



Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish (*Gambusia holbrooki*)

Francesc Rubio-Gracia¹  | Emili García-Berthou¹  | Dani Latorre¹ |
Ramon Moreno-Amich¹ | Pao Srean^{1,2} | Yiping Luo^{1,3} | Anna Vila-Gispert¹

¹GRECO, Institute of Aquatic Ecology, University of Girona, Girona, Spain

²Faculty of Agriculture and Food Processing, University of Battambang, Battambang, Cambodia

³Key Laboratory of Freshwater Fish Reproduction and Development, Ministry of Education, School of Life Science, Southwest University, Chongqing, China

Correspondence

Francesc Rubio-Gracia, GRECO, Institute of Aquatic Ecology, University of Girona, Girona, Spain.
Email: francescmanel.rubio@udg.edu

Funding information

Spanish Ministry of Science, Innovation and Universities, Grant/Award Number: CGL2013-43822-R and CGL2016-80820-R; University of Girona, Grant/Award Number: IFUdG17; Generalitat de Catalunya, Grant/Award Number: 2017 SGR 548

Abstract

Swimming performance is a key feature that mediates fitness and survival in many fish species. Using a swim tunnel respirometer, we compared prolonged swimming performance and energy use for two competing species: an endangered, endemic toothcarp (*Aphanius iberus*) and a worldwide invasive mosquitofish (*Gambusia holbrooki*). Critical (U_{crit}) and optimal swimming speeds, standard and maximal metabolic rates, absolute aerobic scope, as well as the minimum cost of transport were estimated and compared between species and sexes. Body streamlining and caudal peduncle depth were also measured to explain the differences in swimming performance and efficiency. Both sexes of *A. iberus* presented similar swimming capacity and metabolic traits, whereas males of *G. holbrooki* showed higher critical swimming speeds, maximal metabolic rate and absolute aerobic scope than females. We also found marked differences between species in most of the response variables examined. *Aphanius iberus* showed lower swimming capacity (U_{crit} mean $<10\text{ cm s}^{-1}$), higher maximal metabolic rate and absolute aerobic scope than the invasive species. By contrast, *G. holbrooki* swam faster and had lower cost of transport at a given fish mass and speed, thereby leading to a higher swimming efficiency. The observed differences in swimming efficiency were closely related to differences in morphological characteristics and therefore to drag pressures and propulsion. Our results add a mechanistic basis to the ecological understanding of these two species and suggest that although both are poor swimmers compared to many other similarly sized species, the native species likely has more restricted water flow tolerance and dispersal capacities.

KEYWORDS

cost of transport, fish, metabolic rates, morphology, Spanish toothcarp, swimming efficiency

1 | INTRODUCTION

Swimming performance has long been seen as ecologically relevant for the determination of fitness and survival of species in aquatic ecosystems (Plaut, 2001). Predator-prey interactions (e.g. food capture, competition with other predators and escapement from

predators), reproduction and migration are processes that depend on the swimming ability of the individual (Fisher, Bellwood, & Job, 2000; Reidy, Kerr, & Nelson, 2000). Similarly, energetic costs of swimming are also directly linked to several ecological processes (Videler, 1993; Webb, 1994), and consequently, the understanding of the energy budget of individuals has become increasingly important

for developing bioenergetics models in ecological fish studies (e.g. Ohlberger, Staaks, Van Dijk, & Hölker, 2005).

In nature, steady swimming is commonly used by fish during foraging, reproduction and seeking favourable environmental conditions (Blake, 2004; Plaut, 2001). When swimming faster, fish can reach critical swimming speed (U_{crit}), which is defined as the highest swimming speed that a fish can maintain for a time period (Brett, 1964). In general, U_{crit} is a standard measurement for evaluating the swimming capabilities of fish (Plaut, 2001), and it involves aerobic and anaerobic metabolism (Hammer, 1995; Videler, 1993). Oxygen consumption measurements over time are usually used to estimate metabolic rates (Bell & Terhune, 1970). Two important metabolic traits can be estimated using the U_{crit} protocol, from which the respiratory capacity of a fish is generally described. Maximal metabolic rate (MMR) is traditionally defined as the maximum oxygen consumption rate during swimming exercise (Norin & Clark, 2016), whereas standard metabolic rate (SMR) is the baseline energy required for maintenance measured in postabsorptive, resting state (Chabot, Steffensen, & Farrell, 2016). Variation in SMR and MMR across species can be due to different growth strategies and contrasting lifestyles (Killen, Atkinson, & Glazier, 2010; Stoffels, 2015).

Based on fish locomotor theory, energetic costs of swimming are positively correlated to drag forces, which are determined by size or frontal area of a fish and swimming speed (Webb, 1975). Increased energetic costs are generally associated with stabilising body posture and maintaining direction at lower speeds, and when fish face perturbing forces while swimming at higher speeds. However, there is a range of intermediate speeds at which fish can optimally swim. Thus, physiologists define optimal swimming speed (U_{opt}) as the speed at which the energetic cost of transport (COT) is the lowest (Tucker, 1970). Cost of transport and optimal swimming speed are commonly used to compare energetic costs between species (Ohlberger, Staaks, & Hölker, 2006; Tudorache, Viaene, Blust, Vereecken, & De Boeck, 2008; Videler, 1993).

Body morphology is an important factor to be considered when determining swimming performance of fish, because performance depends on drag forces and propulsion (Webb, 1984). Previous studies have explored the relationship between body morphology and swimming capacity among a wide range of fish species (Fisher & Hogan, 2007; Leavy & Bonner, 2009; Walker, Alfaro, Noble, & Fulton, 2013). Morphological traits that maximise steady swimming are an increased depth of the anterior body and head, a shallow caudal region and a streamlined body shape (Fisher & Hogan, 2007; Walker et al., 2013). In particular, a streamlined or fusiform body shape can also influence energetic costs of swimming due to a decrease in drag pressures along the body and a reduction in recoil energy losses (Langerhans, 2009; Langerhans & Reznick, 2010; Scarnecchia, 1988; Webb, 1975). Therefore, it is generally assumed that fish with different morphological traits would display variation in swimming performance and energy metabolism (Pettersson & Bronmark, 1997).

The present study compares the swimming capacity and metabolic rates of the Spanish toothcarp, *Aphanius iberus* (Valenciennes,

1846), and the eastern mosquitofish *Gambusia holbrooki* Girard, 1859, in relation to their body shape. Spanish toothcarp (*A. iberus*, hereinafter) is an endemic cyprinodontiform fish of the Iberian Peninsula, whose current populations are restricted to a few isolated areas in salt marshes, coastal lagoons and river mouths along the Spanish Mediterranean coastline (Alcaraz & García-Berthou, 2007a; Oliva-Paterna, Torralva, & Fernández-Delgado, 2006). Human pressure, water pollution, habitat fragmentation and, especially, the presence of the invasive mosquitofish (*G. holbrooki*, hereinafter) are some of the most pressing threats for its conservation (Oliva-Paterna et al., 2006). So far, some research has been focused on assessing ecological and behavioural aspects that could mediate the interspecific competition between these two species (Alcaraz, Bisazza, & García-Berthou, 2008a; Carmona-Catot, Magellan, & García-Berthou, 2013; Magellan & García-Berthou, 2016; Rincón, Correias, Morcillo, Risueño, & Lobón-Cerviá, 2002). In relation to swimming capabilities and metabolism, a previous study reported data on swimming performance and metabolic traits of *G. holbrooki* (Srean, Almeida, Rubio-Gracia, Luo, & García-Berthou, 2016), yet little is known about *A. iberus* on this issue. As with the invasive competitor, *A. iberus* is a so-called "limnophilic" species, that is preferring standing waters, so it may be of interest to compare their physiological response to constant water flow.

The main objectives of this study were to (a) determine prolonged swimming speeds and metabolic traits of *A. iberus* and (b) compare swimming performance and energy use between *A. iberus* and *G. holbrooki* in relation to morphological aspects. We hypothesised that: (i) *A. iberus* would display lower swimming speeds and higher cost of transport than *G. holbrooki*, given the differences in habitat use and migration rates between these two species (Alemadi & Jenkins, 2008; Díez-del-Molino et al., 2018); and (ii) differences in swimming capacity and efficiency would be determined by their morphological attributes, because of its intimate relationship with swimming ability and kinematics (Leavy & Bonner, 2009).

2 | MATERIALS AND METHODS

2.1 | Study area and experimental fish

Specimens of *Aphanius iberus* were collected using a dip net from La Rubina salt marshes in Alt Empordà, north-eastern Iberian Peninsula (42°15'38.7" N, 3°8'38.9" E) in 2017. Further information of the sampling area can be found elsewhere (García-Berthou & Moreno-Amich, 1992). Animal research and fieldwork were authorised by the Autonomous Government of Catalonia (Expedient PNAE 2017PNATAAEAUT075), the Commission of Animal Experimentation (Ref.: CEA-OH/9673/1) and by the Aiguamolls de l'Empordà Natural Park. Sampling was performed until obtaining up to twenty individuals of each sex. In total, thirteen females (20–44-mm total length and 70–1400-mg fish mass) and fifteen males (20–40-mm total length and 40–750-mg fish mass) of *A. iberus* were used for the comparison with 30 females and 30 males of *Gambusia holbrooki* (15–43-mm total length and 40–650-mg fish mass; 21–34-mm total length and

80–370-mg fish mass, females and males respectively). After sampling, fish were immediately transported to the laboratory, where they were acclimated to experimental conditions for 2 weeks prior to the experiment. Fish were placed into glass aquariums (90 L) at a temperature of $25 \pm 1^\circ\text{C}$ under a natural photoperiod cycle. Vigorous aeration was provided to all aquariums ensuring at least 90% of air saturation. From the second day of acclimation, fish were fed once a day with frozen bloodworms (*Chironomus* sp.) to ensure that most individuals had begun feeding. Feeding was interrupted for 24 hr prior to the experiment to avoid postprandial effects during experiment. No mortalities occurred during the acclimation period, and visually all fish remained in a good health. The experiment carried out in this study was conducted in early spring to avoid the breeding season of this fish species (García-Berthou & Moreno-Amich, 1992; Oliva-Paterna et al., 2006). After swimming performance trials, fish were kept in a quarantine tank for 1 week and were returned to the same place where they were caught.

2.2 | Determination of swimming performance and metabolic traits

Data on swimming capacity and metabolism for *Gambusia holbrooki* were obtained from our previous study (Srean et al., 2016), where details on the experimental fish and methods can be found. As for *Aphanius iberus*, we used essentially the same methodology in order to ensure that both species were tested under the same experimental conditions. Briefly, respirometry was conducted using a modified Bläzka-type swimming tunnel, with a nonturbulent laminar flow and equipped with a continuous-flow respirometer (Loligo® Systems). Flow was generated by a propeller connected to the motor outside of the respirometer. The tubular test section (170-ml volume, 100-mm length \times 26.4-mm internal diameter) was laterally covered with nonreflecting white screens to avoid disturbing fish by “mirror effects.” An external buffer tank was connected to the respirometer, and an automated flush pump flushed the air-saturated water at a rate of 5 L min^{-1} inside the respirometer to ensure well-mixed water. The temperature in the swim tunnel was kept constant at $25 \pm 1^\circ\text{C}$ by using a heater (Eheim Jäger Model 3,613; EHEIM GmbH & Co KG) placed in the buffer tank.

Rate of oxygen consumption (\dot{M}_{O_2}) was measured using computerised, intermittent-flow respirometry. The respirometer was periodically flushed with aerated water for 2 min followed by a 1 min closed mixing period and then 20 min of closed respirometry. Measurements of oxygen consumption were recorded for a period of 20 min due to it has been shown to be sufficient to detect changes in dissolved oxygen concentration (Plaut, 2000). An optical fibre oxygen instrument (Witrox 1; Loligo® Systems) was used to measure dissolved oxygen concentration in water. Atmospheric pressure on the oxygen probe was adjusted based on the actual pressures before the oxygen consumption measurement. For calibration purposes, two-point calibration with the oxygen sensor was used to record the highest concentration value as 100% air-saturated and the

lowest concentration value as 0% using a solution of sodium sulphite (Na_2SO_3 , 0.159 M).

Fish were placed into the respirometer and allowed to acclimatise for 1 hr to an initial velocity of ca. $0.5 \text{ BL}\cdot\text{s}^{-1}$ (body length, taken as the standard length of the fish, per second). After that, a critical swimming speed (U_{crit}) test was performed with step-wise increases in flow speed of approximately $1 \text{ BL}\cdot\text{s}^{-1}$ until fatigue. Fatigue was defined as occurring when a fish could no longer swim against the current continuously (Farrell, 2008). Following Brett (1964), the critical swimming speed (U_{crit} , cm s^{-1}) was calculated as:

$$U_{\text{crit}} = U_f + U_i T_f T_i^{-1},$$

where U_f is the highest speed maintained for a full-time period (in cm s^{-1}), T_f is the time swum at the last speed increment (in min), T_i is the set interval time (i.e. 20 min) and U_i is the speed increment (in cm s^{-1}). Swimming speeds were not corrected by the “solid-blocking effect” because the cross-sectional area of the fish never exceeded 10% of that of the respirometer (Bell & Terhune, 1970). Background microbial respiration inside the respirometer was calculated with no fish for 10 min at the end of each trial (i.e. blank run) to correct metabolic rates.

Measurements of oxygen consumption of individual fish were recorded during the U_{crit} swimming test. Oxygen consumption was calculated by fitting linear regression of the oxygen concentration decline over time. The resulting slope or regression coefficient was used to calculate oxygen consumption rates (\dot{M}_{O_2} , $\text{mg O}_2 \text{ h}^{-1}$):

$$\dot{M}_{\text{O}_2} = -(\Delta\text{Of} - \Delta\text{Ob}) \times V,$$

where ΔOf and ΔOb are the oxygen consumption rates ($\text{mg O}_2 \text{ l}^{-1} \text{ min}^{-1}$) due to fish respiration and microbial respiration respectively, and V is the volume of the respirometer (after subtracting the fish volume). \dot{M}_{O_2} was then expressed in $\text{mg O}_2 \text{ h}^{-1}$. The exponential function was used to describe the relationship between \dot{M}_{O_2} (log-transformed) and swimming speed through linear regression (Beamish, 1978; Brett, 1964; Korsmeyer, Steffensen, & Herskin, 2002; Pettersson & Hedenström, 2000; Tudorache et al., 2008; Webb, 1975):

$$\dot{M}_{\text{O}_2} = \text{SMR} \times e^{bU},$$

where SMR is the standard metabolic rate extrapolated to zero activity and b , an estimated constant that can be used as an index of the swimming efficiency. The higher the value of b , the more marked the increase in the swimming \dot{M}_{O_2} values with an increased swimming speed. In addition to SMR, two metabolic traits were obtained from the measurement of \dot{M}_{O_2} : maximal metabolic rate (MMR) was determined as the highest value of \dot{M}_{O_2} during the U_{crit} swimming test, which was usually close to the fastest speed (Keen & Farrell, 1994; Nelson, 2016); and absolute aerobic scope (AAS) was calculated as the difference between MMR and SMR as an indicator of the physiological capacity of fish to perform several oxygen-demanding processes simultaneously (Clark, Sandblom, & Jutfelt, 2013).

2.3 | Determination of fish swimming efficiency

The optimal swimming speed (U_{opt} , cm s^{-1}), that is the swimming speed with the lowest energy cost per unit distance, was calculated from the exponential function according to Pettersson and Hedenström (2000):

$$U_{opt} = \frac{1}{b}$$

Consequently, the minimum cost of transport (COT, J m^{-1}) was estimated as:

$$\text{COT} = \frac{\dot{M}_{O_{2opt}}}{U_{opt}}$$

where $\dot{M}_{O_{2opt}}$ is the oxygen consumption rate obtained at U_{opt} . In addition, the relationship between COT and swimming speed was evaluated by dividing the obtained \dot{M}_{O_2} values by the corresponding speed. COT values were then converted into energy units using an oxycaloric value of $14.1 \text{ J} \cdot \text{mg O}_2^{-1}$ (Hepher, 1988) and corrected for size effects by dividing with fish mass. Mass-specific cost of transport (MCOT) was expressed in $\text{J} \cdot \text{m}^{-1} \cdot \text{kg}^{-1}$.

2.4 | Fish morphology

After the experimental procedure, each individual was measured to the nearest ($\pm 1 \text{ mm}$) and weighed to the nearest ($\pm 0.1 \text{ mg}$). To analyse morphological traits, some morphometric measurements were obtained from digital images of the individuals. These measurements included standard body length, total body length, maximum body depth and least depth of caudal peduncle. Two morphometric ratios were then obtained from these measurements: fineness ratio (ratio between standard body length and maximum body depth) and caudal peduncle depth factor (ratio between least depth of caudal peduncle and maximum body depth). Both morphological ratios have been previously used as predictors of swimming ability and efficiency (Fisher & Hogan, 2007; Ohlberger et al., 2006; Scarnecchia, 1988; Walker et al., 2013; Webb, 1975).

2.5 | Statistical analyses

All statistical analyses were performed with the R software (Development Core Team, 2018). Analysis of covariance (two-way ANCOVA) was used to test for differences in swimming performance and energetic costs between species and sexes as categorical factors. Fish mass was the best predictor of all metrics (i.e. r^2 values were always higher for mass than for total body length) and was thus used as covariate for further analyses. Additionally, swimming speed was used as covariate to test for differences in MCOT between species. The assumption of parallelism of standard ANCOVA was tested analysing the interactions between the covariate and the categorical factors. If such interactions are not significant, they indicate that the slopes are homogeneous and the

assumption of parallelism is thus satisfied. Then, the interactions were removed from the model in order to improve the statistical power of the ANCOVA (García-Berthou & Moreno-Amich, 1993). Afterwards, if the interaction between species and sex was found significant, ANCOVA was separately performed for each species to elucidate the differences between sexes within each species. Finally, the effects of sex were tested within each species when the assumption of parallelism was not satisfied. Consequently, if slopes were homogeneous and differences between sexes were not significant, ANCOVA was then applied to test for differences between species (both sexes pooled).

When response variables were not correlated to fish size, an analysis of variance (two-way ANOVA) was performed to test for differences between species and sex. After that, a Tukey's multiple comparison test (Tukey's HSD) was conducted to compare the means of the four groups (i.e. species by sex). Finally, relations between morphological traits and critical swimming capacity (U_{crit}) and swimming efficiency (U_{opt} and COT) were evaluated from Pearson product-moment correlation coefficients (r). All variables were \log_{10} -transformed to satisfy the assumptions of the parametric statistical methods (i.e. normality, homoscedasticity and linearity).

3 | RESULTS

3.1 | Swimming performance and metabolic traits

The interactions between fish mass and the categorical factors (i.e. species and sex) in the ANCOVAs were not significant for U_{crit} , MMR and AAS ($p > .05$), and hence homogeneous slopes among groups were assumed. U_{crit} , MMR and AAS were significantly different between the two species (Table 1), with marked differences after accounting for fish mass (i.e. different intercepts or adjusted means) (Table 2). *Gambusia holbrooki* had higher swimming capacity (Figure 1a). Values of U_{crit} for the 28 *Aphanius iberus* ranged from 5.04 to 18.90 $\text{cm} \cdot \text{s}^{-1}$, while for the 60 *G. holbrooki* ranged from 4.85 to 22.26 $\text{cm} \cdot \text{s}^{-1}$. Concerning metabolic traits, *A. iberus* had higher MMR (Figure 1b) and AAS (Figure 1c) compared to *G. holbrooki*. Values of MMR and AAS for *A. iberus* ranged from 0.10 to 1.21 $\text{mg O}_2 \text{ h}^{-1}$ and from 0.06 to 0.52 $\text{mg O}_2 \text{ h}^{-1}$ respectively. As for *G. holbrooki*, MMR and AAS ranged from 0.05 to 0.55 $\text{mg O}_2 \text{ hr}^{-1}$ and from 0.03 to 0.40 $\text{mg O}_2 \text{ h}^{-1}$ respectively. The species \times sex interaction was found to be significant for U_{crit} , MMR and AAS (Table 1), thereby showing that differences among sexes depend on species. Males of *G. holbrooki* had higher U_{crit} ($F_{1,57} = 20.51, p < .001$), MMR ($F_{1,57} = 5.62, p = .021$) and AAS ($F_{1,57} = 5.59, p = .022$) than females, whereas sexes of *A. iberus* had similar U_{crit} ($F_{1,25} = 0.002, p = .965$), MMR ($F_{1,25} = 2.86, p = .104$) and AAS ($F_{1,25} = 1.77, p = .195$), after accounting for the effects of fish mass.

The assumption of parallelism was not satisfied for SMR, and therefore the effects of sex were tested within each species. There were no differences in SMR between sexes in *A. iberus* ($F_{1,25} = 2.33, p = .140$) and *G. holbrooki* ($F_{1,56} = 0.13, p = .718$). SMR was not significantly different between species (Table 1, Figure S1).

TABLE 1 Analyses of covariance (ANCOVA) of swimming performance and energetic costs for Spanish toothcarp (*Aphanius iberus*) ($N = 28$) and mosquitofish (*Gambusia holbrooki*) ($N = 60$)

	U_{crit}			MMR			AAS			SMR			COT		
	$R^2_{adj} = 0.607$			$R^2_{adj} = 0.579$			$R^2_{adj} = 0.444$			$R^2_{adj} = 0.238$			$R^2_{adj} = 0.603$		
	SS	df	p	SS	df	p	SS	df	p	SS	df	p	SS	df	p
M	0.570	1	***	1.605	1	***	1.369	1	***	2.678	1	***	0.615	1	***
Species	0.372	1	***	0.773	1	***	0.808	1	***	0.207	1	0.193	3.085	1	***
Sex	0.000	1	0.954	0.109	1	0.052	0.046	1	0.293	-	-	-	-	-	-
Species × Sex	0.090	1	**	0.159	1	*	0.178	1	*	-	-	-	-	-	-
Residuals	0.978	83		2.295	83		3.342	81		10.10	84		2.909	72	

Note: Models test differences between species and sexes for U_{crit} , MMR and AAS with fish mass (M) as a covariate. Models test differences between species for SMR and COT with fish mass as a covariate. The assumption of parallelism was not satisfied for U_{opt} (optimal swimming speed), and therefore ANCOVA was not applied. All variables were \log_{10} -transformed.

Abbreviations: AAS, absolute aerobic scope; COT, minimum cost of transport; MMR, maximal metabolic rate; SMR, standard metabolic rate; U_{crit} , critical swimming speed.

* $p \leq .05$.

** $p \leq .01$.

*** $p \leq .001$.

TABLE 2 Linear regression functions ($y = a + bx$) of the swimming performance and energetic costs with fish mass for Spanish toothcarp (*Aphanius iberus*) and mosquitofish (*Gambusia holbrooki*)

Species	Sex	Independent variable	a	95% CI	b	95% CI	R^2_{adj}	N	p
<i>A. iberus</i>	Pooled	U_{crit}	1.078	(0.990, 1.166)	0.291	(0.163, 0.419)	0.434	28	***
<i>G. holbrooki</i>	Female	U_{crit}	1.282	(1.189, 1.375)	0.288	(0.158, 0.417)	0.404	30	***
<i>G. holbrooki</i>	Male	U_{crit}	1.410	(1.172, 1.648)	0.279	(0.008, 0.550)	0.107	30	*
<i>A. iberus</i>	Pooled	MMR	-0.199	(-0.376, -0.023)	0.390	(0.132, 0.648)	0.243	28	**
<i>G. holbrooki</i>	Female	MMR	-0.290	(-0.403, -0.176)	0.612	(0.453, 0.771)	0.679	30	***
<i>G. holbrooki</i>	Male	MMR	-0.209	(-0.489, 0.072)	0.606	(0.287, 0.926)	0.328	30	**
<i>A. iberus</i>	Pooled	AAS	-0.431	(-0.580, -0.281)	0.378	(0.159, 0.597)	0.300	28	**
<i>G. holbrooki</i>	Female	AAS	-0.559	(-0.760, -0.358)	0.593	(0.297, 0.889)	0.363	30	***
<i>G. holbrooki</i>	Male	AAS	-0.377	(-0.826, 0.073)	0.636	(0.127, 1.145)	0.166	30	*
<i>A. iberus</i>	Pooled	SMR	-0.604	(-0.956, -0.251)	0.595	(0.080, 1.111)	0.146	28	*
<i>G. holbrooki</i>	Pooled	SMR	-0.718	(-0.933, -0.502)	0.585	(0.314, 0.856)	0.233	60	***
<i>A. iberus</i>	Pooled	U_{opt}	1.151	(0.967, 1.336)	0.393	(0.123, 0.663)	0.228	28	**
<i>G. holbrooki</i>	Pooled	U_{opt}	1.376	(1.262, 1.490)	0.335	(0.195, 0.476)	0.292	60	***
<i>A. iberus</i>	Pooled	COT	-1.728	(-1.929, -1.526)	0.202	(-0.093, 0.497)	0.035	28	0.17
<i>G. holbrooki</i>	Pooled	COT	-2.020	(-2.192, -1.848)	0.378	(0.162, 0.594)	0.20	60	***

Note: A single linear regression function is shown when differences between sexes were not significant ($p > .05$). All variables were \log_{10} -transformed. Abbreviations: AAS, absolute aerobic scope; COT, minimum cost of transport; MMR, maximal metabolic rate; SMR, standard metabolic rate; U_{crit} , critical swimming speed; U_{opt} , optimal swimming speed.

* $p \leq .05$.

** $p \leq .01$.

*** $p \leq .001$.

3.2 | Swimming efficiency

The effects of sex were also tested for U_{opt} and COT within each species. There were no differences in COT between sexes of *Aphanius iberus* ($F_{1,25} = 3.38$, $p = .078$) and between sexes of *Gambusia holbrooki* ($F_{1,45} = 0.43$, $p = .517$). ANCOVA showed significant differences in COT between species (Table 1). *Aphanius iberus*

generally displayed higher COT (Figure 2a) compared to *G. holbrooki*, for a given body mass. Within each species, the slope (b) of COT was found to be higher in *G. holbrooki* (Table 2), although *A. iberus* generally displayed more variability (i.e. a wider 95% CI). COT ranged from 0.004 to 0.034 $J \cdot m^{-1}$ and from 0.001 to 0.012 $J \cdot m^{-1}$ in *A. iberus* and *G. holbrooki* respectively. In addition, MCOT was negatively related with swimming speed ($p < .001$), suggesting that both species swam

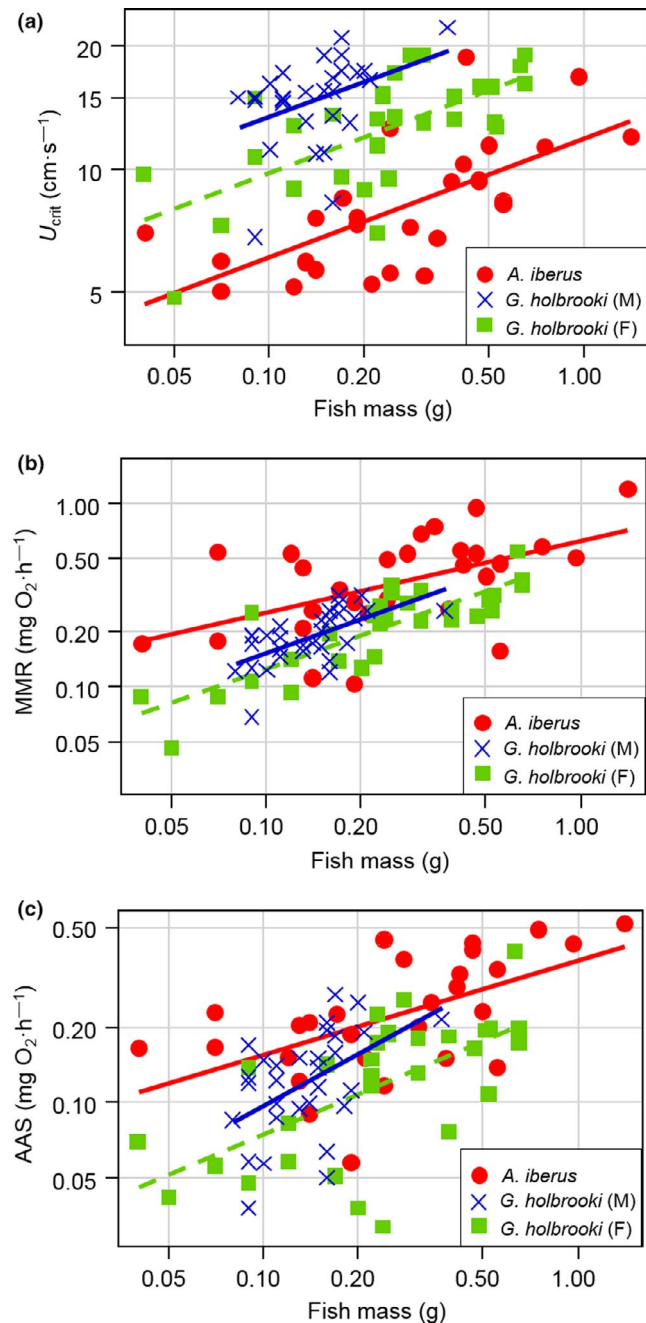


FIGURE 1 Relationships of critical swimming speed U_{crit} (a), maximal metabolic rate MMR (b) and absolute aerobic scope AAS (c) with fish mass for Spanish toothcarp, *Aphanius iberus* ($N = 28$) and mosquitofish, *Gambusia holbrooki* ($N = 60$). Regressions are significantly different between species (red circles for both sexes of *A. iberus*) and between sexes of mosquitofish (green squares for females and blue multiplication signs for males) for U_{crit} , MMR and AAS (Table 1). For clarity, regression of females of *G. holbrooki* is represented by a dashed line. Regression coefficients are shown in Table 2. Note both axes are on a log scale

more efficiently with increasing swimming speed. Most individuals of *A. iberus* generally had higher MCOT than *G. holbrooki* as a function of swimming speed (Figure 2b). ANCOVA showed interaction between swimming speed and species ($p = .014$), indicating thus

that variation in the energetic cost in relation to swimming speed was different between species ($F_{1,483} = 21.73$, $p < .001$). As for U_{opt} , the assumption of parallelism was not satisfied, that is slopes were not homogeneous between sexes of *G. holbrooki*, and therefore a ANCOVA was not applied. *Aphanius iberus* generally displayed lower U_{opt} than *G. holbrooki* (Figure S2).

3.3 | Relationship between fish morphology and swimming efficiency

Morphological ratios were not significantly related to fish size. ANOVA showed significant differences in fineness ratio between species ($F_{1,77} = 55.19$, $p < .001$). When accounting for both sexes, *Gambusia holbrooki* had higher values of fineness ratio than *Aphanius iberus* (Tukey test, $p < .05$; Figure 3a). In addition, we found significant positive correlations of fineness ratio with U_{crit} ($r = 0.39$, $df = 79$, $p < .001$) and U_{opt} ($r = 0.29$, $df = 76$, $p < .01$). Instead, fineness ratio had a significant negative correlation with COT ($r = -0.45$, $df = 72$, $p < .001$). As for the caudal peduncle depth factor, sex as factor ($F_{1,78} = 101.61$, $p < .001$) and the species \times sex interaction ($F_{1,78} = 38.27$, $p < .001$) were significant; males of *G. holbrooki* had the highest values, followed by *A. iberus* (without differences between sexes) and finally, females of *G. holbrooki* showing the lowest values (Tukey test, $p < .05$; Figure 3b). In addition, caudal peduncle depth factor was not significantly correlated with U_{crit} ($r = 0.11$, $df = 80$, $p = .307$) and U_{opt} ($r = -0.05$, $df = 77$, $p = .693$). However, we found a significant negative correlation of caudal peduncle depth factor with COT ($r = -0.24$, $df = 73$, $p = .041$).

4 | DISCUSSION

The present study reports the first data on the swimming performance and energy metabolism of the endangered Spanish toothcarp (*Aphanius iberus*). Concerning swimming speeds, *A. iberus* displayed low critical swimming capacity reaching the fatigue levels nearby 3–5 $BL \cdot s^{-1}$ (U_{crit} mean = $8.64 \text{ cm} \cdot \text{s}^{-1}$). This swimming capacity is far below the average of similar small-bodied species (Li, Lin, Xu, & Sun, 2017; Plaut, 2002) and other cyprinodontiforms (Plaut, 2000). Swimming capacity and metabolic rates increased significantly with fish mass in *A. iberus*, showing that heavier fish performed best. Additionally, we investigated the effects of sex on swimming capacity and metabolism of this native toothcarp. Results suggest that both sexes present a similar steady locomotor performance and energy use, since no differences were detected for any of the response variables studied. In general, species that present a distinct sexual dimorphism are expected to display differences in swimming performance between sexes (Conradsen & McGuigan, 2015; Oufiero & Garland, 2007; Royle, Metcalfe, & Lindström, 2006). However, that is not likely the case as for *A. iberus* despite the fact that females are generally larger than males. Even though we did not measure specific body traits in *A. iberus*, the lack of differences in some morphometric ratios (i.e. caudal peduncle depth factor and fineness ratio) might not lead to

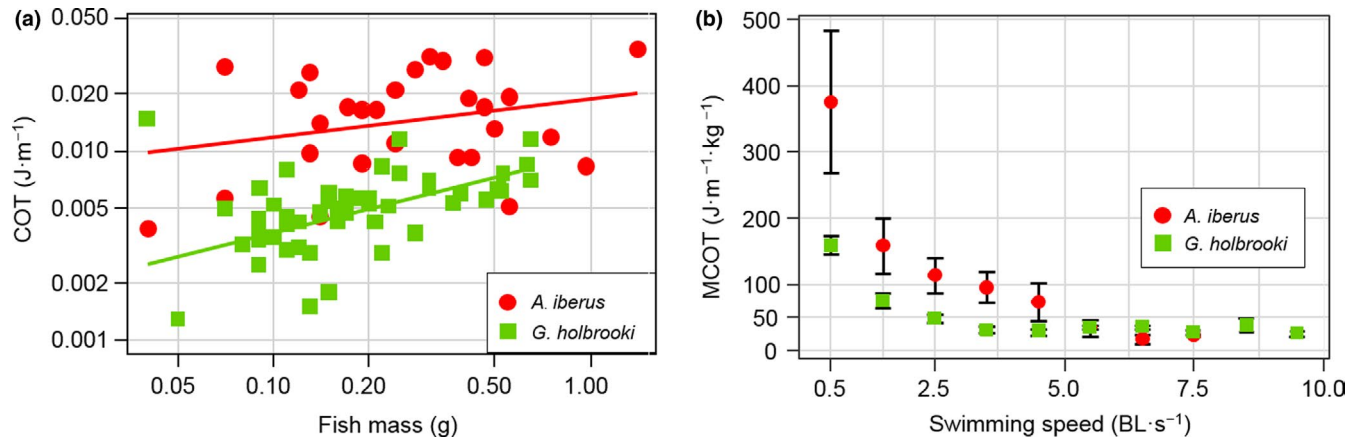


FIGURE 2 Relationships of minimum cost of transport COT with fish mass (a) and mass-specific cost of transport MCOT with swimming speed (b) for Spanish toothcarp, *Aphanius iberus* ($N = 28$) and mosquitofish, *Gambusia holbrooki* ($N = 60$). COT was significantly different between Spanish toothcarp and mosquitofish (Table 1). Regression coefficients are shown in Table 2. MCOT was derived from the swimming speed and metabolic rates (mean \pm SE). Note both axes in Figure 2a and y-axis in Figure 2b are on a log scale

sex-dependent differences in swimming performance. In contrast, the effects of sex in *G. holbrooki* are more clear since males displayed higher critical swimming speeds and maximal metabolic rates than females, as previously observed in Srean et al. (2016). However, while Srean et al. (2016) did not find significant differences in the factorial aerobic scope (FAS) between sexes, in this study males had higher absolute aerobic scope than females. This finding is not surprising since calculations of factorial aerobic scope (i.e. MMR/SMR) could be strongly influenced by variation in the ratio's denominator, whereas absolute aerobic scope (i.e. $\text{MMR}-\text{SMR}$) is generally less sensitive to variation in standard metabolism among individuals (Clark et al., 2013; Halsey, Killen, Clark, & Norin, 2018).

We observed large differences in swimming performance and metabolism between *A. iberus* and *G. holbrooki*, being the invasive species which swam faster (U_{crit} mean = $14.11 \text{ cm}\cdot\text{s}^{-1}$). However, this swimming capacity can still be considered low, especially if compared to rheophilic species (Tudorache et al., 2008). Despite this, the reduced swimming capacity of *G. holbrooki* has not stopped it from living also in downstream rivers and streams (Benejam, Alcaraz, Sasal, Simon-Levert, & García-Berthou, 2009; Carmona-Catot, Benito, & García-Berthou, 2011), where water velocity is much reduced (Díez-del-Molino et al., 2018). In fact, the invasion success of certain species is not always related to a high steady swimming capacity (e.g. Tierney, Kasurak, Zielinski, & Higgs, 2011). On the other hand, the endangered *A. iberus* is mainly found in calm waters (Alcaraz, Pou-Rovira, & García-Berthou, 2008b), and recent research (Gonzalez et al., 2018) pointed out that habitat fragmentation appears to reduce the migration of individuals causing increased genetic drift across its geographical range. So, the preference for slow-moving habitats agrees with the poor swimming capacity shown by this fish species. In fact, there is a close link between habitat characteristics (e.g. water flow) and swimming performance of a fish (Alexandre, Quintella, Ferreira, Romão, & Almeida, 2014; Langerhans, 2009; Oufiero & Whitlow, 2016).

After accounting for fish size, the minimum cost of transport was more favourable in *G. holbrooki*, since it displayed less

energy-consumption per meter swum. This means that Spanish toothcarp used distinctly more energy than mosquitofish of the same mass swimming at the same speed. In addition, when comparing energetic costs at different swimming speeds, differences between species are also remarkable. We found evidence that *A. iberus* showed high mass-specific cost of transport at lower speeds due to high metabolism required for locomotion, obtaining thus poorer swimming efficiency. In contrast, *G. holbrooki* showed similar mass-specific cost of transport in a wider range of swimming speeds, indicating that this species can optimally swim without excessively increasing the energetic costs, a fact which has also been observed in cyprinids (e.g. Ohlberger et al., 2006). However, the partial contribution of the anaerobic metabolism was not quantitatively assessed in this study. For instance, Svendsen et al. (2010) found that up to 25% of the energetic costs were associated with anaerobic metabolism in a labriform species. So, it is likely that total energetic costs might be increased further in Spanish toothcarp and mosquitofish after accounting for anaerobic power output. In addition, optimal swimming speeds were generally higher in *G. holbrooki* (U_{opt} mean = $6.32 \text{ BL}\cdot\text{s}^{-1}$), suggesting that this species can swim faster at optimal speeds than *A. iberus* (U_{opt} mean = $4.58 \text{ BL}\cdot\text{s}^{-1}$). Killen, Croft, Salin, and Darden (2016) found similar optimal swimming speeds in females of *Poecilia reticulata* (Peters, 1859), which were on average about $5 \text{ BL}\cdot\text{s}^{-1}$. Surprisingly, optimal swimming speeds were higher than critical swimming speeds in some individuals examined. This result suggests that because of the exponential fit, the accuracy of optimal swimming speed values might be more difficult to obtain in species with low swimming capacity, since the optimal swimming speed is only affected by the speed exponent (b) from the equation (Pettersson & Hedenström, 2000).

There was no evidence of differences in standard metabolism between species. However, the standard metabolic rate varied substantially in individuals of similar size in *A. iberus*. For instance, two fish of 70 mg had a standard metabolic rate of 0.32 and 0.01 mg

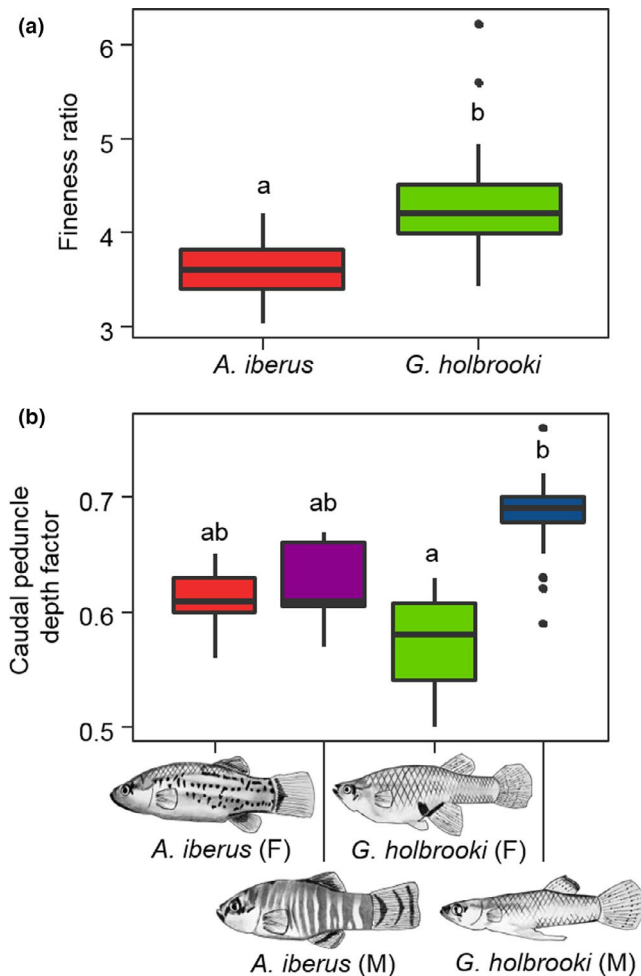


FIGURE 3 Fineness ratio (a) and caudal peduncle depth factor (b) for Spanish toothcarp, *Aphanius iberus* and mosquitofish, *Gambusia holbrooki*. Boxplots show median (line), quartiles (box) and ranges excluding outliers (error bars). Significant difference represented by different lowercase letter from Tukey's HSD ($p < .05$)

O_2 hr^{-1} respectively. However, individual fish with a higher standard metabolism rate also had a higher maximal metabolic rate (0.55 and 0.18 $mg O_2 \cdot h^{-1}$ respectively), suggesting that there is a cost of maintaining the machinery that supports high aerobic capacity (Biro & Stamps, 2010). Intraspecific variation in metabolic traits of fish within a population is a current concern (Pettersen, Marshall, & White, 2018), and standard metabolic rates, in particular, are thought to have important effects on some fitness correlates such as growth, reproduction and survival (Burton, Killen, Armstrong, & Metcalfe, 2011).

Interestingly, when comparing metabolic rates between species we found that *A. iberus* had higher maximal metabolic rate, after controlling for the effects of fish mass. This finding is, however, contrary with the notion that species reaching lower swimming speeds are expected to have more limited capacity for oxygen uptake (Norin & Clark, 2016; Tudorache et al., 2008). Even so, most of this research has been focused on comparing metabolic rates among rheophilic species such as cyprinids (Yan, He, Cao, & Fu, 2013) and among species with

contrasting lifestyle or migratory rates (Stoffels, 2015; Tudorache et al., 2008). It is likely, therefore, that a "weak" swimmer may attain higher metabolic rates at a specific speed if we compare it with other one of similar swimming capacity. Additional support for such a link might be found in the observed correlation between gait transitions (i.e. the changing use of certain body fins while swimming) with metabolic rates and locomotor performance in several fish species (Kendall, Lucey, Jones, Wang, & Ellerby, 2007; Korsmeyer et al., 2002; Svendsen, Banet, Christensen, Steffensen, & Aarestrup, 2013). Thus, we cannot rule out that the observed differences in metabolic rates could be partly attributed to swimming gait or swimming mechanics. In addition, absolute aerobic scope was also found to be higher for *A. iberus* (AAS mean = 0.26 $mg O_2 \cdot h^{-1}$). Taken together, our results suggest that *G. holbrooki* is able to use a lower proportion of their total aerobic capacity for swimming, thereby leading to a better swimming efficiency. For instance, *A. iberus* used on average about 67% of their total aerobic scope at 2.5 $BL \cdot s^{-1}$, while *G. holbrooki* only used about 32% at the same speed. So, the invasive species had about 68% of their total aerobic scope remaining for other tasks than locomotion.

Body form is strongly linked to locomotion and swimming efficiency of species (Pettersson & Hedenström, 2000; Wainwright, Bellwood, & Westneat, 2002; Webb, 1984). Species with a thinner caudal peduncle can produce thrust for cruising at high speed using an axially oscillating caudal fin (Moran, Ferry, & Gibb, 2016; Webb & Weihs, 1986). In our study, both sexes of *G. holbrooki* had a narrower caudal peduncle than *A. iberus*, and even males of *G. holbrooki* had the highest caudal peduncle depth factor. Further, the significant negative correlation between the minimum cost of transport and the caudal peduncle depth factor suggests that this morphological trait seems to be especially relevant to obtain higher swimming efficiency. On the other hand, fineness ratios between 2 and 6 result in a decrease of the drag produced by the fish, with the optimum ratio for efficient swimming at about 4.5 (Langerhans & Reznick, 2010; Webb, 1975). In this study, fineness ratio was found to be higher for *G. holbrooki*, suggesting that this fish species has a more hydrodynamic shape for swimming (Ohlberger et al., 2006). In line with this hypothesis, we found a significant negative correlation of minimum cost of transport with fineness ratio, whereas there was a positive correlation with critical and optimal swimming speeds. In addition, it has been shown that fineness ratio is related to microhabitat and water flow (Scarnecchia, 1988), and therefore differences in body shape between these two species may reveal a functional trade-off between morphology and habitat preference. The more streamlined body may allow mosquitofish to swim in a variety of microhabitats (Alemadi & Jenkins, 2008), whereas the deeper body of the native toothcarp may enhance its manoeuvrability (Webb, 1994) in environments with abundant vegetation (Alcaraz & García-Berthou, 2007b).

In conclusion, we found that *G. holbrooki* performed better than *A. iberus* in the swim performance tests. Results suggest that *A. iberus* is not able to maintain steady swimming at relatively low speeds. Furthermore, energetic costs of swimming measured as cost of transport were higher in this native toothcarp, which suggest that this species requires additional energetics per unit of mass and

distance compared to the invasive mosquitofish. These differences in swimming performance and efficiency can partly be explained by the habitat preferences and morphological features. This study would represent a significant contribution to address some physiological traits of these two species, and this information might be useful for ecological studies of species interactions.

ACKNOWLEDGEMENTS

This research was supported by the Spanish Ministry of Science, Innovation and Universities (projects CGL2013-43822-R and CGL2016-80820-R) and the Government of Catalonia (ref. 2017 SGR 548). F. Rubio-Gracia benefitted from a predoctoral fellowship from the University of Girona (IFUG17). We wish to thank D. Ispan for his help with field and laboratory work at the University of Girona. Authors deeply thank P. Domenici for his valuable and constructive comments on an earlier version of the manuscript. Finally, we would like to thank two anonymous reviewers for their helpful comments on the manuscript.

CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceived and designed the investigation: FRG, AVG. Performed field work: FRG, DL, PS. Analysed the data: FRG, AVG. Contributed materials and/or tools for analysis: EGB, DL, RMA, YL. Wrote the paper: FRG, AVG, EGB.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ORCID

Francesc Rubio-Gracia  <https://orcid.org/0000-0002-0991-5341>

Emili García-Berthou  <https://orcid.org/0000-0001-8412-741X>

REFERENCES

- Alcaraz, C., Bisazza, A., & García-Berthou, E. (2008a). Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia*, *155*, 205–213. <https://doi.org/10.1007/s00442-007-0899-4>
- Alcaraz, C., & García-Berthou, E. (2007a). Life history variation of invasive mosquitofish (*Gambusia holbrooki*) along a salinity gradient. *Biological Conservation*, *139*, 83–92. <https://doi.org/10.1016/j.biocon.2007.06.006>
- Alcaraz, C., & García-Berthou, E. (2007b). Food of an endangered cyprinodont (*Aphanius iberus*): Ontogenetic diet shift and prey electivity. *Environmental Biology of Fishes*, *78*, 193–207. <https://doi.org/10.1007/s10641-006-0018-0>
- Alcaraz, C., Pou-Rovira, Q., & García-Berthou, E. (2008b). Use of a flooded salt marsh habitat by an endangered cyprinodontid fish (*Aphanius iberus*). *Hydrobiologia*, *600*, 177–185. <https://doi.org/10.1007/s10750-007-9230-y>
- Alemadi, S. D., & Jenkins, D. G. (2008). Behavioral constraints for the spread of the eastern mosquitofish, *Gambusia holbrooki* (Poeciliidae). *Biological Invasions*, *10*, 59–66. <https://doi.org/10.1007/s10530-007-9109-x>
- Alexandre, C. M., Quintella, B. R., Ferreira, A. F., Romão, F. A., & Almeida, P. R. (2014). Swimming performance and ecomorphology of the Iberian barbel *Luciobarbus bocagei* (Steindachner, 1864) on permanent and temporary rivers. *Ecology of Freshwater Fish*, *23*, 244–258. <https://doi.org/10.1111/eff.12073>
- Beamish, F. W. H. (1978). Swimming capacity. In: W. S. Hoar, & D. J. Randall (Eds.), *Fish physiology, locomotion*. Vol. VII. London, UK: Academic Press.
- Bell, W. H., & Terhune, L. D. B. (1970). Water tunnel design for fisheries research. *Fisheries Research Board of Canada Technical Report*, *195*, 1–69.
- Benejam, L., Alcaraz, C., Sasal, P., Simon-Levert, G., & García-Berthou, E. (2009). Life history and parasites of the invasive mosquitofish (*Gambusia holbrooki*) along a latitudinal gradient. *Biological Invasions*, *11*, 2265–2277. <https://doi.org/10.1007/s10530-008-9413-0>
- Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology and Evolution*, *25*, 653–659. <https://doi.org/10.1016/j.tree.2010.08.003>
- Blake, R. W. (2004). Review paper: Fish functional design and swimming performance. *Journal of Fish Biology*, *65*, 1193–1222. <https://doi.org/10.1111/j.1095-8649.2004.00568.x>
- Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada*, *21*, 1183–1226. <https://doi.org/10.1139/f64-103>
- Burton, T., Killen, S. S., Armstrong, J. D., & Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences*, *278*, 3465–3473. <https://doi.org/10.1098/rspb.2011.1778>
- Carmona-Catot, G., Benito, J., & García-Berthou, E. (2011). Comparing latitudinal and upstream-downstream gradients: Life history traits of invasive mosquitofish. *Diversity and Distributions*, *17*, 214–224. <https://doi.org/10.1111/j.1472-4642.2011.00743.x>
- Carmona-Catot, G., Magellan, K., & García-Berthou, E. (2013). Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. *PLoS ONE*, *8*, e54734. <https://doi.org/10.1371/journal.pone.0054734>
- Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016). The determination of standard metabolic rate in fishes. *Journal of Fish Biology*, *88*, 81–121. <https://doi.org/10.1111/jfb.12845>
- Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *Journal of Experimental Biology*, *216*, 2771–2782. <https://doi.org/10.1242/jeb.084251>
- Conradsen, C., & McGuigan, K. (2015). Sexually dimorphic morphology and swimming performance relationships in wild-type zebrafish *Danio rerio*. *Journal of Fish Biology*, *87*, 1219–1233. <https://doi.org/10.1111/jfb.12784>
- Development Core Team, R. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from www.r-project.org.
- Diez-del-Molino, D., García-Berthou, E., Araguas, R. M., Alcaraz, C., Vidal, O., Sanz, N., ... García-Marín, J. L. (2018). Effects of water pollution and river fragmentation on population genetic structure of invasive mosquitofish. *Science of the Total Environment*, *637–638*, 1372–1382. <https://doi.org/10.1016/j.scitotenv.2018.05.003>

- Farrell, A. P. (2008). Comparisons of swimming performance in rainbow trout using constant acceleration and critical swimming speed tests. *Journal of Fish Biology*, 72, 693–710. <https://doi.org/10.1111/j.1095-8649.2007.01759.x>
- Fisher, R., Bellwood, D. R., & Job, S. D. (2000). Development of swimming abilities in reef fish larvae. *Marine Ecology Progress Series*, 202, 163–173. <https://doi.org/10.3354/meps202163>
- Fisher, R., & Hogan, J. D. (2007). Morphological predictors of swimming speed: A case study of pre-settlement juvenile coral reef fishes. *Journal of Experimental Biology*, 210, 2436–2443. <https://doi.org/10.1242/jeb.004275>
- García-Berthou, E., & Moreno-Amich, R. (1992). Age and growth of an Iberian cyprinodont. *Journal of Fish Biology*, 40, 929–937. <https://doi.org/10.1111/j.1095-8649.1992.tb02638.x>
- García-Berthou, E., & Moreno-Amich, R. (1993). Multivariate analysis of covariance in morphometric studies of the reproductive cycle. *Canadian Journal of Fisheries and Aquatic Sciences*, 50, 1394–1399. <https://doi.org/10.1139/f93-159>
- Gonzalez, E. G., Cunha, C., Ghanavi, H. R., Oliva-Paterna, F. J., Torralva, M., & Doadrio, I. (2018). Phylogeography and population genetic analyses in the Iberian toothcarp (*Aphanius iberus* Valenciennes, 1846) at different time scales. *Journal of Heredity*, 109, 253–263. <https://doi.org/10.1093/jhered/esx076>
- Halsey, L. G., Killen, S. S., Clark, T. D., & Norin, T. (2018). Exploring key issues of aerobic scope interpretation in ectotherms: Absolute versus factorial. *Reviews in Fish Biology and Fisheries*, 28, 405–415. <https://doi.org/10.1007/s11160-018-9516-3>
- Hammer, C. (1995). Fatigue and exercise tests with fish. *Comparative Biochemistry and Physiology A*, 112, 1–20. [https://doi.org/10.1016/0300-9629\(95\)00060-K](https://doi.org/10.1016/0300-9629(95)00060-K)
- Hepher, B. (1988). *Nutrition of pond fishes*. Cambridge, UK: Cambridge University Press. <https://doi.org/10.1017/CBO9780511735455>
- Keen, J. E., & Farrell, A. P. (1994). Maximum prolonged swimming speed and maximum cardiac performance of rainbow trout, *Oncorhynchus mykiss*, acclimated to two different water temperatures. *Comparative Biochemistry and Physiology A*, 108, 287–295. [https://doi.org/10.1016/0300-9629\(94\)90097-3](https://doi.org/10.1016/0300-9629(94)90097-3)
- Kendall, J. L., Lucey, K. S., Jones, E. A., Wang, J., & Ellerby, D. J. (2007). Mechanical and energetic factors underlying gait transitions in bluegill sunfish (*Lepomis macrochirus*). *Journal of Experimental Biology*, 210, 4265–4271. <https://doi.org/10.1242/jeb.009498>
- Killen, S. S., Atkinson, D., & Glazier, D. S. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on life-style and temperature. *Ecology Letters*, 13, 184–193. <https://doi.org/10.1111/j.1461-0248.2009.01415.x>
- Killen, S. S., Croft, D. P., Salin, K., & Darden, S. K. (2016). Male sexually coercive behaviour drives increased swimming efficiency in female guppies. *Functional Ecology*, 30, 576–583. <https://doi.org/10.1111/1365-2435.12527>
- Korsmeyer, K. E., Steffensen, J. F., & Herskin, J. (2002). Energetics of median and paired fin swimming, body and caudal fin swimming, and gait transition in parrotfish (*Scarus schlegelii*) and triggerfish (*Rhinecanthus aculeatus*). *Journal of Experimental Biology*, 205, 1253–1263. PMID:11948202.
- Langerhans, R. B. (2009). Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *Journal of Evolutionary Biology*, 22, 1057–1075. <https://doi.org/10.1111/j.1420-9101.2009.01716.x>
- Langerhans, R., & Reznick, D. (2010). Ecology and evolution of swimming performance in fishes: Predicting evolution with biomechanics. In: P. Domenici, & B. G. Kapoor (Eds.), *Fish locomotion: An ethoecological perspective*. Enfield, UK: Science Publishers.
- Leavy, T. R., & Bonner, T. H. (2009). Relationships among swimming ability, current velocity association, and morphology for freshwater lotic fishes. *North American Journal of Fisheries Management*, 29, 72–83. <https://doi.org/10.1577/M07-040.1>
- Li, J., Lin, X., Xu, Z., & Sun, J. (2017). Differences in swimming ability and its response to starvation among male and female *Gambusia affinis*. *Biology Open*, 6, 625–632. <https://doi.org/10.1242/bio.022822>
- Magellan, K., & García-Berthou, E. (2016). Experimental evidence for the use of artificial refugia to mitigate the impacts of invasive *Gambusia holbrooki* on an endangered fish. *Biological Invasions*, 18, 873–882. <https://doi.org/10.1007/s10530-016-1057-x>
- Moran, C. J., Ferry, L. A., & Gibb, A. C. (2016). Why does *Gila elegans* have a bony tail? A study of swimming morphology convergence. *Zoology*, 119, 175–181. <https://doi.org/10.1016/j.zool.2016.03.002>
- Nelson, J. A. (2016). Oxygen consumption rate v. rate of energy utilization of fishes: A comparison and brief history of the two measurements. *Journal of Fish Biology*, 88, 10–25. <https://doi.org/10.1111/jfb.12824>
- Norin, T., & Clark, T. D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology*, 88, 122–151. <https://doi.org/10.1111/jfb.12796>
- Ohlberger, J., Staaks, G., & Hölker, F. (2006). Swimming efficiency and the influence of morphology on swimming costs in fishes. *Journal of Comparative Physiology B*, 176, 17–25. <https://doi.org/10.1007/s00360-005-0024-0>
- Ohlberger, J., Staaks, G., Van Dijk, P. L. M., & Hölker, F. (2005). Modelling energetic costs of fish swimming. *Journal of Experimental Zoology Part A Comparative Experimental Biology*, 303, 657–664. <https://doi.org/10.1002/jez.a.181>
- Oliva-Paterna, F. J., Torralva, M., & Fernández-Delgado, C. (2006). Threatened fishes of the world: *Aphanius iberus* (Cuvier & Valenciennes, 1846) (Cyprinodontidae). *Environmental Biology of Fishes*, 75, 307–309. <https://doi.org/10.1007/s10641-006-0016-2>
- Oufiero, C. E., & Garland, T. (2007). Evaluating performance costs of sexually selected traits. *Functional Ecology*, 21, 676–689. <https://doi.org/10.1111/j.1365-2435.2007.01259.x>
- Oufiero, C. E., & Whitlow, K. R. (2016). The evolution of phenotypic plasticity in fish swimming. *Current Zoology*, 62, 475–488. <https://doi.org/10.1093/cz/zow084>
- Petterson, A. K., Marshall, D. J., & White, C. R. (2018). Understanding variation in metabolic rate. *Journal of Experimental Biology*, 221. <https://doi.org/10.1242/jeb.166876>
- Petterson, L. B., & Bronmark, C. (1997). Density-dependent costs of an inducible morphological defense in crucian carp. *Ecology*, 78, 1805–1815. [https://doi.org/10.1890/0012-9658\(1997\)078\[1805:DDCOA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[1805:DDCOA]2.0.CO;2)
- Petterson, L. B., & Hedenström, A. (2000). Energetics, cost reduction and functional consequences of fish morphology. *Proceedings of the Royal Society B: Biological Sciences*, 267, 759–764. <https://doi.org/10.1098/rspb.2000.1068>
- Plaut, I. (2000). Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline cyprinodontid, *Aphanius dispar*, acclimated to a wide range of salinities. *Physiological and Biochemical Zoology*, 73, 590–596. <https://doi.org/10.1086/317746>
- Plaut, I. (2001). Critical swimming speed: Its ecophysiological relevance. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 126, 121. [https://doi.org/10.1016/S1095-6433\(00\)80239-1](https://doi.org/10.1016/S1095-6433(00)80239-1)
- Plaut, I. (2002). Does pregnancy affect swimming performance of female Mosquitofish, *Gambusia affinis*? *Functional Ecology*, 16, 290–295. <https://doi.org/10.1046/j.1365-2435.2002.00638.x>
- Reidy, S. P., Kerr, S. R., & Nelson, J. A. (2000). Aerobic and anaerobic swimming performance of individual Atlantic cod. *Journal of Experimental Biology*, 203, 347–357. PMID:10607544.
- Rincón, P. A., Correas, A. M., Morcillo, F., Risueño, P., & Lobón-Cerviá, J. (2002). Interaction between the introduced eastern mosquitofish

- and two autochthonous Spanish toothcarps. *Journal of Fish Biology*, 61, 1560–1585. <https://doi.org/10.1006/jfbi.2002.2175>
- Royle, N. J., Metcalfe, N. B., & Lindström, J. (2006). Sexual selection, growth compensation and fast-start swimming performance in Green Swordtails, *Xiphophorus helleri*. *Functional Ecology*, 20, 662–669. <https://doi.org/10.1111/j.1365-2435.2006.01147.x>
- Scarnecchia, D. L. (1988). The importance of streamlining in influencing fish community structure in channelized and unchannelized reaches of a prairie stream. *Regulated Rivers: Research & Management*, 2, 155–166. <https://doi.org/10.1002/rrr.3450020209>
- Srean, P., Almeida, D., Rubio-Gracia, F., Luo, Y., & García-Berthou, E. (2016). Effects of size and sex on swimming performance and metabolism of invasive mosquitofish *Gambusia holbrooki*. *Ecology of Freshwater Fish*, 26, 424–433. <https://doi.org/10.1111/eff.12286>
- Stoffels, R. J. (2015). Physiological trade-offs along a fast-slow lifestyle continuum in fishes: What do they tell us about resistance and resilience to hypoxia? *PLoS ONE*, 10, 1–19. <https://doi.org/10.1371/journal.pone.0130303>
- Svendsen, J. C., Banet, A. I., Christensen, R. H. B., Steffensen, J. F., & Aarestrup, K. (2013). Effects of intraspecific variation in reproductive traits, pectoral fin use and burst swimming on metabolic rates and swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *Journal of Experimental Biology*, 216, 3564–3574. <https://doi.org/10.1242/jeb.083089>
- Svendsen, J. C., Tudorache, C., Jordan, A. D., Steffensen, J. F., Aarestrup, K., & Domenici, P. (2010). Partition of aerobic and anaerobic swimming costs related to gait transitions in a labriform swimmer. *Journal of Experimental Biology*, 213, 2177–2183. <https://doi.org/10.1242/jeb.041368>
- Tierney, K. B., Kasurak, A. V., Zielinski, B. S., & Higgs, D. M. (2011). Swimming performance and invasion potential of the round goby. *Environmental Biology of Fishes*, 92, 491–502. <https://doi.org/10.1007/s10641-011-9867-2>
- Tucker, V. A. (1970). Energetic cost of locomotion in animals. *Comparative Biochemistry and Physiology*, 34, 841–846. [https://doi.org/10.1016/0010-406X\(70\)91006-6](https://doi.org/10.1016/0010-406X(70)91006-6)
- Tudorache, C., Viaene, P., Blust, R., Vereecken, H., & De Boeck, G. (2008). A comparison of swimming capacity and energy use in seven European freshwater fish species. *Ecology of Freshwater Fish*, 17, 284–291. <https://doi.org/10.1111/j.1600-0633.2007.00280.x>
- Videler, J. J. (1993). *Fish swimming*. *Fish and fisheries series*, 1st ed. London, UK: Chapman and Hall.
- Wainwright, P. C., Bellwood, D. R., & Westneat, M. W. (2002). Ecomorphology of locomotion in labrid fishes. *Environmental Biology of Fishes*, 65, 47–62. <https://doi.org/10.1023/A:1019671131001>
- Walker, J. A., Alfaro, M. E., Noble, M. M., & Fulton, C. J. (2013). Body fineness ratio as a predictor of maximum prolonged-swimming speed in coral reef fishes. *PLoS ONE*, 8, e75422. <https://doi.org/10.1371/journal.pone.0075422>
- Webb, P. W. (1975). Hydrodynamics and energetics of fish propulsion. *Bulletin of the Fisheries Research Board of Canada*, 190, 1–158.
- Webb, P. W. (1984). Body form, locomotion and foraging in aquatic vertebrates. *Integrative and Comparative Biology*, 24, 107–120. <https://doi.org/10.1093/icb/24.1.107>
- Webb, P. W. (1994). The biology of fish swimming. In L. Maddock, Q. Bone, & J. Rayner (Eds.), *The Mechanics and physiology of animal swimming*. Cambridge, UK: Cambridge University Press.
- Webb, P. W., & Weihs, D. (1986). Functional locomotor morphology of early life history stages of fishes. *Transactions of the American Fisheries Society*, 115, 115–127. [https://doi.org/10.1577/1548-8659\(1986\)115](https://doi.org/10.1577/1548-8659(1986)115)
- Yan, G. J., He, X. K., Cao, Z. D., & Fu, S. J. (2013). An interspecific comparison between morphology and swimming performance in cyprinids. *Journal of Evolutionary Biology*, 26, 1802–1815. <https://doi.org/10.1111/jeb.12182>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Rubio-Gracia F, García-Berthou E, Latorre D, et al. Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish (*Gambusia holbrooki*). *Ecol Freshw Fish*. 2020;29:230–240. <https://doi.org/10.1111/eff.12509>

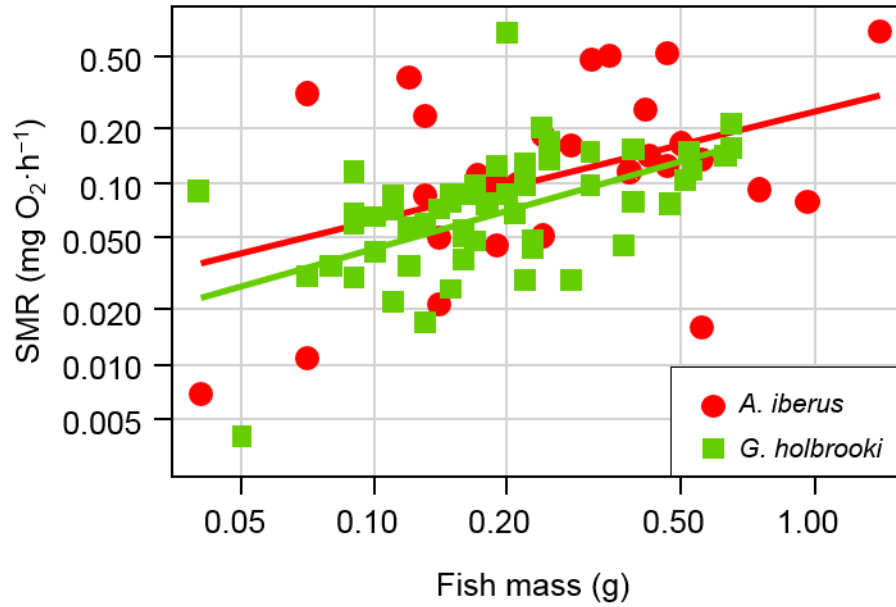


Figure S1. Relationship of standard metabolic rate SMR with fish mass for Spanish toothcarp, *Aphanius iberus* ($N = 28$) and mosquitofish, *Gambusia holbrooki* ($N = 60$). Regressions are not significantly different between species for SMR (Table 1), and thus a single linear regression is shown by species. Regression coefficients are shown in Table 2. Note both axes are on a log scale

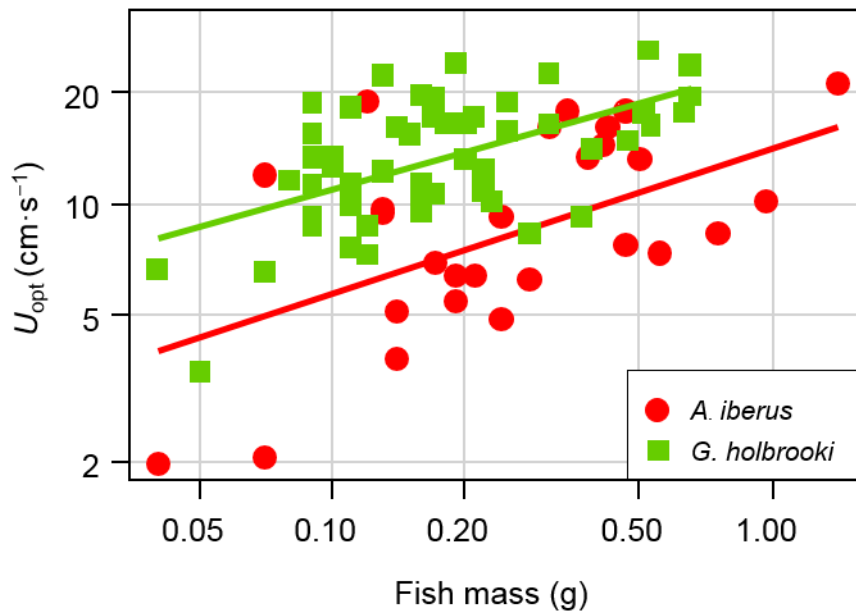


Figure S2. Relationship of optimal swimming speed U_{opt} with fish mass for Spanish toothcarp, *Aphanius iberus* ($N = 28$) and mosquitofish, *Gambusia holbrooki* ($N = 60$). The assumption of parallelism was not satisfied for U_{opt} , and therefore ANCOVA was not applied. Regression coefficients are shown in Table 2. Note both axes are on a log scale