



## Research

**Cite this article:** Aiello BR, Stewart TA, Hale ME. 2016 Mechanosensation in an adipose fin. *Proc. R. Soc. B* 20152794. <http://dx.doi.org/10.1098/rspb.2015.2794>

Received: 19 November 2015

Accepted: 19 February 2016

**Subject Areas:**

biomechanics, evolution, neuroscience

**Keywords:**

appendage, fishes, flow sensing, neurophysiology, Siluriformes

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2015.2794> or via <http://rsob.royalsocietypublishing.org>.

## Mechanosensation in an adipose fin

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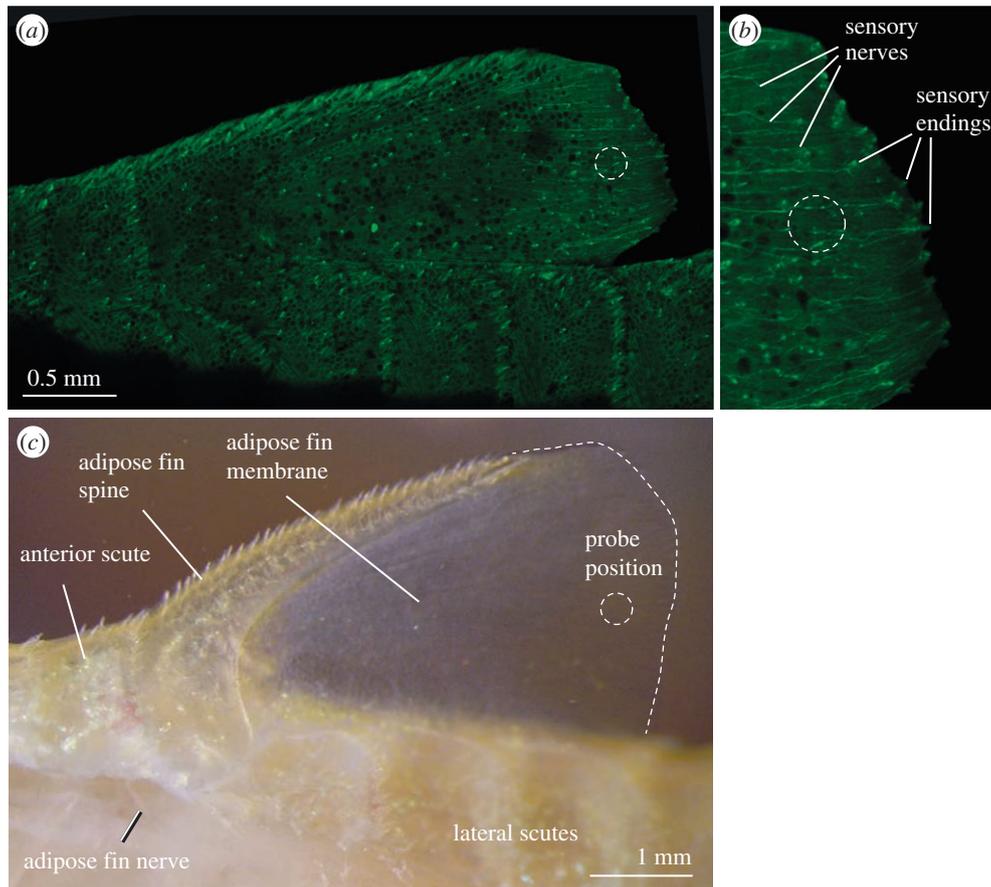
Adipose fins are found on approximately 20% of ray-finned fish species. The apparently rudimentary anatomy of adipose fins inspired a longstanding hypothesis that these fins are vestigial and lack function. However, adipose fins have evolved repeatedly within Teleostei, suggesting adaptive function. Recently, adipose fins were proposed to function as mechanosensors, detecting fluid flow anterior to the caudal fin. Here we test the hypothesis that adipose fins are mechanosensitive in the catfish *Corydoras aeneus*. Neural activity, recorded from nerves that innervate the fin, was shown to encode information on both movement and position of the fin membrane, including the magnitude of fin membrane displacement. Thus, the adipose fin of *C. aeneus* is mechanosensitive and has the capacity to function as a ‘precaudal flow sensor’. These data force re-evaluation of adipose fin clipping, a common strategy for tagging fishes, and inform hypotheses of how function evolves in novel vertebrate appendages.

## 1. Introduction

Adipose fins are enigmatic second dorsal fins found on approximately 6000 species of teleost fishes [1], or about 10% of vertebrate species [2]. In general, adipose fins are smaller than other fins and lack associated musculature and skeleton [3]. The apparently rudimentary anatomy of adipose fins has led to the longstanding hypothesis that they are vestigial [4,5]. However, adipose fins are morphological novelties, newly evolved body parts, which have originated repeatedly across the teleost phylogeny [1] and that have persisted for more than 200 Myr in some lineages [6]. Despite their widespread occurrence, repeated origin and persistence over long evolutionary scales, little is known of their functions.

Recently, it was proposed that adipose fins function as ‘precaudal flow sensors’ [7,8]. This hypothesis was first articulated by Reimchen & Temple [7], who found that the amputation of the adipose fin of the steelhead trout, *Oncorhynchus mykiss* (Walbaum 1792), caused altered caudal fin kinematics during steady swimming that suggest reduced swimming efficiency [7]. Later, consistent with Reimchen & Temple’s hypothesis [7], it was found that the adipose fin of the brown trout, *Salmo trutta* (Linnaeus 1758), has sensory innervation [8]. Furthermore, a comprehensive survey of over 2100 catfish (Siluriformes) species found that species occupying high-flow habitats (streams and rivers) were more likely to possess adipose fins than those species occupying no-flow habitats (lakes and ponds) [9]. Collectively, these data support the hypothesis that adipose fins are functional, and that they are mechanosensitive. While the paired fins of vertebrates have been shown to be mechanosensitive, detecting both dynamic and static information about the fin’s movement and position [10–13], it is unknown whether adipose fins can respond to mechanical stimuli.

To determine if adipose fins have the potential to function as ‘precaudal flow sensors’ [7], we studied adipose fin mechanosensation in the South American armoured catfish, *Corydoras aeneus* (Callichthyidae) (Gill 1858). We determined with immunohistochemistry that the adipose fin of *C. aeneus* is highly innervated. We tested the hypothesis that adipose fins are mechanosensitive by conducting multi-unit recordings from nerves entering the adipose fin while the fin membrane was deflected laterally by a probe attached to a linear actuator. This study, the first to examine the mechanosensitive capability of a median fin, informs the adaptive functions of adipose fins and also the evolution of proprioception in vertebrate fins and limbs.



**Figure 1.** The adipose fin of *C. aeneus* contains sensory innervation. (a) Antibody staining, following published methods [14] and using primary antibody mouse monoclonal anti-acetylated tubulin (Sigma-Aldrich) and the secondary antibody goat anti-mouse antibody conjugated with fluorescein (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), labels nerves in the adipose fin. Sensory fibres that are oriented approximately parallel to the actinotrichia in the adipose fin and enter the fin anteriorly, behind the adipose fin spine. (b) Photograph of dissection that was used for electrophysiology. We recorded from the exposed adipose fin nerves while a probe, at the indicated position, depressed the fin membrane to varying distances. The dashed line indicates fin margin. The dashed circles in (a–c) represent the probe position.

## 2. Material and methods

### (a) Animals

Four *C. aeneus* were used in this study. Their standard lengths ranged from 3.1 to 4.4 cm. The catfish were donated by NBM Aquatics (Chicago, IL, USA) and housed in glass aquaria (10 gallons) equipped with recirculating water filters and water heaters. Fish were fed three times a week with commercial carnivore and algal food pellets (Hikari Hayward, CA, USA). Water temperature was maintained at approximately 23°C and the fish were exposed to a seasonal light/dark cycle. All experimental procedures were carried out under University of Chicago Institutional Animal Care and Use Committee guidelines (protocol 71589 to M.E.H.).

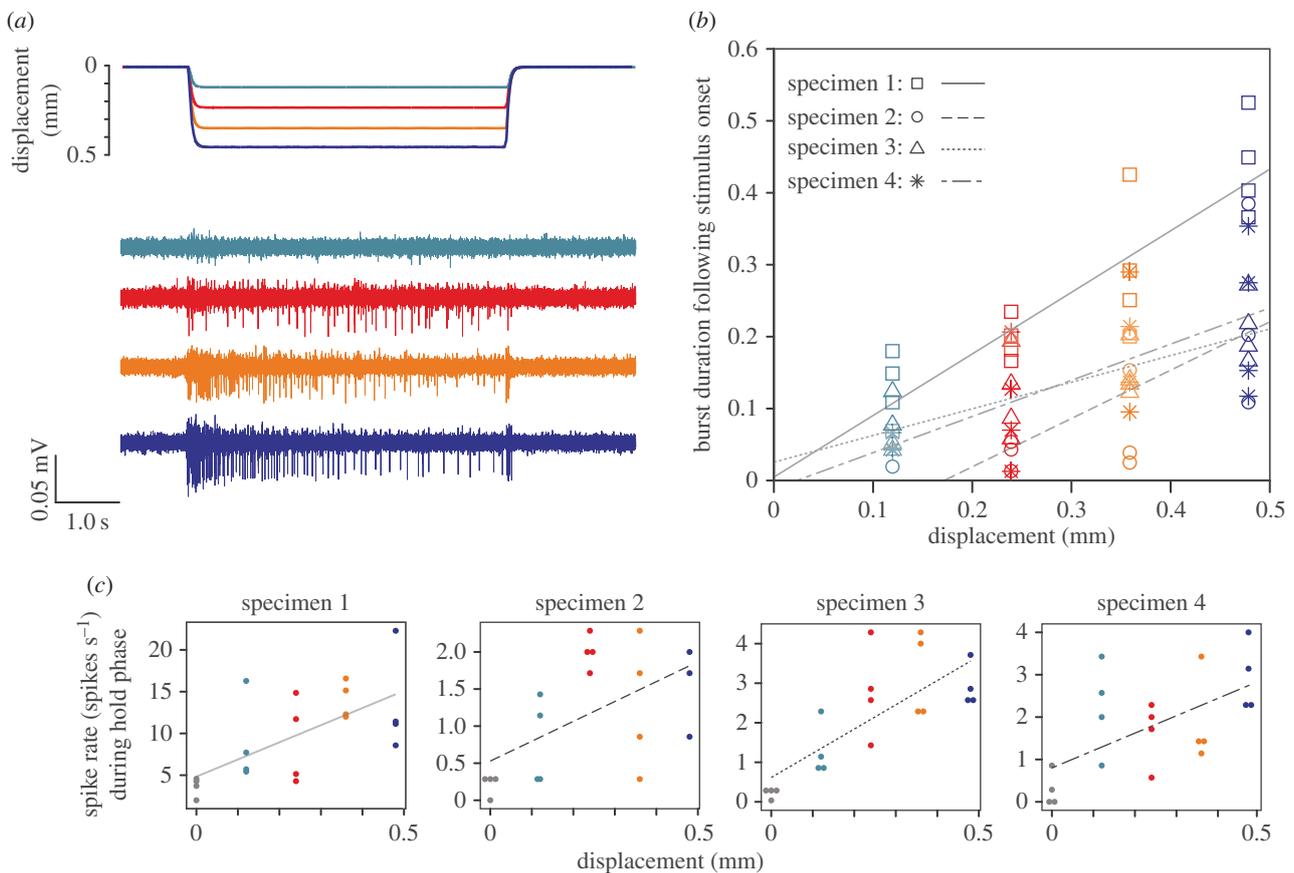
### (b) Electrophysiology

Antibody staining revealed that the adipose fin of *C. aeneus* is highly innervated (figure 1a) [15]. In *C. aeneus*, adipose fin nerves enter the fin posterior to the fin spine (figure 1b) and ramify repeatedly into the adipose fin membrane, with branches organized approximately parallel to the actinotrichia.

We recorded from nerves that enter the adipose fin posterior to the adipose fin spine following published methods [10]. Fish were euthanized with MS222 (Tricaine methanesulfonate, Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 0.5 g l<sup>-1</sup>. Immediately following death, heads were removed, and the caudal portion of the body was bathed in extracellular solution of published ionic concentrations [16]. The solution

was changed regularly throughout the duration of the experiment. The left side of the fish was dissected to remove lateral scutes and skin, and epaxial musculature to expose the nerves leading into the adipose fin, and the specimen was pinned to the extracellular dish. An extracellular glass suction electrode was connected to an adipose fin afferent nerve fibre to record multi-unit responses to fin bending. Electrodes (tip diameter: 15–30 µm) were made by pulling borosilicate glass capillaries (GC150F-7.5 1.5 mm OD, 0.86ID, Harvard Apparatus, Holliston, MA, USA) in a P-97 Flaming/Brown micropipette puller (Sutter Instrument Co., Novato, CA, USA) and fire-polished with a microforge (MF-830, Narishige, East Meadow, NY, USA). Extracellular recording voltages were monitored with a MultiClamp 700B amplifier (Molecular Devices, Foster City, CA, USA). The analogue voltage signals were digitized with a DigiData 1440A digitizing board (Molecular Devices) and acquired using pClamp 10 Clampex software (Molecular Devices).

We recorded from nerves while the fin membrane was pushed by a probe to deflect it laterally. The probe, a pinhead, was mounted to the end of a voice coil positioning stage (VCS10-023-BS-01-M, H2W Technologies Inc., Valencia, CA, USA) and controlled by a programmable driver (Intelligent Servo Drive IDM640-8EI, Technosoft, Canton, MI, USA). Lateral trunk displacement at the position of the adipose fin does not exceed 1.0 mm in *C. aeneus* [17]; these data were used to estimate a range of behaviourally relevant stimuli. A series of four randomly ordered step-and-hold stimuli (0.12, 0.24, 0.36 and 0.48 mm) of 5 s in duration were applied to the fin to assess nerve response to lateral deformation of the fin membrane. This was repeated four times for each



**Figure 2.** Adipose fin nerves are mechanosensitive. (a) Data from a representative experiment. Upper panel shows traces the probe position, and lower panel shows nerve recordings from one individual (Specimen 1) as the fin was displaced to varying distances. A burst of afferent activity is observed immediately following the fin membrane deflection, and nerves continue to fire while the fin membrane is held in a depressed position. (b) Burst duration is positively correlated with magnitude of fin membrane displacement. Regression statistics are detailed in electronic supplementary material, table S1. (c) Spike rate during the hold phase is positively correlated with the magnitude of fin displacement. Overlapping data points were slightly displaced from each other along the x-axis for visual aid. Regression statistics are detailed in electronic supplementary material, table S2.

specimen. For individual 2, the afferent nerve was dislodged from the electrode during the experiment, and therefore, we recorded responses for only 15 out of the 16 stimuli.

Data were sampled at 100 kHz, down-sampled to 10 kHz, and analysed in MATLAB v. 7.10.0 (Mathworks, Natick, MA, USA). To determine if adipose fin afferents could encode the amplitude of fin movement (the dynamic portion of the step-and-hold stimulus), we analysed the bursts of action potentials associated with onset of the stimulus. A burst was defined as three or more spikes within 50 ms of each other. The first action potential after the start of the stimulus was considered the first spike in the burst at the onset of the stimulus, and the burst duration was ongoing so long as three or more spikes were recorded within 50 ms of one another. We tested for correlation between magnitude of fin membrane deflection and the duration of bursting using linear regressions.

To determine if *C. aeneus* is capable of sensing the static position of the adipose fin membrane, we compared the spike rate over the hold period to the spike rate of a 0.5 s prestimulus baseline. The burst duration associated with the onset of the stimulus never exceeded 0.6 s. Therefore, to exclude afferent activity associated with fin movement, we compared afferent responses from 1.0 to 4.5 s after the onset of the stimulus. We tested for correlation between magnitude of fin membrane deflection and spike rate during the hold phase using linear regressions. Two-tailed *t*-tests were performed to compare the spike rate between the hold period and the prestimulus baseline, and to determine the stimulus magnitude needed to elicit a hold period firing rate significantly greater ( $p < 0.025$ ) than the spike rate over the prestimulus interval.

The burst duration associated with the stimulus onset and the spike rate over the hold period of the stimulus were calculated using a custom MATLAB routine (R. Williams IV, University of Chicago). All statistical analyses were performed on each individual separately because the inter-individual differences in afferent response level do not allow us to assume that the error variance is the same for all individuals. Furthermore, in order to control for inflated type one error associated with making multiple statistical tests, we used conservative Bonferroni-corrected *p*-values at an  $\alpha = 0.0125$  for each statistical test. All statistical analyses were performed in MATLAB v. 8.1.0 (MathWorks, Natick) or JMP v. 9.0.1 (SAS, Cary, NC, USA).

### 3. Results

Adipose fin nerves responded to lateral deflection of the fin membrane during step-and-hold stimuli (figure 2). The mechanosensory system of the adipose fin responded to deflection amplitudes as small as 0.12 mm (figure 2, table 1). Afferents exhibited a burst of activity at the onset and offset of the stimulus (figure 2a). Sustained afferent activity was observed over the hold phase of the stimulus, while the fin was being held in a bent position (figure 2a). Collectively, afferents provided information about both the dynamic and static qualities of a stimulus (i.e. movement and position).

The adipose fin afferent response increased with increasing amplitude of fin membrane deflection. The average duration

**Table 1.** A comparison of spike rate during the hold period of a stimulus and baseline. The mean  $\pm$  s.d. are reported for each value of spike rate (spike  $s^{-1}$ ). The hold period activity threshold is defined as the minimum bending amplitude (mm) needed to elicit a significant difference between the hold period spike rate and the prestimulus baseline spike rate. Paired  $t$ -tests were run on the data from each individual's four trials to produce each  $p$ -value, which are all significant to the Bonferroni-corrected  $\alpha = 0.0125$ .

individual	baseline spike rate	hold period activity threshold	spike rate at minimum bending amplitude	d.f.	$p$ -value
1	$3.64 \pm 1.15$	0.36	$14.00 \pm 2.23$	3	0.007
2	$0.20 \pm 0.10$	0.24	$2.00 \pm 0.23$	3	0.002
3	$0.21 \pm 0.14$	0.48	$2.93 \pm 0.54$	3	0.001
4	$0.29 \pm 0.40$	0.48	$2.92 \pm 0.82$	3	0.010

of the bursts associated with the onset of the stimuli were  $0.079 \pm 0.049$ ,  $0.117 \pm 0.076$ ,  $0.182 \pm 0.101$  and  $0.278 \pm 0.129$  s (average  $\pm$  s.d.) for bending amplitudes of 0.12, 0.24, 0.36 and 0.48 mm, respectively. The durations of the bursts associated with stimulus onset were positively correlated with fin deflection amplitude, and significant for three or four individuals at a Bonferroni corrected  $\alpha = 0.0125$  (figure 2b; electronic supplementary material, table S1). Additionally, the average spike rate was  $1.089 \pm 1.620$  spikes  $s^{-1}$  for the prestimulus baseline, and  $3.268 \pm 4.087$ ,  $3.857 \pm 3.903$ ,  $5.089 \pm 5.5099$  and  $5.429 \pm 5.744$  spikes  $s^{-1}$  over the hold period at bending amplitudes of 0.12, 0.24, 0.36 and 0.48 mm, respectively. Spike rate during the hold period was positively and significantly correlated with fin bending amplitude in all four individuals at a Bonferroni corrected  $\alpha = 0.0125$  (figure 2c; electronic supplementary material, table S2). The spike rate over the hold period was significantly greater (Bonferroni corrected  $\alpha = 0.0125$ ) than the spike rate over the prestimulus baseline at bending amplitudes greater than or equal to 0.36, 0.24, 0.48 and 0.48 for individuals 1, 2, 3 and 4, respectively (table 1). Therefore, adipose fin afferents can encode information on the static position of the fin as well as the magnitude of fin membrane deflection.

## 4. Discussion

The purpose of this study was to determine if adipose fins are capable of mechanosensation, as previously proposed [7,8]. The adipose fin of *C. aeneus* is innervated (figure 1a,b), and these nerves are able to sense adipose fin movement and position (figure 2). In combination with previous work in the pectoral fin [18,19], the discovery that adipose fins, which are passive median fins, can function as mechanosensors suggests that mechanosensation might be a general feature of fins. These results broaden our understanding of adipose fin function and also inform the evolution of proprioception in vertebrate appendages.

### (a) Mechanosensation and adipose fin function

The adipose fin of *C. aeneus* is highly sensitive and can detect small amplitude displacements. Thus, consistent with the hypothesis of Reimchen & Temple [7], these fins have the potential to serve as 'precaudal flow sensors'. Afferent nerves in the adipose fin of *C. aeneus* could be stimulated by lateral fin deflection experienced during steady swimming. Our results show sensitivity to magnitudes of deflection as low as 0.12 mm, likely within the range experienced by *C. aeneus*

during steady swimming. In *C. aeneus*, adults exhibit carangiform locomotion (i.e. thrust producing waves passed along the body are of small magnitude anteriorly, while high amplitude lateral excursions are restricted caudally) [17]. Kinematic analysis of adult *C. aeneus* over a range of speeds (3.07–4.87 total lengths  $s^{-1}$ ) found that lateral excursion of the trunk at the position of the adipose fin did not exceed 1.0 mm [17].

Vortices and turbulent flow could also stimulate adipose fins. In the steelhead trout (*O. mykiss*), the effect of adipose fin amputation on caudal fin kinematics is most pronounced under turbulent conditions, suggesting fin function is related to altered flows [7]. Further, experiments in trout possessing adipose fins found that fish were able to hold position in a vortex sheet even when information from the visual and lateral line systems were not available to the animal [20]. One proposed explanation of these results is that additional sensory mechanisms allow these fishes to use a Kármán gait [20]. Our data and those of others [7,8] suggest that adipose fins could provide such a mechanism for detecting vortical flows in the environment. Indeed, an analysis of catfishes revealed significant correlation between adipose fin presence and environmental flow regime [9].

Additional hypotheses of adipose fin function have been proposed (reviewed in [8,21]) and these are not-mutually exclusive with mechanosensation. For example, Drucker & Lauder [22] proposed that adipose fins might have an effect on precaudal flow dynamics that allows for increased thrust through enhancement of the leading edge vortex of the caudal fin. Regardless of additional functions, these data show that mechanosensation should be considered when examining the form, function, and evolution of adipose fins. Discovering how adipose fin sensation impacts behaviour and how the fin affects the dynamics of precaudal flow will shed light on the ecology and morphological diversity of the vast diversity of teleost fishes that have these appendages.

The findings raise important questions about the impact of adipose fin removal in salmonids, questions both of economics and of animal welfare. Adipose fins are routinely amputated from millions of hatchery-raised fishes [23], because it offers a cheap and straightforward means of discerning a fish's provenance, which is crucial for management of fish stocks. The consequences of adipose fin amputation on swimming performance [7] are just beginning to be probed. However, it is known that adipose fins are not vestigial, but newly evolved [1] and, at least in some species, highly sensitive appendages. Therefore, consistent with Buckland-Nicks *et al.* [8], we argue that the practice of adipose fin clipping should be re-evaluated. Future work should evaluate the mechanosensitive capabilities

of adipose fins in salmonids. Additionally, particle imaging velocimetry [22] and nerve transections [18] can be used to tease apart whether the reduced swimming performance in salmonids after adipose fin amputation [7] is due to altered hydrodynamics or the loss of sensory feedback.

## (b) Proprioception and the evolution of active control in vertebrate appendages

Proprioception is the ability to sense the movement and position of one's body and its parts. The adipose fin of *C. aeneus* is proprioceptive; its fin afferents encode information on both fin movement and on the fin membrane's static position. This is, to our knowledge, the first description of proprioception in a passive vertebrate appendage (i.e. an appendage whose position and properties are not actively controlled). In actively controlled appendages, proprioceptive feedback is crucial for the precise control of movement. This is true both in fins [18] and limbs [24,25]. The active control of fins, and therefore limbs, is thought to be secondarily derived, because fins primitively lack endoskeleton or evidence of muscular attachment [1,26]. Therefore, in the light of the discovery that passive fins can be proprioceptive, we propose that

proprioception generally precedes the evolution of active control in vertebrate appendages.

**Ethics.** All experimental procedures were carried out under various Institutional Animal Care and Use Committee guidelines (protocol 71589 to M.E.H.).

**Data accessibility.** All electrophysiological data will be included as electronic supplementary material, table S3, upon the potential acceptance of this manuscript.

**Author contributions.** T.A.S. and B.R.A. contributed to this study equally. T.A.S. conceived the project, helped with data collection and wrote the paper. B.R.A. designed, performed, and analysed neurophysiological experiments, and helped to write the paper. M.E.H. helped with design of experiments, guided data analysis, and helped to write the paper.

**Competing interests.** The authors of this paper declare no competing interests.

**Funding.** This work was supported by the National Science Foundation under grants DGE-0903637 (a traineeship that supported T.A.S. and B.R.A.), a GRFP (to T.A.S) and IOS 1257886 (to M.E.H.), and the Office of Naval Research under grant N00014-0910352 (to M.E.H.). Funding also came from the American Society for Ichthyologists and Herpetologists through the Raney Award (to T.A.S.) and The University of Chicago through the Hinds Fund (to T.A.S).

**Acknowledgements.** Thank you to Michael Coates, Michael LaBarbera, Wenli Dai, Dallas Krentzel, Andrew Gehrke, Katie Henderson and Talia Karasov for helpful discussion and feedback on the manuscript.

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