Swimming with a sword: tail beat kinematics in relation to sword length in *Xiphophorus*

Christopher E. Oufiero*,†, Kristine Jugo and Theodore Garland Jr

Department of Biology, University of California, Riverside, CA 92521, USA

Summary

1. Exaggerated morphological structures that evolve under sexual selection have the potential to alter functional relationships and hence affect aspects of movement. These effects may be more pronounced when the exaggerated morphological trait is coupled to the propulsive system.

2. Many studies have examined the effects of sexually selected traits on whole-organism performance, but few have documented their effects on the kinematics of locomotion.

3. Using four swordtail (*Xiphophorus*) species that vary naturally in their expression of the sexually selected sword, and an experimental manipulation for the species in our sample with the longest sword (*X. alvarezi*), we examined how variation in sword length affects the kinematics of swimming.

4. Among the four species, we found few differences in tail beat kinematics, despite the large variation in sword length among species. In particular, the two species with long swords did not differ from the species lacking a sword, suggesting no locomotor 'cost' of having long swords.

5. Using experimental manipulation, sword removal significantly increased tail beat amplitude, but not frequency, suggesting a potential increase in thrust production.

6. Our comparative results suggest that swimming kinematics do not vary much with sword length, despite the variation in this sexually selected trait among the four species. This result suggests that other physiological mechanisms may be compensating for sword length, or as has been suggested recently, the sword may not impose a significant swimming cost.

Key-words: kinematics, locomotion, sexual selection, swordtail

Introduction

A unique aspect of sexual selection is the evolution of exaggerated morphological structures that may interfere with the functional relationships of the organism. Sexual selection is the selection for increased reproductive success and often results in the evolution of behaviours and morphologies that give the bearer (typically males) a reproductive advantage (Andersson & Simmons 2006). Many sexually selected traits are exaggerations of morphological structures that are beneficial in male–male combat or are preferred by females (Emlen 2001). However, the exaggeration of these structures may interfere with the functional abilities of the bearer, altering the biomechanics or kinematics of movement. The alteration of functional relationships may result in a 'cost' of the sexually selected trait

with respect to whole-organism performance (Kotiaho 2001; Oufiero & Garland 2007). Conversely, the kinematics during a movement may be altered in response to the sexually selected trait to compensate for its potential negative effect (Balmford, Jones & Thomas 1994; Husak & Swallow 2011). Adverse effects of exaggerated structures may be more prominent for sexually selected traits that are directly coupled to the propulsive system of the organism. Many studies have examined the effect of sexually selected traits on whole-organism performance (Oufiero & Garland 2007); yet, despite the potential for exaggerated structures to affect functional relationships, few have examined the kinematics of a movement, such as locomotion, in relation to sexually selected traits (Husak *et al.* 2011; McCullough & Tobalske 2013).

The purpose of the present study was to determine the effect of a sexually selected exaggerated morphological trait on the kinematics of locomotion using *Xiphophorus*, a group of live bearing, freshwater fish found in streams and

**Correspondence author. E-mail: coufiero@towson.edu
†Present address. Department of Biological Sciences, Towson University, Towson, MD 21252, USA.*

ponds throughout Mexico and in Central America. Males among several species of *Xiphophorus* possess an elongation along the lower margin of the caudal fin, termed the sword. This structure has been shown to be the result of a pre-existing female bias for larger males and males with longer swords (Basolo 1990, 1996). However, in some species, the preference for longer swords is reduced (Rosenthal, Wagner & Ryan 2002; Wong & Rosenthal 2006; Kang *et al.* 2013). Therefore, expression of the sword varies dramatically among the 26 recognized species of *Xiphophorus*. Unlike some other sexually selected exaggerated morphological traits (e.g. eye stalks of stalk-eyed flies, horns of beetles and enlarged claw of male fiddler crabs), the sword is directly coupled to one of the main propulsive systems, the caudal peduncle and caudal fin. Because of this direct relationship, the potential effect of the sword on drag during locomotion and whole-organism performance abilities has been studied extensively, with mixed results (Ryan 1988; Basolo & Alcaraz 2003; Royle, Metcalfe & Lindstrom 2006; Kruesi & Alcaraz 2007; Oufiero 2010; Baumgartner *et al.* 2011; Oufiero *et al.* 2012b).

Part of the discrepancy in the results for the effect of the sword on locomotor abilities may be attributable to a lack of understanding of the functional relationships between variation in sword length and the kinematics during swimming. For example, during steady swimming, *Xiphophorus* undulate the caudal part of their body and their caudal fin (body-caudal fin locomotion). However, there could be variation in the speed of the undulation (tail beat frequency) or the amount the tail is moved (tail beat amplitude). Longer swords may increase drag and hinder a male's ability to move the caudal region, resulting in a decrease in the amplitude or frequency of tail movement, which might cause a reduction in (impose a cost on) whole-organism performance. Conversely, males with longer swords may alter their kinematics via neuromuscular mechanisms to compensate for the potential drag and increased mass associated with the exaggerated structure. They may therefore compensate for the potential drag by increasing thrust production through an increase in tail beat amplitude and/or frequency compared with males that have shorter swords. An increase in the amplitude and/or frequency associated with long swords should increase the thrust produced (Bainbridge 1958; Webb 1982). However, the increased muscular power required to compensate and maintain tail beat frequency or amplitude would require greater energy expenditure, that is, be costly (Steinhausen, Steffensen & Andersen 2005). These relationships have been examined in gravid female fish; Plaut (2002) found no effect of pregnancy on tail beat kinematics, and suggested a decrease in swimming performance was due to constraints on aerobic performance. However, during pregnancy, females only change in body shape. The kinematics of swimming has not been examined in fish with varying shape caudal fins. It is therefore unknown how variation in fins, which may be due to sexual selection, affects the functional abilities of the bearer. Using a

series of comparative and experimental tests, we examined the effect of variation in sword length on the kinematics of steady swimming in *Xiphophorus*. Our results demonstrate that despite the variation in the sexually selected trait, there is little variation in the kinematics among species and that experimental reduction in sword length allows greater displacement of the tail, but does not affect the frequency of tail beats.

Materials and methods

To determine the effect of variation in sword length on the kinematics of swimming, we measured four tail beat kinematic traits including, and based on, tail beat amplitude and timing of a tail beat among four species that vary naturally in their expression of the sword (Fig. 1); as well as within a single species (*X. alvarezi*) using experimental manipulation of sword length. We used 10–19 males from each of the species (Fig. 1, Table 1): a platyfish without a sword (*X. meyeri*), a swordtail with a medium-length sword (*X. clemenciae*) and two species from separate clades with relatively long swords (from the northern clade, *X. nigrensis*; from the southern clade *X. alvarezi*) (see phylogenetic information in Oufiero *et al.* In revision). We also experimentally manipulated sword length in *X. alvarezi*, similar to earlier studies in other species of swordtails (Basolo & Alcaraz 2003; Kruesi & Alcaraz 2007; Baumgartner *et al.* 2011) to test the effects of sword removal on swimming kinematics. We examined 19 males with full swords, then surgically removed the sword from nine of those males and performed a sham procedure where only 1 mm of sword length was removed from the other 10, similar to previous studies (Basolo & Alcaraz 2003; Kruesi & Alcaraz 2007). Fish were anaesthetized in a non-lethal dose of MS-222 (Tricaine Methanesulfonate), had their sword (or portion of their sword) removed and returned to their housing tank. Polyaqua was added to housing tanks to aid in the healing process, and males were allowed to heal for 1 week after surgical procedures and before swimming trials.
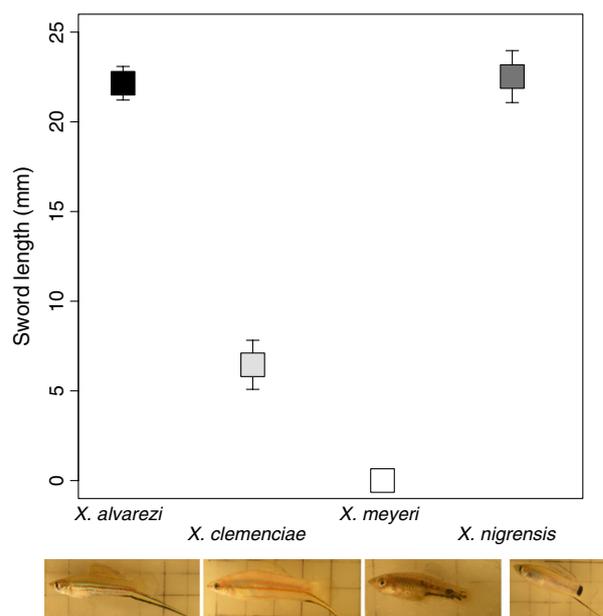

Fig. 1. Mean sword length ± standard errors (mm) for the sample of species used in the study. Representative photos for each species below the species name, images not to scale, grid represents 1 cm.

Table 1. Means and standard errors for standard length (mm) and sword length (mm). Speeds at which each species was swum are also presented. One hundred percentage critical swimming speed (U_{crit}) values were obtained previously (see: Oufiero 2010) and were used to compute 25%, 50% and 75% U_{crit} values, allowing the species to be tested at various speeds corresponding to each species' swimming ability

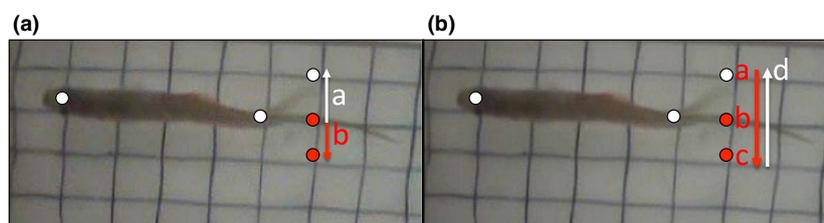| Species	N	Standard length (mm)	Sword length (mm)	25% U_{crit} (cm s^{-1})	50% U_{crit} (cm s^{-1})	75% U_{crit} (cm s^{-1})	100% U_{crit} (cm s^{-1})*
X. alvarezi	19	36·55 ± 0·90	22·15 ± 0·94	6·95	13·90	20·82	27·79 ± 3·42
X. clemenciae	10	31·41 ± 0·75	6·45 ± 1·37	3·78	7·55	11·33	15·10 ± 0·81
X. meyeri	10	23·59 ± 0·37	–	3·62	7·23	10·85	14·46 ± 1·71
X. nigrensis	10	37·21 ± 0·39	22·52 ± 1·45	6·30	12·60	18·90	25·19 ± 1·39

*U_{crit} values are from Oufiero (2010).

Fig. 2. Points digitized for five consecutive tail beats per fish. Points in white represent current frame, points in red represent position of the dorsal and posterior edge of the caudal fin in previous frames. (a) Tail beat amplitude was the average of a1–5, representing maximum lateral extension of the caudal fin on the right side of the fish, and b1–5, representing the maximum lateral extension of the caudal fin on the left side of the fish. (b) Tail beat time was the time to complete one full cycle from points a–d, red arrow + white arrow, averaged over the five tail beats.

Each male from each species and experimental treatment was swum at 25%, 50%, 75% and 100% critical swimming speed (Table 1), which is an estimate of capacity of endurance swimming, and was based on previously obtained values for these species (Oufiero 2010). Prior to measurements, fish were starved for 24 h. Individual fish were then acclimated to a flow tunnel for 15 min at a low flow velocity (3 cm s^{-1}) to orient them to the swim chamber and flow. For details on flow tunnel and acclimation procedures, see Oufiero & Garland (2009) and Oufiero *et al.* (2011). Individual fish were then swum at each speed for several minutes. Using a Sony Handycam HDR-CX110 camera (Sony Corp., New York, NY), fish were recorded dorsally at 120 frames per second. Several seconds of recording were obtained for each fish at each speed when its swimming was stabilized, in view and in focus.

To obtain tail beat kinematics, videos were later digitized using ImageJ (U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/). The head, base of the caudal fin and posterior dorsal tip of the caudal fin were digitized for five consecutive full tail beats taken from portions of the swim trails with minimal movement of the fish. The three points were digitized when the tail was in the centre, and extended maximally laterally, on either side for each of the five full tail beats (Fig. 2). X, Y coordinates for each video and trial were further processed using custom Matlab (Mathworks Inc., Natick, MA, USA) script to obtain the average tail beat amplitude and timing averaged over the five consecutive tail beats. Tail beat amplitude (cm) was the difference in position of the tip of the dorsal edge of the tail between the centre position and the most lateral position on each side and is represented in cm (Fig. 2a). Position of the head was used to correct for any movement of the fish, and left- and right-side tail beat amplitudes were averaged. Tail beat time (ms) was the time it took for one complete tail beat cycle, from maximal lateral position on the right side of the fish, through the centre position, to the maximum lateral position on the left side of the fish, and back to the original starting position on the maximum lateral right side (Fig. 2b). From these two traits, we then calculated tail beat frequency by dividing 1 by tail beat time (converted

to seconds), giving us an estimate of the number of tail beats per second. We also examined a composite trait we refer to as total tail displacement, which was calculated as the tail beat amplitude (cm) multiplied by the tail beat frequency (beats/second). This is an estimate of how much and how fast the tail is moving per second.

These data were then used in repeated-measures statistical models to assess the effect of species, sword length and body size. Specifically, we used the lme function in the nlme package in R. In the comparative experiment, we used species and flow speed as fixed factors, standard length as a covariate, and individual as a random subject factor. We also included the interaction between flow speed and species, as well as the interaction between flow speed and standard length. In analyses of tail beat time and frequency, we also used the residual tail beat amplitude as a covariate to account for the effect the displacement of the tail on how long it takes to move the tail (residual tail beat amplitude was obtained from the repeated-measures model of tail beat amplitude). In the experimental manipulation, we used flow speed and treatment (full sword, sham and no sword) as fixed effects, standard length as a covariate and individual as a random subject factor. Residual tail beat amplitude was also used as a covariate in the model for tail beat time and frequency. In the comparative experiment, we used the Akaike Information Criterion for small sample sizes (AICc) to compare models with interactions among predictors for all four kinematic traits. In the comparative experiment, we also compared models with sword length versus models with species as a factor that subsumed variation in sword length. In all but one trait (tail beat frequency), models with species included as a fixed effect provided better fits to the data (as indicated by lower AICc, see Table 2). For those traits, we used species as our indicator of natural variation in sword length, recognizing that it encompasses any other species differences that may exist (e.g. see Garland & Adolph 1994). We present results from best-fitting models only and provide summaries of the less-supported models in Supplementary Material. All analyses were performed in R (v2.15; R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org).

Table 2. Comparison of models with different interactions included, as well as species versus sword length, for the repeated-measures models of the four species of swordtail for tail beat amplitude, timing, frequency and total tail displacement. Models are listed from best model (lowest AICc) to worst (highest AICc). Tail beat residuals used in tail beat time and frequency models were obtained from the best-fitting tail beat amplitude model, which included no interactions among predictors

Model	$-2 \times$ log likelihood	Parameters	N*	AICc	△ AICc	AICc weight
Tail beat amplitude (cm)						
Speed + Species + SL	−358·15	11	142	−334·12	0·00	0·7511
Speed × SL + Species	−362·03	14	142	−330·73	3·40	0·1374
Speed + Species × SL	−359·81	14	142	−328·50	5·62	0·0451
Speed × Species + SL × Speed	−382·47	23	142	−327·11	7·01	0·0226
Speed + SW × SL	−348·63	10	142	−326·96	7·17	0·0208
Speed × Species + SL	−372·67	20	142	−325·73	8·39	0·0113
Speed + SW + SL	−344·51	9	142	−325·14	8·98	0·0084
Speed × SL + SW	−348·44	12	142	−322·02	12·11	0·0018
Speed × SW + SL × Speed	−354·67	15	142	−320·86	13·26	0·0010
Speed × SW + SL	−345·87	12	142	−319·45	14·67	0·0005
Tail beat time (ms)						
Speed × Species + SL × Speed + TBAr	1205·79	24	142	1264·04	0·00	0·8584
Speed × Species + SL + TBAr	1218·64	21	142	1268·34	4·29	0·1003
Speed × SL + Species + TBAr	1236·80	15	142	1270·61	6·57	0·0322
Speed × SL + SW + TBAr	1245·58	13	142	1274·42	10·38	0·0048
Speed × SW + SL + TBAr	1246·67	13	142	1275·52	11·47	0·0028
Speed × SW + SL × Speed + TBAr	1240·27	16	142	1276·63	12·58	0·0016
Speed + Species + SL + TBAr	1271·01	12	142	1297·42	33·38	0·0000
Speed + SW × SL + TBAr	1275·05	11	142	1299·08	35·04	0·0000
Speed + SW + SL + TBAr	1279·09	10	142	1300·77	36·72	0·0000
Speed + Species × SL + TBAr	1269·99	15	142	1303·80	39·76	0·0000
Tail beat frequency (beats/second)						
Speed × SW + SL + TBAr	532·83	13	142	561·68	0·00	0·5991
Speed × SW + SL × Speed + TBAr	528·55	16	142	564·90	3·22	0·1195
Speed × SL + Species + TBAr	531·53	15	142	565·34	3·66	0·0959
Speed × SL + SW + TBAr	536·69	13	142	565·53	3·86	0·0871
Speed × Species + SL + TBAr	516·36	21	142	566·06	4·38	0·0671
Speed × Species + SL × Speed + TBAr	509·32	24	142	567·58	5·90	0·0313
Speed + Species + SL + TBAr	567·95	12	142	594·37	32·70	0·0000
Speed + SW + SL + TBAr	573·08	10	142	594·76	33·09	0·0000
Speed + Species × SL + TBAr	571·07	11	142	595·10	33·42	0·0000
Speed + SW × SL + TBAr	567·57	15	142	601·38	39·71	0·0000
Total tail displacement (cm × beats/second)						
Speed + Species + SL	253·67	11	142	277·70	0·00	0·7476
Speed × SL + Species	250·01	14	142	281·32	3·62	0·1225
Speed × Species + SL	236·58	20	142	283·52	5·81	0·0408
Speed + Species × SL	252·44	14	142	283·75	6·05	0·0364
Speed × Species + SL × Speed	228·42	23	142	283·77	6·07	0·0360
Speed + SW × SL	265·22	10	142	286·90	9·20	0·0075
Speed + SW + SL	267·98	9	142	287·34	9·64	0·0060
Speed × SW + SL × Speed	256·10	15	142	289·91	12·20	0·0017
Speed × SL + SW	264·64	12	142	291·06	13·35	0·0009
Speed × SW + SL	265·62	12	142	292·04	14·34	0·0006

SL, standard length; SW, sword length; TBAr, tail beat amplitude residuals.
*Sample sizes obtained by the number of individuals × the number of trials (39 × 4 = 156 – missing values for some individuals that had a negative tail beat amplitude due to the body of the fish moving more than the tail), see Oufiero *et al.* 2012b for more information on sample sizes in repeated-measures models.

Results

The best model for tail beat amplitude for the four species combined did not include any interactions (Table 2). Among the four species, there was a significant effect of flow speed ($F_{3,100} = 44·86$, $P < 0·0001$), with an increase in tail beat amplitude with increasing speed for all species (Fig. 3a, Table 1). There was also a significant effect of species ($F_{3,34} = 8·72$, $P = 0·0002$) on tail beat amplitude,

but no effect of standard length ($F_{1,34} = 0·67$, $P = 0·42$). Tukey's *post hoc* comparisons revealed that the effect of species was caused by *X. clemenciae* having a lower tail beat amplitude than all other species (Fig. 3a, Table 3).

The best-fitting model for tail beat time included an interaction between flow speed and species ($F_{9,87} = 3·43$, $P = 0·012$), as well as between standard length and flow speed ($F_{3,87} = 3·97$, $P = 0·0105$, Table 2, Table S2, Supporting informatoin). In this model, there were significant
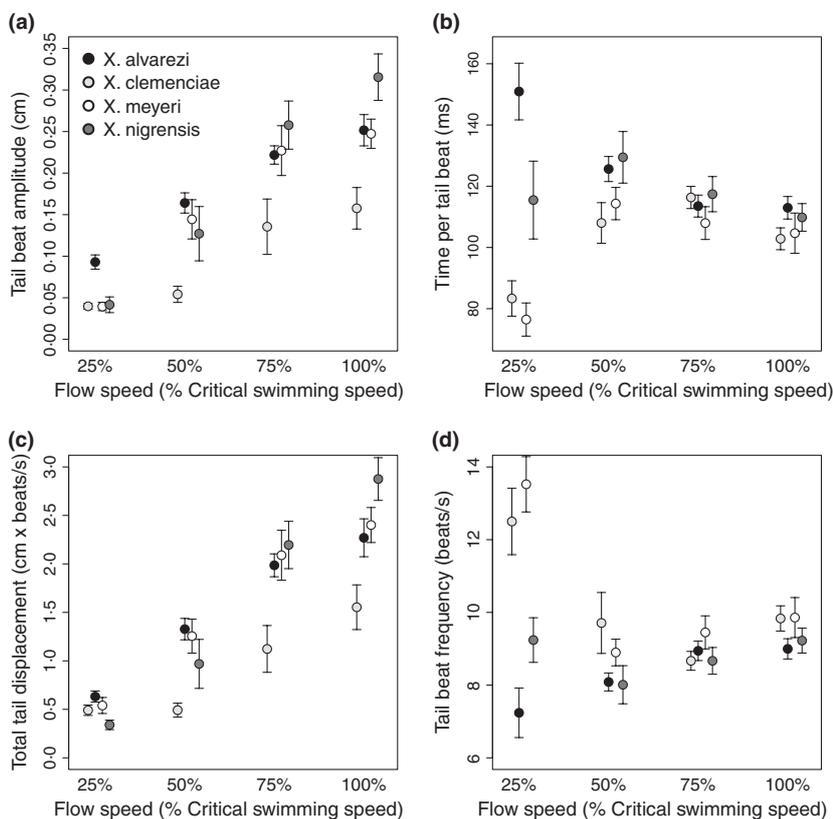
Fig. 3. Mean tail beat amplitude (a), tail beat time (b), total tail displacement (c) and tail beat frequency (d) among the four species across flow speeds. Points represent means for each species at each flow speed ± standard error.

Table 3. *Post hoc* comparison of tail beat amplitude and total tail displacement among the four species. *Xiphophorus clemenciae* differed significantly from all other species, having a lower tail beat amplitude and total tail displacement. Statistically significant ($P < 0.05$) comparisons are in bold

Comparison	Estimate	SE	Z-value	P-value
Post hoc tail beat amplitude species comparisons				
X. clemenciae–*X. alvarezi*	**−0·077**	**0·026**	**−2·997**	**0·012**
X. meyeri–*X. alvarezi*	0·015	0·044	0·334	0·985
X. nigrensis–*X. alvarezi*	0·004	0·021	0·208	0·996
X. meyeri–*X. clemenciae*	**0·092**	**0·031**	**2·950**	**0·014**
X. nigrensis–*X. clemenciae*	**0·081**	**0·027**	**3·016**	**0·012**
X. nigrensis–*X. meyeri*	−0·011	0·046	−0·227	0·995
Post hoc total tail displacement species comparisons				
X. clemenciae–*X. alvarezi*	**−0·677**	**0·217**	**−3·119**	**0·008**
X. meyeri–*X. alvarezi*	0·0114	0·375	0·030	0·999
X. nigrensis–*X. alvarezi*	0·080	0·175	0·457	0·962
X. meyeri–*X. clemenciae*	**0·688**	**0·263**	**2·618**	**0·037**
X. nigrensis–*X. clemenciae*	**0·757**	**0·228**	**3·322**	**0·004**
X. nigrensis–*X. meyeri*	0·068	0·390	0·175	0·998

effects of speed ($F_{3,87} = 2.78$, $P = 0.045$), standard length ($F_{1,34} = 19.21$, $P = 0.0001$), residual tail beat amplitude ($F_{1,87} = 10.01$, $P = 0.0021$) and species ($F_{3,87} = 10.34$, $P = 0.0001$) on tail beat frequency (Fig. 3b).

To further disentangle the effects of speed and determine how the species differed in tail beat time, we used an ANCOVA within each speed and Tukey's *post hoc* comparisons. At a flow speed of 25% critical swimming speed, *X. clemenciae* tail beat frequency differed significantly from *X. alvarezi*

($t = 3.325$, $P = 0.013$) and *X. meyeri* ($t = 4.009$, $P = 0.003$). There was a significant effect of species ($F_{3,24} = 18.59$, $P < 0.0001$), standard length ($F_{1,24} = 13.07$, $P = 0.0014$) and residual tail beat amplitude ($F_{1,24} = 11.93$, $P = 0.0021$) at this speed. At 50% critical swimming speed, there was no statistical difference among species, but there was a significant effect of residual tail beat amplitude ($F_{1,31} = 5.65$, $P = 0.024$). At 75% critical swimming speed, there was again no significant difference among species and a significant size effect ($F_{1,31} = 9.84$, $P = 0.004$). Finally, at 100% critical swimming speed, there was again no significant difference among species and no other significant effects on tail beat time. Therefore, the main difference in tail beat frequency is at lower speeds (Fig. 3b), whereas at higher speeds, the species all swim with similar tail beat times.

The best-fitting model for tail beat frequency contained sword length instead of species, as well as an interaction between sword length and speed (Table 2, Table S3, Supporting information). In this model, there was significant effect of speed ($F_{3,96} = 22.56$, $P < 0.0001$, Fig. 3d), sword length ($F_{1,96} = 24.66$, $P < 0.0001$), tail beat residuals ($F_{1,96} = 6.79$, $P = 0.0106$) and the speed × sword length interaction ($F_{3,24} = 15.12$, $P < 0.0001$). Sword length had a negative effect on tail beat frequency (coefficient = −0.219); therefore, individuals with longer swords had fewer tail beats per second. When we examined the effect of sword length at each speed, sword length was no longer significant (all $P > 0.05$).

The best-fitting model for total tail displacement was the same as for tail beat amplitude, with species instead of
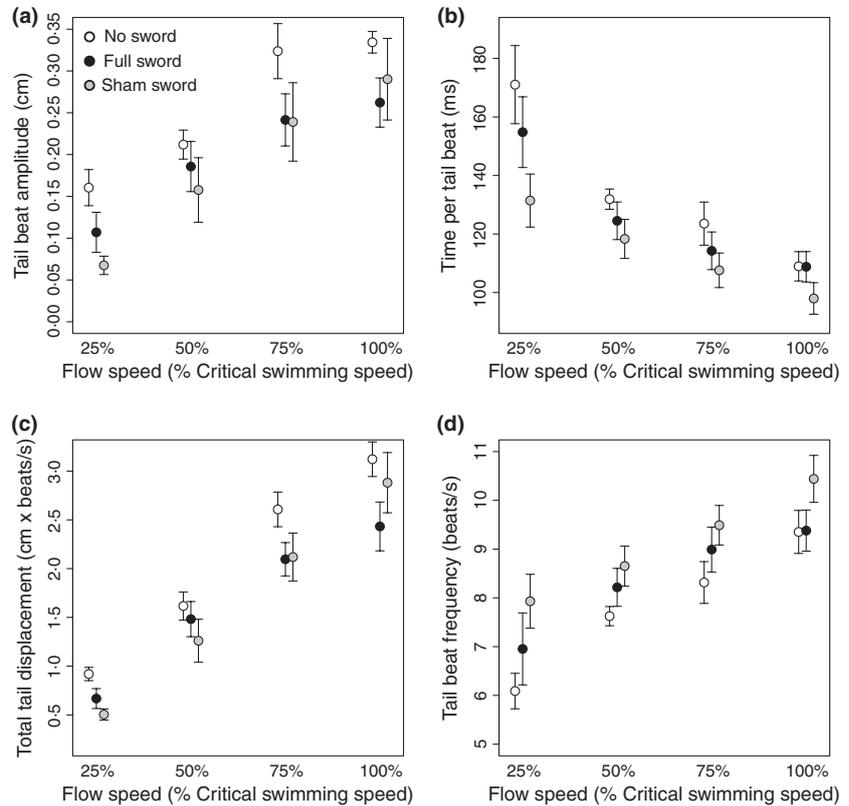
Fig. 4. Mean tail beat amplitude (a), tail beat time (b), total tail displacement (c) and tail beat frequency (d) among experimental treatment of sword length within *X. alvarezi* males across flow speeds. Points represent means for each experimental group at each flow speed ± standard error.

sword length and no interaction among predictors (Table 2, Table S4, Supporting information). There was a significant effect of flow speed ($F_{3,100} = 54.17$, $P < 0.0001$), with greater total tail displacement at higher speeds (Fig. 3c). The effect of species was also significant ($F_{3,34} = 8.57$, $P = 0.0002$), but similar to tail beat amplitude, *post hoc* analysis revealed that this was due to *X. clemenciae* exhibiting lesser total tail displacement than all other species (all *P*-values < 0.05, Table 3).

EXPERIMENTAL MANIPULATIONS

Using *X. alvarezi*, we examined the effect of experimentally reducing sword length on tail beat kinematics (Fig. 4). There was no significant interaction between flow speed and experimental treatment for tail beat amplitude; therefore, we examined a model without any interactions. Flow speed ($F_{3,110} = 89.63$, $P < 0.0001$), experimental treatment ($F_{2,110} = 11.49$, $P < 0.0001$) and standard length ($F_{1,110} = 8.12$, $P = 0.005$) all had significant effects on tail beat amplitude. Tukey's *post hoc* comparisons revealed a significant difference between full swords and removed swords ($P < 0.0001$) and between the sham group and removed sword group ($P = 0.0001$), but no difference between the sham and intact-sword groups ($P = 0.6898$).

There was no significant interaction between speed and treatment for tail beat time, so it was not included in final analyses. Tail beat time (Fig. 4b) was affected by standard length ($F_{1,109} = 73.08$, $P < 0.0001$), flow speed ($F_{3,109} = 46.58$, $P < 0.0001$), experimental treatment ($F_{2,109} = 4.74$, $P = 0.011$) and residual tail beat amplitude ($F_{1,109} = 15.14$, $P = 0.0002$) (Fig. 4b). *Post hoc* analysis revealed that the significant effect was attributable to a significant difference between the sham and sword-removed group ($P = 0.007$), with no significant difference between the full-sword and removed-sword group ($P = 0.386$) or between the sham and full-sword group ($P = 0.0543$).

There was no interaction for tail beat frequency, so it was not included in the final model. Similar to tail beat time, there was a significant effect of standard length ($F_{1,109} = 41.04$, $P < 0.0001$), flow speed ($F_{3,109} = 31.39$, $P < 0.0001$), residual tail beat amplitude ($F_{1,109} = 10.08$, $P = 0.0019$) and experimental treatment on tail beat frequency ($F_{2,109} = 4.95$, $P = 0.0088$). Tukey's *post hoc* analysis revealed a significant difference between the sham and sword-removed group ($P = 0.006$), but no difference between the full-sword and removed-sword ($P = 0.325$) or full-sword and sham ($P = 0.056$, Fig. 4d).

Like the three other kinematic traits, there was no significant interaction between flow speed and treatment for total tail displacement, so it was not included in final analyses. There was no effect of standard length on total tail displacement ($F_{1,110} = 1.93$, $P = 0.1679$), but there was a significant effect of flow speed ($F_{3,110} = 123.28$, $P < 0.0001$) and experimental manipulation ($F_{2,110} = 5.86$, $P = 0.0038$). Tukey's *post hoc* analysis revealed a significant difference between the full-sword and sword-removed group ($P = 0.002$) as well as the sham and sword-removed group ($P = 0.048$), but no difference between the full-sword and sham ($P = 0.937$).

Discussion

Our results demonstrate that the exaggeration of a morphological structure, which is used for propulsion, through sexual selection does not necessarily affect the kinematics of locomotion across species that vary naturally in their expression of the sexually selected trait. In particular, we found that species with longer swords had similar tail beat amplitude and total tail displacement at all speeds, compared to the species with no sword (*X. meyeri*). Furthermore, the only statistically significant difference in time per tail beat and tail beat frequency was at lower speeds. This suggests that species with longer swords are not incurring a cost to swimming and are also not compensating for the structure through an alteration of kinematics (Fig. 3). The sexually selected sword was one of the first examples of sexual selection proposed by Darwin (1871), and has been studied extensively, including the effects of sword length on whole-organism swimming performance (Royle, Metcalfe & Lindstrom 2006; Baumgartner *et al.* 2011; Oufiero *et al.* 2012b). However, the results regarding swimming performances have been mixed. Experimental manipulations of sword length have shown an increase in steady-swimming ability (Kruesi & Alcaraz 2007) and a decrease in routine oxygen consumption (Basolo & Alcaraz 2003), whereas studies of natural variation have found no differences in swimming endurance among males within a species with varying length swords (Ryan 1988; Oufiero *et al.* 2012b). Our results provide a possible mechanistic explanation for these discrepancies. Among species with naturally varying sword lengths, species with longer swords did not exhibit differences in kinematics (i.e. decreased tail beat amplitude or frequency to suggest a cost or increased tail beat amplitude or frequency to suggest compensation). However, when the sword was experimentally removed, we found an increase in the tail beat amplitude and total tail displacement and no significant effect on tail beat time or frequency.

An increase in tail beat amplitude and frequency should increase the amount of thrust produced (Bainbridge 1958; Webb 1982; Plaut 2002), and therefore, the ability for steady swimming. Similar to previous studies, we found an increase in tail beat amplitude as speed increased, but an inconsistent pattern for tail beat time and frequency. An increase in tail beat frequency has been shown to increase oxygen consumption (Steinhausen, Steffensen & Andersen 2005). At low speeds, the two species with the longest swords tended to have increased time per tail beat and thus decreased tail beat frequencies (Fig. 3b). Although *post hoc* analyses revealed a significant difference only between *X. alvarezi* and *X. clemenciae* for tail beat time, these results suggest less consumption of oxygen at more routine swimming speeds, which is inconsistent to some previous results (Basolo & Alcaraz 2003). Furthermore, the only difference in tail beat amplitude and total tail displacement we observed was in *X. clemenciae*, the species with a medium sword length (Figs. 1, 3), and which is of hybrid origin

(Meyer, Salzburger & Schartl 2006). More studies are warranted to tease apart these relationships. Nevertheless, if species are naturally varying in their thrust production due to changes in tail beat amplitude and frequency, irrespective of sword length then they will vary in their swimming abilities. These difference could be caused by any number of factors that contribute to swimming performance, including variation in such suborganismal traits as heart size, ventilation rate, muscle fibre type or overall morphological shape (Garland 1984; Careau & Garland 2012; Oufiero *et al*. In revision). At an ultimate level, species differences could be related to variation in the past selective regime caused by ecological variation, such as differences in flow velocity among native streams (Nelson, Gotwalt & Snodgrass 2003). Therefore, differences in tail kinematics among species may reflect differences in underlying traits and selective regimes, while the evolution and development of the sword may have little impact on a male's ability to swim (Oufiero & Garland 2007; Oufiero *et al.* 2012b).

In contrast to our comparative analysis, complete removal of the sword in *X. alvarezi* resulted in an increased tail beat amplitude and total tail displacement and no significant difference in tail beat time or frequency, suggesting that the males are able to generate more thrust when the sword is removed (Fig. 4). This finding is consistent with results of Kruesi & Alcaraz (2007), who demonstrated an increase in critical swimming speed (i.e. an increase in endurance performance) when the sword was completely removed in *X. montezumae*. Because males develop the sword for several weeks (Marcus & McCune 1999; Oufiero *et al.* 2012b), presumably they are training with the potential-added burden, similar to resistance training (Bird, Tarpenning & Marino 2005). However, once the trait is removed, the males are able to perform better, presumably because of the physiological adaptations that have occurred to swim with the elongated structure. Our results demonstrate that one of the mechanisms that enables them to perform better is an increase in how much the tail moves to generate more thrust, without an alteration in how fast the tail moves; they are therefore producing more thrust for a given tail beat, potentially without increasing their energetic demands by an appreciable amount. Results also suggest that the sword does not affect contractile velocity of muscle fibres. Future studies should examine training effects on muscle composition and type to determine whether there are physiological adaptions of muscle physiology that may help to explain how the development of the sword affects whole-organism performance and the kinematics of locomotion.

Our results are among the first to examine the mechanistic effects of a sexually selected structure that is also used for locomotion. However, some evidence for the effect of sexually selected structures on kinematics and biomechanics is available in other organisms. Recent work in stalk-eyed flies has demonstrated a reduction in size-corrected wing beat frequency of males compared to females within a species, as well as a reduction in size-corrected wing beat

frequency among males in species with longer eye spans. These results suggest that the evolution of longer wings for compensation of the longer eye stalks constrains the kinematics of flight (Husak *et al.* 2011). In the rhinoceros beetle *Trypoxylus dichotomus*, recent studies have employed biomechanical techniques to visualize the air-flow patterns around the sexually selected horns (McCullough & Tobalske 2013). Using digital particle image velocimtery, the authors found that based on the minimal added weight of the horns, as well as the flight angle of the males, the horns presented little impact on the drag during flying. Even though in both of these studies, the sexually selected structure is not linked to the propulsive system, unlike in *Xiphophorus*, they highlight the importance of incorporating kinematic and biomechanic techniques to understand how the evolution of sexually selected traits affects the diversification of functional abilities. The sexually selected, enlarged claw of fiddler crabs, which is used in male–male competition and displays instead of feeding, have also provided excellent examples of incorporating biomechanical and kinematic studies to understand the functional consequences of exaggerated structures (Valiela *et al.* 1974; Caravello & Cameron 1987; Levinton, Judge & Kurdziel 1995; McLain & Pratt 2008; Darnell & Munguia 2011; Dennenmoser & Christy 2013).

Among the more than 27 000 species of acanthomorph fish, fin shape is tremendously diverse, with some of this diversity resulting from sexual selection (Nicoletto 1991; Basolo 1995; Langerhans, Layman & DeWitt 2005; Wilson *et al.* 2010; Trappett *et al.* 2013). Despite this diversity, little comparative work has examined the effects of fin shape on whole-organism performance. An increasing number of studies are examining the effect of morphological diversity that results from sexual selection on whole-organism performance in fish, often with mixed results, both within and among taxa (Ryan 1988; Nicoletto 1991; Basolo 1995; Basolo & Alcaraz 2003; Langerhans, Layman & DeWitt 2005; Royle, Metcalfe & Lindstrom 2006; Kruesi & Alcaraz 2007; Wilson *et al.* 2010; Baumgartner *et al.* 2011; Oufiero *et al.* 2012b; Trappett *et al.* 2013). However, less work has examined the biomechanical and kinematic variation associated with the morphological diversity in fins among fish (Lauder 1990, 2000; Lauder & Drucker 2004). Our results demonstrate – at a very fine taxonomic scale – little variation in the kinematics of swimming despite large difference in caudal fin shape. How diversity in other fin shapes affects swimming performance has yet to be tested vigorously, but may lead to interesting discoveries of form-function relationships.

The study of sexually selected traits has a long history; their seemingly un-natural appearance has led to many studies investigating the presumed negative impact these structures have on the bearer (Kotiaho 2001; Oufiero & Garland 2007). However, few studies have investigated the mechanistic functional consequences of these traits to understand how their evolution and development affect the overall evolution and development of the bearer. For example, recent work on the effects of sexual dimorphism on the feeding kinematics of threespine stickleback (*Gasterosteus aculeatus*) has suggested that this sexual dimorphism within a single species may have played a role in the radiation of this group (McGee & Wainwright 2013). Examining the kinematics and biomechanics of exaggerated traits that are evolving under the competing demands of natural and sexual selection may provide further insight into form-function relationships (Oufiero *et al.* 2012a), multiple solutions (Wainwright *et al.* 2005; Garland *et al.* 2011; Holzman *et al.* 2011), ecological impacts (McGee & Wainwright 2013) and evolutionary relationships (Kazancioglu *et al.* 2009; Bondurinasky 2011) of traits that evolve to increase reproductive success while maintaining adequate function.

Acknowledgements

References

Andersson, M. & Simmons, L.W. (2006) Sexual selection and mate choice. *Trends in Ecology & Evolution*, **21**, 296–302.

Bainbridge, R. (1958) The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat. *Journal of Experimental Biology*, **35**, 109.

Balmford, A., Jones, I.L. & Thomas, A.L.R. (1994) How to compensate for costly sexually selected tails: the origin of sexually dimorphic wings in long-tailed birds. *Evolution*, **48**, 1062–1070.

Basolo, A.L. (1990) Female preference predates the evolution of the sword in swordtail fish. *Science*, **250**, 808–810.

Basolo, A.L. (1995) Phylogenetic evidence for the role of a preexisting bias in sexual selection. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **259**, 307–311.

Basolo, A.L. (1996) The phylogenetic distribution of a female preference. *Systematic Biology*, **45**, 290–307.

Basolo, A.L. & Alcaraz, G. (2003) The turn of the sword: length increases male swimming costs in swordtails. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 1631–1636.

Baumgartner, A., Coleman, S., Swanson, B. & Laudet, V. (2011) The cost of the sword: escape performance in male swordtails. *PLoS ONE*, **6**, 205–214.

Bird, S.P., Tarpenning, K.M. & Marino, F.E. (2005) Designing resistance training programmes to enhance muscular fitness: a review of the acute programme variables. *Sports medicine*, **35**, 841–851.

Bondurinasky, R. (2011) Sexual selection and conflict as engines of ecological diversification. *The American Naturalist*, **178**, 729–745.

Caravello, H.E. & Cameron, G.N. (1987) The effects of sexual selection on the foraging behaviour of the Gulf Coast fiddler crab, *Uca panacea*. *Animal Behaviour*, **35**, 1864–1874.

Careau, V.C. & Garland, T. Jr (2012) Performance, personality, and energetics: correlation, causation, and mechanism. *Physiological and Biochemical Zoology*, **85**, 543–571.

Darnell, M.Z. & Munguia, P. (2011) Thermoregulation as an alternate function of the sexually dimorphic fiddler crab claw. *The American naturalist*, **178**, 419–428.

Darwin, C. (1871) *The Decent of Man and Selection in Relation to Sex.* John Murray, London.

Dennenmoser, S. & Christy, J.H. (2013) The design of a beautiful weapon: compensation for opposing sexual selection on a trait with two functions. *Evolution*, **67**, 1181–1188.

Emlen, D.J. (2001) Costs and the diversification of exaggerated animal structures. *Science*, **291**, 1534–1536.

Garland, T. (1984) Physiological correlates of locomotory performance in a lizard: an allometric approach. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology*, **247**, 806–815.

Garland, T. Jr & Adolph, S.C. (1994) Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiological Zoology*, **67**, 797–828.

Garland, T., Kelly, S.A., Malisch, J.L., Kolb, E.M., Hannon, R.M., Keeney, B.K. *et al.* (2011) How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 574–581.

Holzman, R.A., Collar, D.C., Mehta, R.S. & Wainwright, P.C. (2011) Functional complexity can mitigate performance trade-offs. *American Naturalist*, **177**, E69–E83.

Husak, J.F. & Swallow, J.G. (2011) Compensatory traits and the evolution of male ornaments. *Behavior*, **148**, 1–29.

Husak, J.F., Ribak, G.A.L., Wilkinson, G.S. & Swallow, J.G. (2011) Sexual dimorphism in wing beat frequency in relation to eye span in stalk-eyed flies (Diopsidae). *Biological Journal of the Linnean Society*, **104**, 670–679.

Kang, J.H., Schartl, M., Walter, R.B. & Meyer, A. (2013) Comprehensive phylogenetic analysis of all species of swordtails and platies (Pisces: Genus *Xiphophorus*) uncovers a hybrid origin of a swordtail fish, *Xiphophorus monticolus*, and demonstrates that the sexually selected sword originated in the ancestral li. *BMC Evolutionary Biology*, **13**, 25.

Kazancioglu, E., Near, T.J., Hanel, R. & Wainwright, P.C. (2009) Influence of sexual selection and feeding functional morphology on diversification rate of parrotfishes (*Scaridae*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **276**, 3439–3446.

Kotiaho, J.S. (2001) Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biological Reviews*, **76**, 365–376.

Kruesi, K. & Alcaraz, G. (2007) Does a sexually selected trait represent a burden in locomotion? *Journal of Fish Biology*, **70**, 1161–1170.

Langerhans, R.B., Layman, C.A. & DeWitt, T.J. (2005) Male genital size reflects a tradeoff between attracting mates and avoiding predators in two live-bearing fish species. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 7618–7623.

Lauder, G.V. (1990) Functional morphology and systematics: studying functional patterns in an historical context. *Annual Review of Ecology and Systematics*, **21**, 317–340.

Lauder, G.V. (2000) Function of the caudal fin during locomotion in fishes: kinematics, flow visualization, and evolutionary patterns. *Integrative and Comparative Biology*, **40**, 101–122.

Lauder, G.V. & Drucker, E.G. (2004) Morphology and experimental hydrodynamics of fish fin control surfaces. *Ieee Journal of Oceanic Engineering*, **29**, 556–571.

Levinton, J.S., Judge, M.L. & Kurdziel, J.P. (1995) Functional differences between the major and minor claws of fiddler crabs (*Uca*, family Ocypodidae, Order Decapoda, Subphylum Crustacea): a result of selection or developmental constraint? *Journal of Experimental Marine Biology and Ecology*, **193**, 147–160.

Marcus, J.M. & McCune, A.R. (1999) Ontogeny and phylogeny in the northern swordtail clade of Xiphophorus. *Systematic Biology*, **48**, 491–522.

McCullough, E.L. & Tobalske, B.W. (2013) Aerodynamic costs Elaborate horns in a giant rhinoceros beetle incur negligible aerodynamic costs. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 1–5.

McGee, M.D. & Wainwright, P.C. (2013) Sexual dimorphism in the feeding mechanism of threespine stickleback. *The Journal of Experimental Biology*, **216**, 835–840.

McLain, D.K. & Pratt, A.E. (2008) Asymmetry of leg size and differential leg usage in the sand fiddler crab, *Uca pugilator*. *Journal of Crustacean Biology*, **28**, 601–606.

Meyer, A., Salzburger, W. & Schartl, M. (2006) Hybrid origin of a swordtail species (Teleostei: Xiphophorus clemenciae) driven by sexual selection. *Molecular Ecology*, **15**, 721–730.

Nelson, J.A., Gotwalt, P.S. & Snodgrass, J.W. (2003) Swimming performance of blacknose dace (*Rhinichthys atratulus*) mirrors home-stream current velocity. *Canadian Journal of Fisheries and Aquatic Sciences*, **60**, 301–308.

Nicoletto, P.F. (1991) The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology*, **28**, 365–370.

Oufiero, C.E. (2010) *The Cost of Bearing a Sword: Locomotor Costs and Compensations in Relation to a Sexually Selected Trait in Xiphophorus.* University of California, Riverside.

Oufiero, C.E. & Garland, T. (2007) Evaluating performance costs of sexually selected traits. *Functional Ecology*, **21**, 676–689.

Oufiero, C.E. & Garland, T. (2009) Repeatability and correlation of swimming performances and size over varying time scales in the guppy (*Poecilia reticulata*). *Functional Ecology*, **23**, 969–978.

Oufiero, C.E., Walsh, M.R., Reznick, D.N. & Garland, T. Jr (2011) Swimming performance trade-offs across a gradient in community composition in Trinidadian killifish (*Rivulus hartii*). *Ecology*, **92**, 170–179.

Oufiero, C.E., Holzman, R.A., Young, F.A. & Wainwright, P.C. (2012a) New insights from serranid fishes on the role of trade-offs in suction-feeding diversification. *The Journal of Experimental Biology*, **215**, 3845–3855.

Oufiero, C.E., Jugo, K.N., Tran, P. & Garland, T. Jr (2012b) As the sword grows: ontogenetic effects of a sexually selected trait on locomotor performance in *Xiphophorus hellerii*. *Physiological and Biochemical Zoology*, **85**, 683–694.

Oufiero, C.E., Meredith, R.W., Jugo, K., Tran, P., Chappell, M.A., Springer, M.S. *et al.* (In revision) The locomotor benefits of bearing a sword: an increase in the sexually selected sword increases aerobic locomotor performance among *Xiphophorus*.

Plaut, I. (2002) Does pregnancy affect swimming performance of female Mosquitofish, *Gambusia affinis*? *Functional Ecology*, **16**, 290–295.

Rosenthal, G.G., Wagner, W.E. & Ryan, M.J. (2002) Secondary reduction of preference for the sword ornament in the pygmy swordtail *Xiphophorus nigrensis* (Pisces: Poeciliidae). *Animal Behaviour*, **63**, 37–46.

Royle, N.J., Metcalfe, N.B. & Lindstrom, J. (2006) Sexual selection, growth compensation and fast-start swimming performance in Green Swordtails, *Xiphophorus helleri*. *Functional Ecology*, **20**, 662–669.

Ryan, M.J. (1988) Phenotype, genotype, swimming endurance and sexual selection in a swordtail (*Xiphophorus nigrensis*). *Copeia*, **1988**, 484–487.

Steinhausen, M.F., Steffensen, J.F. & Andersen, N.G. (2005) Tail beat frequency as a predictor of swimming speed and oxygen consumption of saithe (*Pollachius virens*) and whiting (*Merlangius merlangus*) during forced swimming. *Marine Biology*, **148**, 197–204.

Trappett, A., Condon, C.H., White, C., Matthews, P. & Wilson, R.S. (2013) Extravagant ornaments of male threadfin rainbowfish (*Iriatherina werneri*) are not costly for swimming. *Functional Ecology*, **27**, 1034–1041.

Valiela, I., Babiec, D.F., Atherton, W., Seitzinger, S. & Krebs, C. (1974) Some consequences of sexual dimorphism: feeding in male and female fiddler crabs, *Uca pugnax* (Smith). *Biological Bulletin*, **147**, 652–660.

Wainwright, P.C., Alfaro, M.E., Bolnick, D.I. & Hulsey, C.D. (2005) Many-to-one mapping of form to function: a general principle in organismal design? *Integrative and Comparative Biology*, **45**, 256.

Webb, P.W. (1982) Locomotor patterns in the evolution of Actinopterygian fishes. *American Zoologist*, **22**, 329–342.

Wilson, R.S., Condon, C.H., David, G., FitzGibbon, S., Niehaus, A.C. & Pratt, K. (2010) Females prefer athletes, males fear the disadvantaged: different signals used in female choice and male competition have varied consequences. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **277**, 1923–1928.

Wong, B.B.M. & Rosenthal, G.G. (2006) Female disdain for swords in a swordtail fish. *American Naturalist*, **167**, 136–140.

Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. Main effects for all models tested for tail beat amplitude among the four species.

Table S2. Main effects for all models tested for tail beat time among the four species.

Table S3. Main effects for all models tested for tail beat frequency among the four species.

Table S4. Main effects for all models tested for total tail beat displacement among the four species.