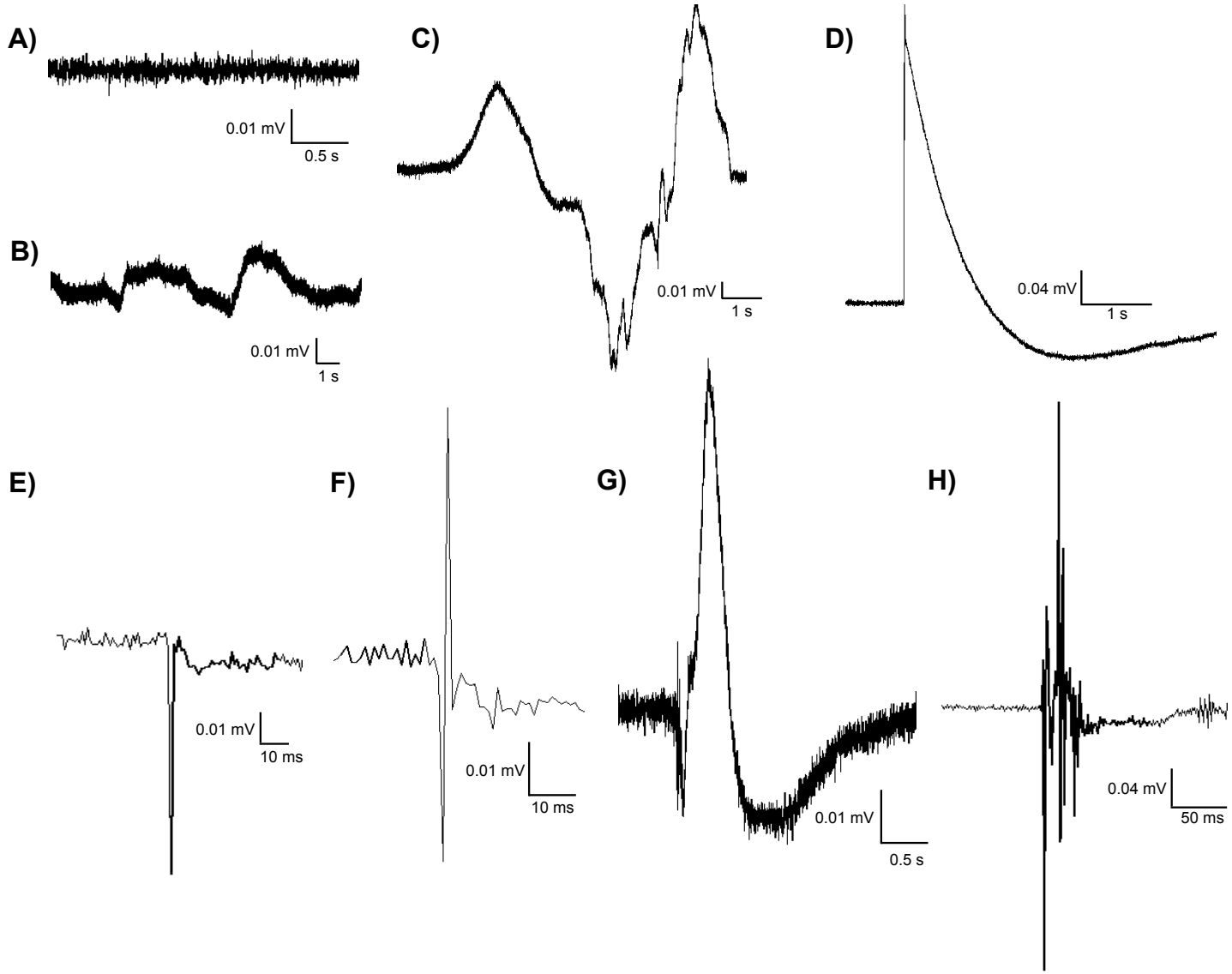

Figure 3.1. Examples of electrical recording traces. Letters correspond to trace type classifications outlined in Table 3.1.

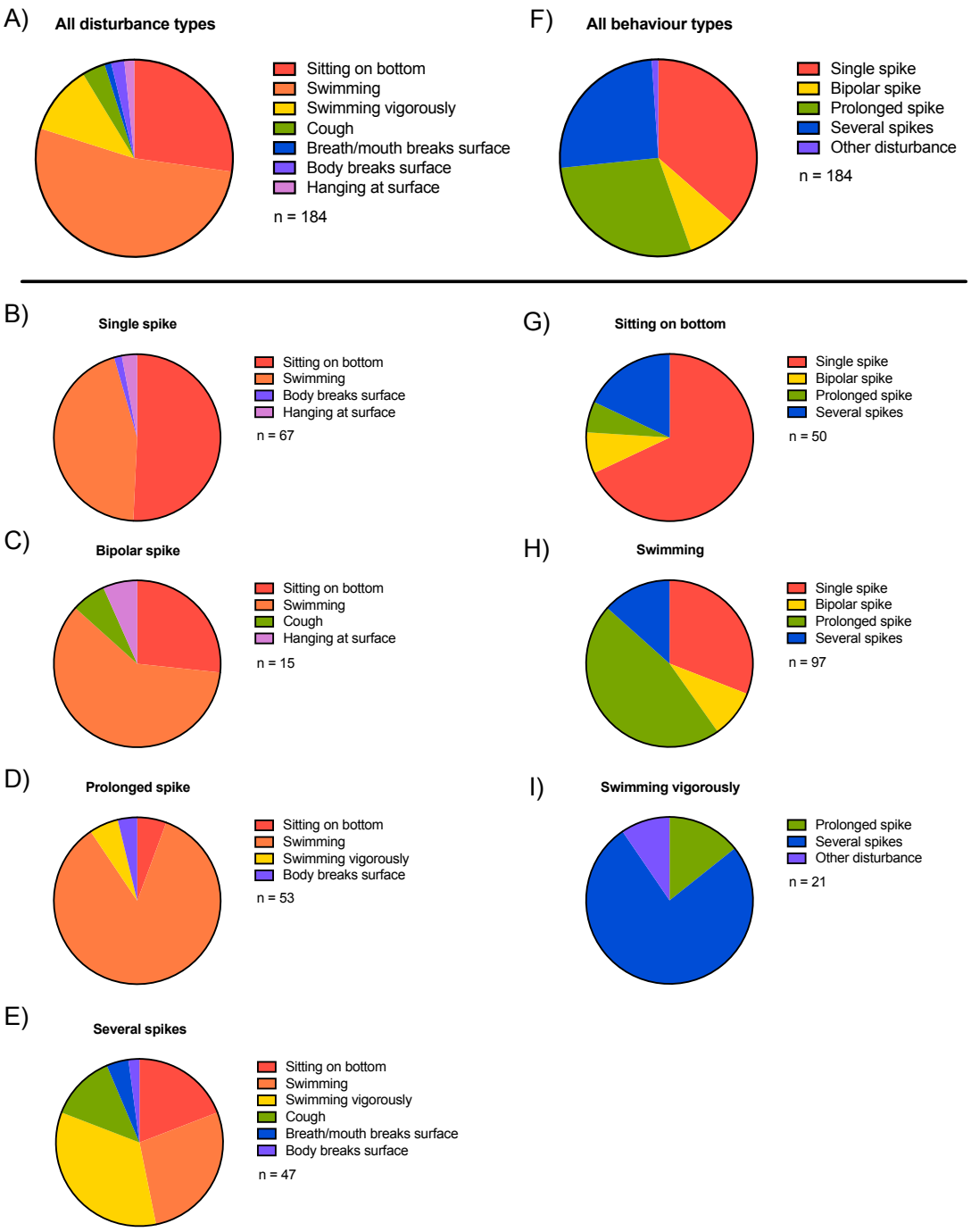

Figure 3.2. Electrical disturbances and corresponding behaviours for all four single-fish trials. The top two charts summarize the fish behaviours observed across all electrical disturbances that might have biological origins (A), and the reverse, the disturbance types observed across all behaviours (F). Below, behaviours are shown subdivided by disturbance type (B-E), and vice versa (G-I). Charts which would contain less than 10 observations are not shown.

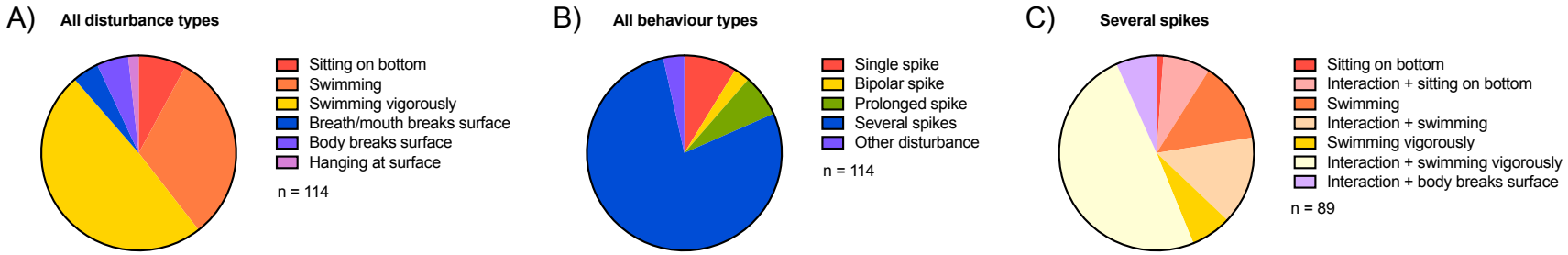

Figure 3.3. Electrical disturbances and corresponding behaviours for paired-fish trial. Behaviours observed across all electrical disturbances (A), disturbance types observed across all behaviours (B), and behaviours observed during "several spikes" disturbance type (C). Electrical disturbances are compared to the behaviour of the fish demonstrating the greater amount of movement.

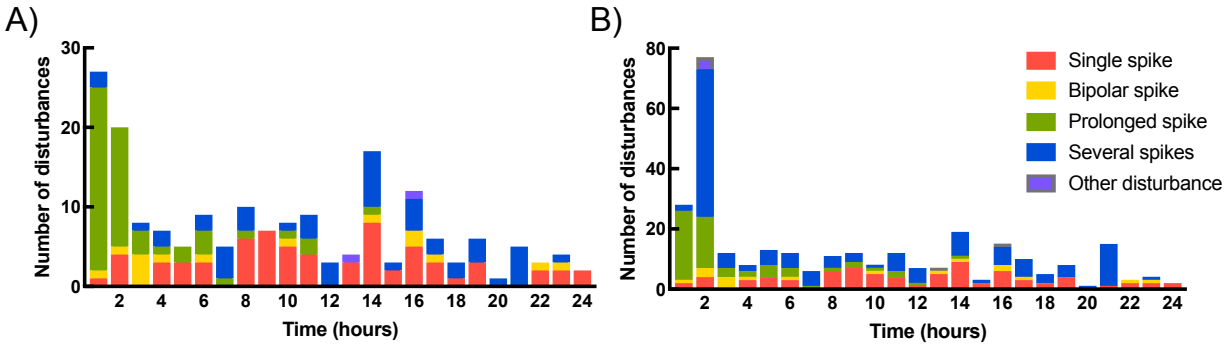

Figure 3.4. Occurrence of electrical disturbances over 24 h period beginning at 9:00 am, for single-fish trials (A), and for all trials, including paired-fish trial (B).

3.6 TABLES

Table 3.1. Classification of electrical recording trace by disturbance shape. See Fig. 3.1 for examples.

Class	Trace type	Description
A	Baseline	Flat trace (amplitude ≤ 0.004 mV)
B	Small irregular deviations	Small (≤ 0.04 mV) disturbances from baseline, irregular and often over extended time period
C	Larger irregular deviations	Large (> 0.04 mV) disturbances from baseline, irregular and often over extended time period
D	Large regular deviations	Large, rapid changes in amplitude followed by slow (> 1 s) return to baseline, consistent in shape
E	Single spike	Single spike exceeding 0.04 mV, lasting less than 50 ms
F	Bipolar spike	Positive and negative spikes exceeding 0.04 mV, lasting less than 50 ms
G	Prolonged spike	Single disturbance exceeding 0.04 mV, lasting 50-500 ms
H	Several spikes	Set of several spikes, at least one of which exceeds 0.04 mV, lasting less than 150 ms
I	Other disturbance	Anything that does not fit in the above categories

Table 3.2. Classification of recorded video by fish behaviour. In classification, brief behaviours (classes 4-6) took precedence over extended behaviours (classes 1-3, 7) when both were occurring simultaneously.

Class	Behaviour	Description
1	Sitting on bottom	Fish is relatively still, sitting on bottom of enclosure
2	Swimming	Fish is swimming at slow to moderate speeds
3	Swimming vigorously	Fish is swimming rapidly, burst of movement
4	Cough	Fish rapidly opens jaws and snaps them closed again
5	Breath/mouth breaks surface	Fish takes breath at surface, and/or the nose breaks the water's surface
6	Body breaks surface	Body of fish breaks water's surface
7	Hanging at surface	Fish is relatively still, at or near the water's surface
8	Interaction (paired fish)	Fish are interacting, are in contact with one another, are chasing or biting the other

Table 3.3. Observations of electrical disturbances deviating from baseline under control conditions. Control conditions were recorded with heater on for 1.9499 days (46.7969 h), and with heater off for 1.1822 days (28.3728 h).

Class	Trace type	Control with heater		Control without heater	
		Number of observations	Rate (observations/day)	Number of observations	Rate (observations/day)
B	Small irregular deviations	0	0	0	0
C	Larger irregular deviations	1	0.5129	3	2.5376
D	Large regular deviations	86	44.1054	2	1.6918
E	Single spike	9	4.6157	0	0
F	Bipolar spike	1	0.5129	0	0
G	Prolonged spike	0	0	0	0
H	Several spikes	0	0	0	0
I	Other disturbance	0	0	0	0

Table 3.4. Observations of electrical disturbances deviating from baseline when one fish is in enclosure. Four fish were recorded for approximately one day each (0.8907±0.0541 d, or 21.3774±1.2980 h). Means are shown ± standard error of the mean.

Class	Trace type	Total number of observations (for all fish, all days)	Rate (observations/day)	Amplitude (mV)	Duration (ms)
E	Single spike	67	18.9111±5.1259	0.1233±0.0154	3.5610±0.7460
F	Bipolar spike	15	4.2717±1.6104	0.1329±0.0137	4.2821±0.4523
G	Prolonged spike	53	15.9064±15.5641	0.0404±0.0124	151.7500±101.7500
H	Several spikes	47	13.5519±4.2182	0.1843±0.0432	72.8303±8.2285
I	Other disturbance	2	0.5096±0.2942	0.0593±0.0007	100.0000±90.0000

Table 3.5. Observations of electrical disturbances deviating from baseline when two fish are in enclosure. The pair of fish were recorded for approximately one day (0.8316 d or 19.9572 h).

Class	Trace type	Total number of observations	Rate (observations/day)	Amplitude (mV)	Duration (ms)
E	Single spike	10	12.0257	0.1364±0.0430	9.9500±4.8497
F	Bipolar spike	3	3.6077	0.9800±0.4884	4.6667±0.3333
G	Prolonged spike	8	9.6206	0.0999±0.0305	288.7500±88.0632
H	Several spikes	89	107.0289	0.3558±0.0292	36.2360±2.4222
I	Other disturbance	4	4.8103	0.2688±0.0861	302.5000±83.7033

Table 3.6. Comparison of disturbance rate and amplitude between disturbance classes E-I. Trial was set as a fixed factor to investigate differences between the single-fish trials and the paired-fish trial. Light refers to whether the enclosure was being lit by room lighting (during the day), or by infrared lighting (at night). Trial*disturbance class interaction could not be included for amplitude model due to insufficient replication. The dependent variable was transformed to meet assumptions where necessary. Significance of terms determined by maximum likelihood ratio test comparing models with and without each variable. When $P>0.05$, term was not included in final model.

Dependent variable	Model (transform)	Significance of term (*P*)						Final model
		Trial* Disturbance	Trial*Light	Light* Disturbance	Trial	Disturbance	Light	
Rate (obs./d)	Linear (sqrt(y))	0.0002 (χ^2_1=43.39)	0.0043 (χ^2_1=15.19)	0.0548 (χ^2_1=9.27)	N/A	N/A	N/A	Rate ~ Trial*Disturbance + Trial*Light
Amplitude (mV)	Linear (log(y))	N/A	0.0556 (χ^2_1=9.29)	0.6421 (χ^2_1= 2.51)	<0.0001 (χ^2_1=29.52)	<0.0001 (χ^2_1=57.15)	0.8950 (χ^2_1=0.02)	Amplitude ~ Disturbance + Trial

Table 3.7. Effects of fish behaviour, trial, and lighting condition on observed disturbance class. Multinomial regression was used, with trial set as a fixed factor to investigate differences between the single-fish trials and the paired-fish trial. Light refers to whether the enclosure was being lit by room lighting (during the day), or by infrared lighting (at night). Significance of terms determined by maximum likelihood ratio test comparing model with and without each variable. When $P>0.05$, term was not included in final model.

Dependent variable	**Model (transform)**	**Significance of term (*P*)**						**Final model**
		Trial* Behaviour	**Trial*Light**	**Light* Behaviour**	**Trial**	**Behaviour**	**Light**	
Disturbance class	Multinomial (none)	0.8919 (χ^2_1=43.34)	0.3160 (χ^2_1=18.14)	0.9572 (χ^2_1=10.54)	<0.0001 (χ^2_1=113.88)	<0.0001 (χ^2_1=141.75)	<0.0001 (χ^2_1=4.12)	Disturbance ~ Trial + Behaviour + Light

CONCLUSION

Overall, we gained an increased understanding of the manner in which *Polypterus* gathers information about its surroundings, and uses this information in locomotor behaviour. We explored the influence of vision and lateral line inputs on the control of locomotion, the visual abilities of the fish, the changes in these between terrestrial and aquatic environments, and the possibility of electrogenesis in *Polypterus*.

Despite decreased function in air, both visual inputs and lateral line inputs influence locomotor behaviour both in water and on land. As expected in a nocturnal fish, with regard to general exploratory behaviour, lateral line input could compensate for vision loss, while the reverse was not true. Several kinematic variables showed large increases in variability when fish could see but not feel, which we hypothesize to be a result of an unexpected conflict in incoming sensory information. In the future, this response to sensory discord could be further investigated by placing fish in a swimming flume set-up in which water flow speed and visual stimulus speed are in conflict. Changes in sensory function in fish reared on land could also be explored. Arrangement of neuromasts may be altered, or desiccation of eyes and neuromasts may impair their function. Extent of reliance on these senses may change as a result.

Other sensory modalities may have a larger impact on swimming and walking. Tactile, proprioceptive, and vestibular inputs are likely to be both important in locomotion and substantially affected by the change in environment. Notably, unlike visual and lateral line information, which might influence descending inputs to spinal central pattern generators (CPGs), proprioception can affect CPGs locally. Although proprioception cannot be disabled as easily as the lateral line system, nerve transections or immunohistological strategies might be employed to assess its relative importance in both types of locomotion.

Polypterus visual acuity, as determined by optokinetic response, is relatively poor. The fish showed some response to large and slow-moving stimuli, but the limits of both spatial and temporal acuity were low compared to many other fishes (Caves et al., 2017). This may reflect the ecology of the animal, since gaze stabilization may be less crucial in a nocturnal ambush predator (Dieringer et al., 1992). Contrast may be maximized at the expense of acuity, as it is in some other fishes inhabiting low-light environments (Brokovich et al., 2010; Ryan et al., 2016). As might be predicted in a fish demonstrating no specialized adaptations for aerial vision (Kröger et al., 2014; Rochon-Duvingneaud, 1943, in Pfeiffer, 1968), vision was poorer in air. Given that the optokinetic response is conserved across aquatic and terrestrial vertebrates (Mueller and Neuhauss, 2010), a change in the characteristics of the saccade itself, namely a decrease in saccade angular displacement, was unexpected. Perhaps a decline in peripheral acuity in air decreases the incentive to rotate the eye as far temporally. This hypothesis could be tested by repeating the experiments using stimulus bars spaced widely, so that only one is in the visual field of the fish at any time, removing the effect of peripheral stimulation.

Although *Polypterus* have been recorded emitting electric discharges (Baron and Pavlov, 2003), we did not find, in our study, clear evidence of electrogenesis. It is possible that discharges could not be detected in our set-up, or were indistinguishable from movement artefacts. In the future, bigger fish, a smaller recording area, and larger electrodes might maximize the probability of discharge detection (Olshansky, 2010). If *Polypterus* are electrogenic, the purpose of these discharges remains unknown. Baron and Pavlov (2003) did not observe changes in discharge rate between solitary and paired fish, so electrogenesis would not seem to function in communication. In addition, as the discharges are infrequent, an electrolocative function seems unlikely as well.

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