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Intraspecific Energetic Trade-Offs and Costs of Encephalization Vary from Interspecific Relationships in Three Species of Mormyrid Electric Fishes

Kimberley V. Sukhum Megan K. Freiler Bruce A. Carlson

Department of Biology, Washington University, Saint Louis, MO, USA

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Abstract
The evolution of increased encephalization comes with an energetic cost. Across species, this cost may be paid for by an increase in metabolic rate or by energetic trade-offs between the brain and other energy-expensive tissues. However, it remains unclear whether these solutions to deal with the energetic requirements of an enlarged brain are related to direct physiological constraints or other evolved co-adaptations. We studied the highly encephalized mormyrid fishes, which have extensive species diversity in relative brain size. We previously found a correlation between resting metabolic rate and relative brain size across species; however, it is unknown how this interspecific relationship evolved. To address this issue, we measured intraspecific variation in relative brain size, the sizes of other organs, metabolic rate, and hypoxia tolerance to determine if intraspecific relationships between brain size and organismal energetics are similar to interspecific relationships. We found that 3 species of mormyrids with varying degrees of encephalization had no intraspecific relationships between relative brain size and relative metabolic rate or relative sizes of other organs, and only 1 species had a relationship between relative brain size and hypoxia tolerance. These species-specific differences suggest that the interspecific relationship between metabolic rate and relative brain size is not the result of direct physiological constraints or strong stabilizing selection, but is instead due to other species level co-adaptations. We conclude that variation within species must be considered when determining the energetic costs and trade-offs underlying the evolution of extreme encephalization.

Introduction

Both within and between species, an enlarged brain is often associated with increased cognitive abilities [Reader and Laland, 2002; Sol et al., 2005; Burns and Rodd, 2008; Kotrschal et al., 2013; Benson-Amram et al., 2016]. However, brain tissue is extremely metabolically expensive [Elliott 1948; Gallagher et al., 1998]. Interspecific studies of taxa with moderate encephalization have found that the energetic cost of an enlarged brain can be

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E-Mail karger@karger.com
www.karger.com/bbe

B.A. Carlson
Washington University
1 Brookings Drive, Campus Box 1137
St. Louis, MO 63130-4899 (USA)
E-Mail carlson.bruce@wustl.edu

mitigated by trade-offs with other energetically expensive organs or traits [Aiello and Wheeler, 1995; Isler and van Schaik, 2006; Isler and van Schaik, 2009; Fonseca-Azevedo and Herculano-Houzel, 2012]. By contrast, in taxa with extreme encephalization, there is often a positive relationship between metabolic rates and relative brain size among species [Armstrong, 1983; Isler and van Schaik, 2006; Pontzer et al., 2016; Sukhum et al., 2016].

Mormyrids are weakly electric African fishes that use electric organ discharges (EODs) for electrolocation and electrocommunication [Carlson and Arnegard, 2011]. They are well known for having large brains [Nilsson, 1996; Striedter, 2005; Sukhum et al., 2016]. We previously found that relative oxygen consumption rate is positively correlated with relative brain size across species, suggesting that mormyrids pay for an increase in brain size with an increase in basal metabolic rate [Sukhum et al., 2016]. Direct physiological constraints are one possible mechanism for this relationship between relative brain size and metabolic rate. Alternatively, strong stabilizing selection may be driving this correlation. In either case, we expect to find the same positive correlation between relative brain size and relative metabolic rate within species that we found across species. A third alternative is that relative brain size and relative metabolic rate have co-adapted across mormyrids but are not directly constraining each other. In this case, we would not expect to find a positive correlation between relative brain size and relative metabolic rate within species. Other intraspecific constraints on brain size could also be present as trade-offs between the relative sizes of the brain and other organs or between the brain and sensitivity to changes in environmental energy availability. In aquatic environments, oxygen concentration can vary greatly [Talling, 1965; Chapman and Chapman, 1998], leading to hypoxic conditions that can restrict metabolic activity [Nilsson, 1996; Chapman et al., 2002]. Measuring an organism's hypoxia tolerance is a way to determine its sensitivity to metabolic restrictions.

We determined intraspecific relationships between relative brain size and metabolic rate, relative sizes of other organs, and hypoxia tolerance in 3 mormyrid species. To include the range of variation in brain size across mormyrids, we chose species that have significantly different relative brain sizes [*Brienomyrus brachyistius* brain residuals: mean = –0.134, SE = 0.0253; *Brevimyrus niger* brain residuals: mean = 0.168, SE = 0.0203; *Gnathonemus petersii* brain residuals: mean = 0.329, SE = 0.0335] [Sukhum et al., 2016].

Materials and Methods

Animal Care

Fish were obtained from the aquarium trade and housed with conspecifics in water with a conductivity of 175–225 μS/cm, a pH of 6–7, and a temperature of 25–29 °C. Fish were kept on a 12-h: 12-h light:dark cycle and fed live black worms 4 times a week.

Specimens

We used 3 focal species for intraspecific comparisons, *B. brachyistius*, *B. niger*, and *G. petersii*, which have relatively small, medium, and large brains, respectively [Sukhum et al., 2016]. We used 14 individuals of *B. brachyistius*, 15 individuals of *B. niger*, and 15 individuals of *G. petersii*. We measured the oxygen consumption and then hypoxia tolerance of each individual before dissection to obtain organ weights.

Oxygen Consumption Rates

Oxygen consumption rates were determined using closed-chamber respirometry following previously described methods [for details see Sukhum et al., 2016]. Clean, filtered water was used for each experiment. Fish were deprived of food for at least 24 h prior to the experiment. Fish mass was determined before the experiment by gently dabbing a fish with a paper towel to remove excess water and then adding the fish to a beaker to measure the change in mass. Fish were acclimated to the respirometry chamber for 3 h with the chamber open and oxygen flowing freely. The chamber was then closed with a rubber stopper, and a polarographic dissolved oxygen probe (Analytical Sensors, Inc.; DOX) was used to measure oxygen concentration throughout the experiment. Oxygen concentrations were recorded using a dO_2 isoPod, e-corder 210 and the program Chart (eDAQ). A stir bar covered with plastic mesh was added to the bottom of the chamber to maintain water circulation. Oxygen measurements were taken every second over the course of 3 h, and a linear slope was fitted to the data to determine the oxygen consumption rate (r^2 = 0.902–0.994; p values < 10^{-16}). We had previously determined variability in oxygen consumption [Sukhum et al., 2016] by comparing the slope of oxygen consumption at half-hour increments. This analysis, which included 2 *B. brachyistius* and 2 *G. petersii* individuals, revealed no significant changes in oxygen consumption rates over time.

Hypoxia Tolerance

Hypoxia tolerance was measured using progressive hypoxia following previously described methods [for details see Sukhum et al., 2016]. Fish mass was determined before the experiment, and then fish were transferred to an 11-L tank. We prevented aquatic surface respiration, a behavior fish exhibit to obtain more oxygen at the surface of the water, by placing fish in a tube covered in netting. Oxygen concentration was measured with the dissolved oxygen probe. We recorded EODs using 2 carbon electrodes placed on opposite ends of the tank. We placed a Logitech HD Webcam c270 in front of the tank to record fish behavior throughout the experiment.

Fish were acclimated for 20 min before starting the experiment. Then, between 45–65 mL of a 500-mM solution of sodium sulfite was added to the tank to decrease the dissolved oxygen concentration at a rate of ~2 ppm per hour. During the experiment, we continuously recorded EODs and oxygen concentration. When the fish reached metabolic failure, defined as the point when a fish could no longer maintain upright swimming, or the oxygen con-

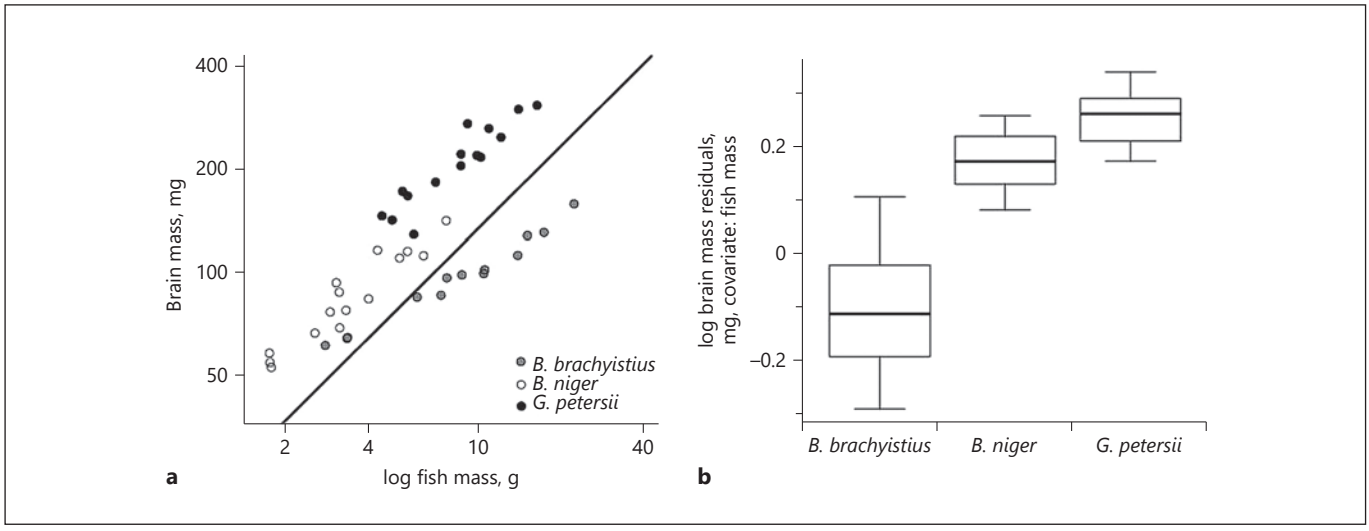

Fig. 1. Relative brain size varied between 3 focal mormyrid species. **a** Total brain mass was plotted against total body mass for all specimens from *B. brachyistius* ($n = 14$), *B. niger* ($n = 15$), and *G. petersii* ($n = 15$). Specimens were compared to the Brownian phylogenetic generalized least-square (PGLS) regression between brain and body mass found for all mormyrid species [Sukhum et al., 2016] ($y = ax^b$, a = 21.53, b = 0.79). **b** Box plot of brain mass residuals calculated from Brownian PGLS regression [Sukhum et al., 2016] for 3 focal species: *B. brachyistius*, *B. niger*, and *G. petersii* (ANOVA: $F_{2, 41} = 76.65$, $p < 10^{-13}$, Tukey HSD: *B. niger-B. brachyistius* $p < 0.01$, *G. petersii-B. brachyistius* $p < 0.01$, *G. petersii-B. niger* $p < 0.05$).

Table 1. Correlative analyses of log-transformed trait versus log-transformed body mass

	Intercept ± SE	Slope ± SE	*p* value	r^2
B. brachyistius				
Brain	1.589±0.017	0.426±0.017	***<10*$^{-11}$**	0.982
Liver	1.446±0.122	0.695±0.122	***<10*$^{-4}$**	0.732
Heart	0.480±0.110	0.753±0.110	***<10*$^{-4}$**	0.797
GI	1.280±0.174	0.985±0.174	***<10*$^{-3}$**	0.729
Kidney	0.530±0.217	0.682±0.218	***<0.01***	0.452
ROB	–0.310±0.003	1.017±0.003	***<10*$^{-15}$**	1.000
Oxygen	0.731±0.115	0.658±0.115	***<10*$^{-4}$**	0.732
B. niger				
Brain	1.596±0.032	0.620±0.057	***<10*$^{-7}$**	0.902
Liver	0.948±0.160	0.753±0.282	0.05	0.355
Heart	0.583±0.127	0.721±0.224	***<0.01***	0.444
GI	1.422±0.184	0.749±0.324	***<0.05***	0.291
Kidney	1.027±0.160	0.215±0.282	0.459	0.043
ROB	–0.028±0.002	1.018±0.003	***<10*$^{-15}$**	1.000
Oxygen	0.752±0.083	0.817±0.147	***<10*$^{-4}$**	0.703
G. petersii				
Brain	1.732±0.065	0.632±0.070	***<10*$^{-6}$**	0.852
Liver	0.982±0.219	0.853±0.235	***<0.01***	0.503
Heart	0.311±0.121	0.942±0.130	***<10*$^{-5}$**	0.802
GI	1.669±0.145	0.560±0.156	***<0.01***	0.500
Kidney	0.758±0.315	0.478±0.337	0.181	0.134
ROB	–0.031±0.004	1.015±0.004	***<10*$^{-15}$**	1.000
Oxygen	0.907±0.209	0.609±0.224	***<0.05***	0.363

SE indicates standard error.

centration remained at 0 ppm for 10 min, the experiment was stopped, and the fish was placed back into freshwater for recovery.

Oxygen concentrations and EOD data were extracted in 20-s recording blocks. EOD rate was calculated as the number of peaks in each recording block divided by 20 s. A running average for EOD rate of 25 points before and after was calculated to obtain a smoothed curve of the EOD rate throughout hypoxia experiments. Baseline EOD activity was calculated as the average EOD rate when the oxygen concentration was between 8 and 4 ppm. A threshold point in EOD activity was calculated as the oxygen concentration at which the running average dropped 1 standard deviation below the baseline EOD rate. A half-threshold point was defined as the oxygen concentration for the point halfway between the threshold point and the lowest EOD rate.

Organ Size Measurements

Fish were euthanized in 300 mg/L MS-222 (tricaine methanesulfonate) and transferred to 4% paraformaldehyde in 0.1 M phosphate buffer for immersion fixation after gilling ceased. Fish were given unique fin clips before fixation to mark individual identity during dissection. After 2 weeks, fish were moved to 70% ethanol.

Approximately 24 h prior to dissection, fish were rehydrated in 0.1 M phosphate buffer. Full wet body mass was measured before dissection. Gonads, heart, liver, gastrointestinal (GI) tract, kidney, and brain were all removed and individually massed. Stomach contents were removed before massing the GI tract. Rest of body (ROB) was determined based on subtracting all organ masses from full wet body mass.

Data and Statistical Analyses

We used a power analysis to determine whether a sample size of 14–15 individuals provided enough statistical power to find an

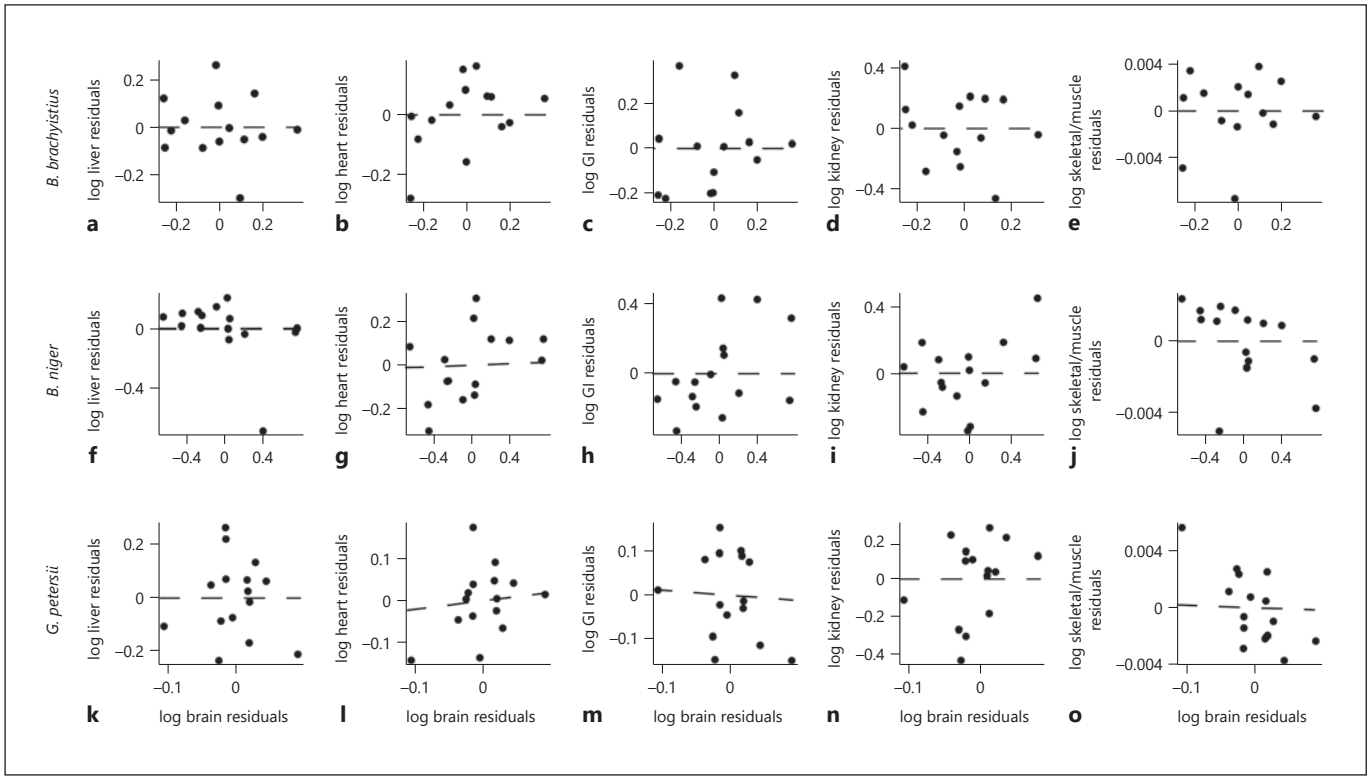

Fig. 2. No relationship between relative brain mass and masses of other organs or ROB mass. Plots of brain residuals against liver (**a**, **f**, **k**), heart (**b**, **g**, **l**), GI (**c**, **h**, **m**), kidney (**d**, **i**, **n**), and ROB mass residuals (**e**, **j**, **o**) for *B. brachyistius* (**a–e**), *B. niger* (**f–j**), and *G. petersii* (**k–o**) (mg; covariate: fish mass). Nonsignificant relationships are plotted as dashed lines.

effect using the pwr package in R [Champely, 2018]. We used the correlation coefficient from the interspecific relationship between relative metabolic rate and relative brain mass [Sukhum et al., 2016] to estimate the expected effect size. With a significance level of 0.05, and an effect size of 0.872, the likelihood of finding an effect that is present is 0.990–0.993.

For each species, we determined the static allometric relationships of each organ against body mass using all available dissection data. We did the same with the allometric relationships of oxygen consumption and log body mass using all available closed chamber respirometry experiments. To determine if scaling coefficients for the allometric relationship between brain and body mass varied between species, we used ANCOVA to compare slopes.

Then we determined log residuals of brain mass, organ masses, and oxygen consumption rates from the species-specific static allometric relationships. Linear regressions using log residual masses of heart, GI tract, liver, kidney, brain, and ROB were used to predict log oxygen consumption rates, point of metabolic failure, EOD threshold, and EOD half-threshold. To account for false positives due to multiple comparisons, we used a Bonferroni correction to determine appropriate α values. All statistical calculations were completed in R 3.0.2 [R Core Team, 2012].

Results

Relative Brain Size Varied among 3 Focal Species

We analyzed relative brain sizes among the 3 species by comparing the brain and body mass of all specimens to the mormyrid evolutionary allometric relationship determined by phylogenetic generalized least-square regression of brain versus body mass found across mormyrid species [Sukhum et al., 2016] (Fig. 1a). We then determined relative brain size from the residuals of each specimen to this evolutionary allometry and confirmed that *B. brachyistius*, *B. niger*, and *G. petersii* have relatively small, medium, and large brains, respectively (Fig. 1b). This analysis confirms that there is significant variation in relative brain size among these species (ANOVA: $F_{2, 41} = 76.65$, $p < 10^{-13}$, Tukey HSD: *B. niger-B. brachyistius* $p < 0.01$, *G. petersii-B. brachyistius* $p < 0.01$, *G. petersii-B. niger* $p < 0.05$). In addition, we used ANCOVA to do a pairwise comparison between species of brain-body mass allometric relationships. We found that the scaling coefficients of the allometric relationships were signifi-

cantly different between *B. brachyistius* and *B. niger* ($p < 10^{-16}$) and *B. brachyistius* and *G. petersii* ($p < 10^{-16}$), but not between *B. niger* and *G. petersii* ($p = 0.654$).

There Were No Correlations between Relative Brain Size and the Sizes of Other Tissues

Next, we wanted to determine whether intraspecific trade-offs between the brain and other organs exist, and whether these trade-offs differ between species with different brain sizes. We determined residuals from the species-specific static allometric relationships between body mass and each organ mass as well as ROB (Table 1). We found no significant correlations between any of the relative organ masses and relative brain mass in any species (Fig. 2; Table 2).

There Were No Correlations between Relative Brain Size and Oxygen Consumption Rates

Next, we determined whether there are intraspecific relationships between relative metabolic rate and relative brain size, and whether these relationships vary for species with different relative brain sizes. We measured oxygen consumption rates in all 3 species. There was a species-specific static allometric relationship between oxygen consumption and body mass (Table 1). Before Bonferroni correction, we found a positive correlation between relative oxygen consumption and relative brain mass in *B. brachyistius* (Fig. 3a; Table 2). After Bonferroni correction, however, there were no significant relationships between relative brain size and relative oxygen consumption in any species (Fig. 3; Table 2).

There Were No Negative Relationships between Hypoxia Tolerance and Relative Brain Size within Species

To determine whether there are relationships between hypoxia tolerance and relative brain size, we measured hypoxia tolerance in *B. brachyistius*, *B. niger*, and *G. petersii*. We looked at 3 different measurements of hypoxia tolerance: oxygen at metabolic failure, which was defined as losing the ability to remain upright and generate EODs, plus EOD threshold and half-threshold, measurements that quantified the dependence of EOD rate decreases on oxygen concentration. Both before and after Bonferroni correction, we found a negative correlation between oxygen at metabolic failure and relative brain mass within *B. brachyistius* (Fig. 3b; Table 2), suggesting a positive relationship between relative brain size and hypoxia tolerance. Within *B. niger* and *G. petersii*, we found no relationship between oxygen at metabolic failure and rela-

Table 2. Linear regression of log-transformed relative organ size and ROB size to predict relative brain size, oxygen consumption, metabolic failure, EOD threshold, and EOD half-threshold

	Brain size			Oxygen			Metabolic failure			EOD threshold			EOD half–threshold		
	slope ± SEM	*p* value	adj. α	slope ± SEM	*p* value	adj. α	slope ± SEM	*p* value	adj. α	slope ± SEM	*p* value	adj. α	slope ± SEM	*p* value	adj. α
B. brachyistius															
Brain	NA	NA	NA	**4.381±1.516**	**0.014**	0.008	***–5.330±1.488***	***0.004***	0.008	–5.337±12.834	0.683	0.008	–5.193±4.868	0.307	0.008
Liver	–0.014±0.040	0.732	0.01	–0.007±0.273	0.980	0.008	–0.180±0.291	0.548	0.008	–3.732±1.425	0.022	0.008	–1.247±0.605	0.061	0.008
Heart	0.065±0.040	0.132	0.01	–0.043±0.302	0.889	0.008	**–0.665±0.266**	**0.028**	0.008	–1.048±1.957	0.602	0.008	–0.658±0.757	0.402	0.008
GI	0.019±0.027	0.494	0.01	0.129±0.187	0.506	0.008	–0.105±0.205	0.618	0.008	0.808±1.229	0.524	0.008	0.389±0.480	0.433	0.008
Kidney	–0.016±0.022	0.470	0.01	**–0.310±0.124**	**0.028**	0.008	0.257±0.148	0.108	0.008	0.051±1.001	0.960	0.008	0.144±0.392	0.719	0.008
ROB	0±0.017	0.998	0.01	0±0.113	0.999	0.008	–0.096±0.119	0.435	0.008	–0.043±0.740	0.954	0.008	–0.064±0.291	0.828	0.008
B. niger															
Brain	NA	NA	NA	1.136±0.646	0.102	0.008	–1.936±0.735	0.021	0.008	–2.017±5.396	0.715	0.008	–2.252±2.048	0.291	0.008
Liver	–0.079±0.051	0.151	0.01	–0.167±0.137	0.245	0.008	0.177±0.177	0.336	0.008	0.463±1.085	0.677	0.008	0.162±0.429	0.998	0.008
Heart	0.106±0.064	0.120	0.01	0.297±0.162	0.090	0.008	–0.253±0.220	0.271	0.008	0.500±1.368	0.721	0.008	–0.577±0.518	0.286	0.008
GI	0.083±0.043	0.074	0.01	0.051±0.125	0.689	0.008	**–0.353±0.126**	**0.015**	0.008	–0.808±0.922	0.397	0.008	***–0.883±0.283***	***0.008***	0.008
Kidney	0.078±0.051	0.151	0.01	–0.006±0.144	0.968	0.008	–0.099±0.181	0.593	0.008	–0.903±1.061	0.410	0.008	–0.001±0.430	0.998	0.008
ROB	–0.001±0.056	0.987	0.01	–0.002±0.144	0.987	0.008	–0.342±0.157	0.336	0.008	–2.391±0.866	0.016	0.008	–0.741±0.378	0.072	0.008
G. petersii															
Brain	NA	NA	NA	0.526±0.874	0.557	0.008	1.216±0.707	0.109	0.008	1.899±3.010	0.539	0.008	2.580±1.596	0.130	0.008
Liver	–0.017±0.083	0.835	0.01	–0.482±0.228	0.054	0.008	0.154±0.230	0.515	0.008	–0.034±0.911	0.971	0.008	0.488±0.337	0.171	0.008
Heart	0.192±0.140	0.194	0.01	–0.278±0.472	0.566	0.008	–0.268±0.417	0.531	0.008	0.827±1.636	0.622	0.008	0.591±0.931	0.536	0.008
GI	–0.120±0.120	0.337	0.01	–0.271±0.392	0.502	0.008	0.122±0.351	0.735	0.008	0.006±1.377	0.997	0.008	–0.020±0.788	0.980	0.008
Kidney	0.071±0.054	0.209	0.01	–0.217±0.174	0.234	0.008	**0.356±0.129**	**0.016**	0.008	–0.109±0.634	0.867	0.008	0.488±0.337	0.171	0.008
ROB	–0.003±0.069	0.971	0.01	0.004±0.220	0.987	0.008	–0.163±0.190	0.405	0.008	–0.499±0.748	0.516	0.008	0.161±0.433	0.715	0.008

Relative traits were calculated from linear allometric models (Table 1). α values have been corrected using a Bonferroni correction. Bold cells indicate significant relationships that did not hold after Bonferroni correction. Bold, italicized cells indicate significant relationships that did hold after Bonferroni correction.

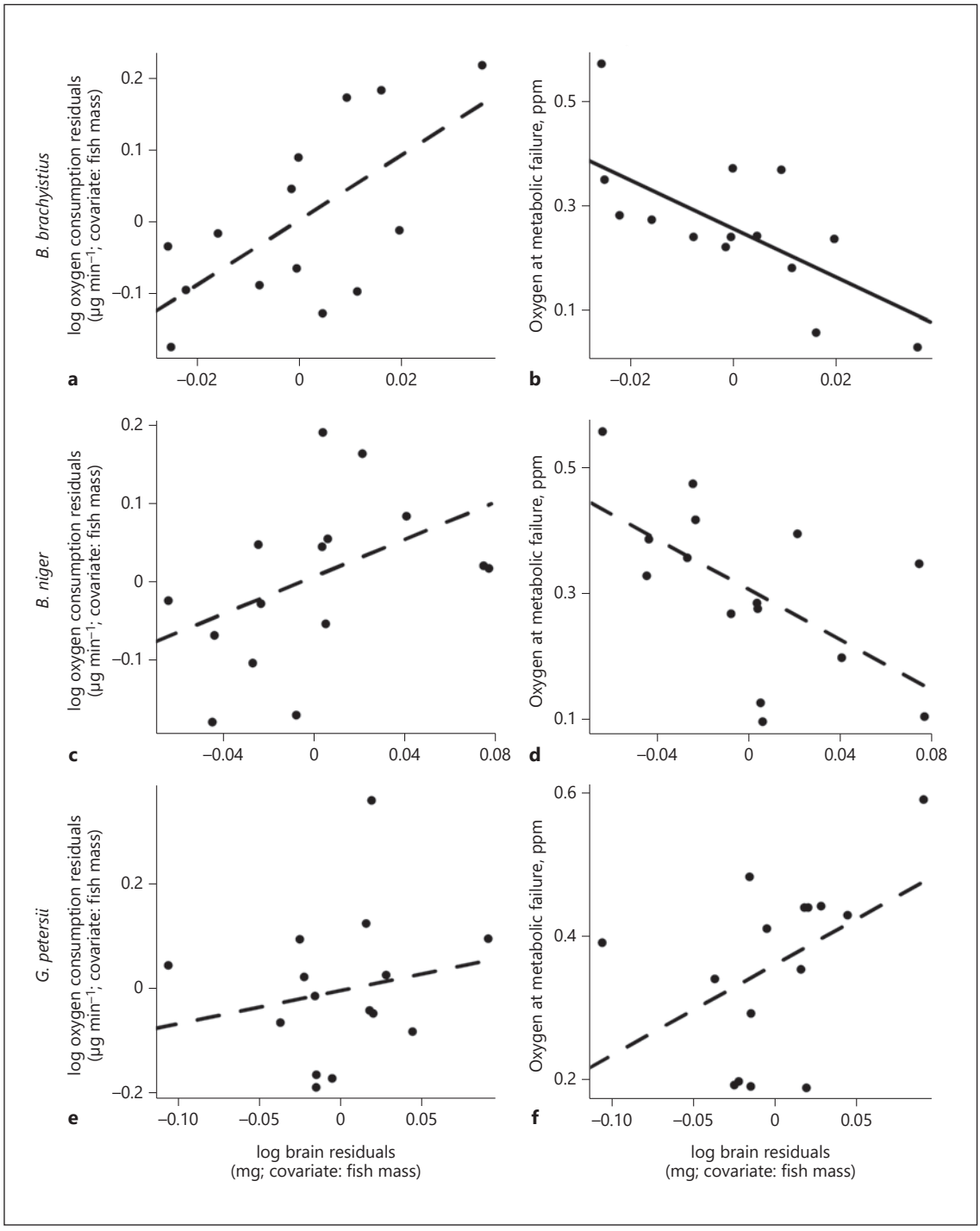

Fig. 3. There was no relationship between relative brain size and metabolic rate within species; a relationship between relative hypoxia tolerance and relative brain size was only evident in a small-brained species. Plots of brain residuals against oxygen consumption residuals (**a**, **c**, **e**) and oxygen at metabolic failure (**b**, **d**, **f**) for *B. brachyistius* (**a**, **b**), *B. niger* (**c**, **d**), and *G. petersii* (**e**, **f**). Nonsignificant relationships are plotted as dashed lines.

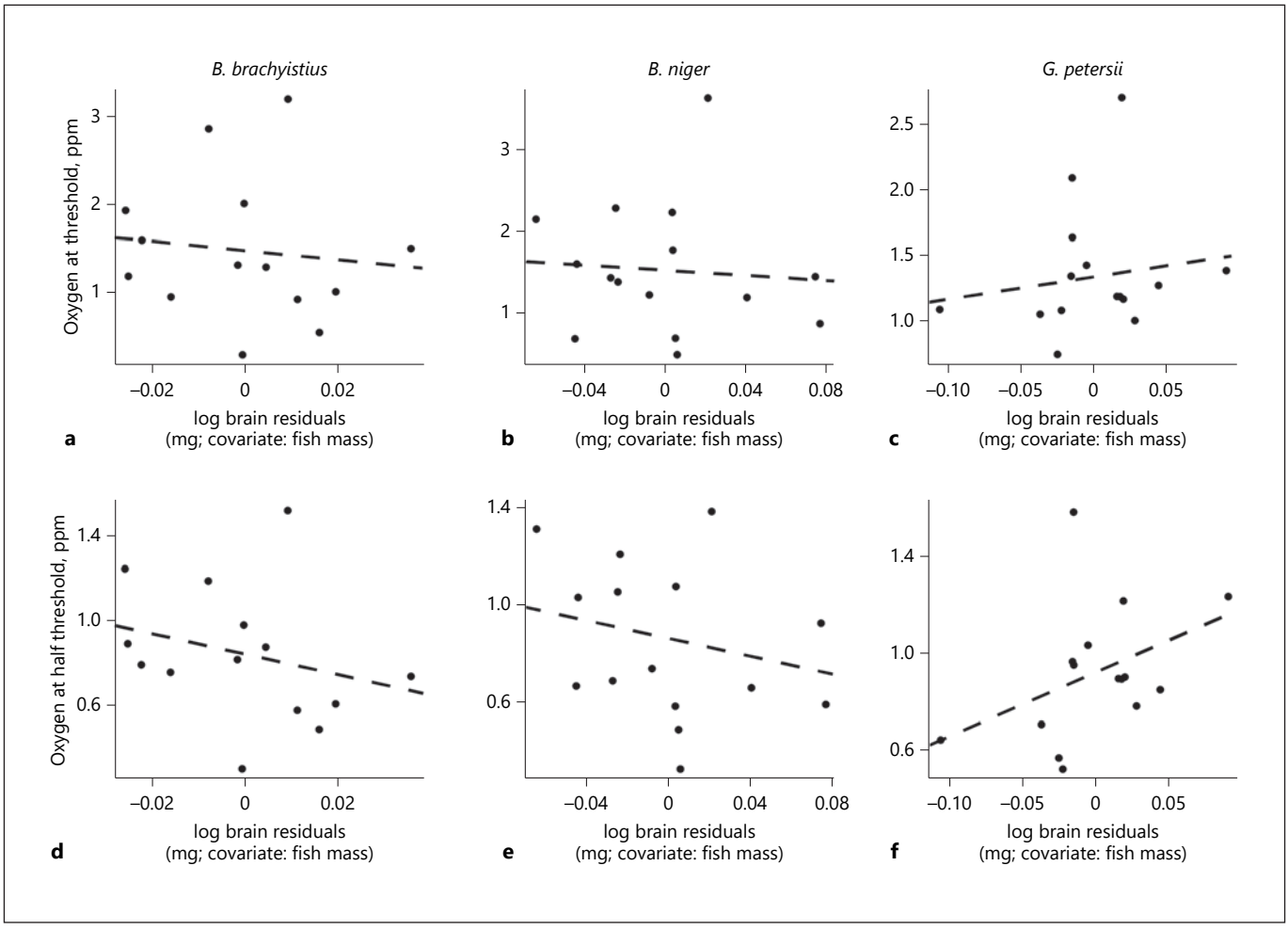

Fig. 4. There was no relationship between relative brain size and EOD hypoxia tolerance measurements within species. Plots of brain residuals against oxygen at threshold, where EOD rate decreased 1 standard deviation below baseline EOD activity (**a–c**), and oxygen at half-threshold, where EOD rate was halfway between the threshold and lowest EOD rate (**d**, **e**) for *B. brachyistius*, *B. niger*, and *G. petersii*. Nonsignificant relationships are plotted as dashed lines.

tive ROB mass (Fig. 3d, f; Table 2). We also found no correlation between EOD threshold/half-threshold and relative brain size within any species (Fig. 4; Table 2).

Discussion

We used mormyrid electric fishes from Africa to study the intraspecific metabolic costs and energetic trade-offs of increasing brain size. Previously, we found a positive interspecific relationship between oxygen consumption and relative brain size [Sukhum et al., 2016]. This relationship supported the metabolic constraint hypothesis that relative brain size is constrained by metabolic rate. In our current study, all 3 species had a positive slope estimate for the relationship between metabolic rate and relative brain size. Although these relationships were not significant in any species after correcting for multiple hypothesis testing, the p value was <0.05 in *B. brachyistius*, suggesting a trend between these variables. However, such a trend was not observed in the other 2 species. Previous studies have also found an interspecific negative correlation between hypoxia tolerance and relative brain size in mormyrids [Chapman and Hulen, 2001; Sukhum et al., 2016]. When we looked at hypoxia tolerance within species, we did not find a negative correlation with relative brain size. These data demonstrate that the relationships between brain size and organismal energetics with-

in mormyrid species do not conform to the same patterns that occur between species. Further, the narrow-sense constraint hypothesis would predict that the scaling exponents for the allometric relationship between brain and body mass are similar across species [Martin, 1981]; however, we found that *B. brachyistius* has a significantly different slope from both *B. niger* and *G. petersii*. These different scaling coefficients suggest that there are not narrow-sense constraints between brain and body size across mormyrids.

Because the patterns observed between species are not found within species, we conclude that the interspecific correlation between relative brain size and metabolic rate is not due to a direct physiological constraint or strong stabilizing selection. Although metabolic rate is not directly tied to relative brain size within a species, it may indirectly restrict the size of the brain for a given species. For example, *B. brachyistius*, our smallest-brained species, has the lowest average metabolic rate of the species studied. This low metabolic rate may restrict the maximum relative brain size in *B. brachyistius*. If relative brain size is always at the maximum size possible for a given individual's metabolic rate, then there would still be a relationship between relative brain size and metabolic rate within species, such as seen in *B. brachyistius*. However, other energetic trade-offs could also exist. Rather than having the maximum possible relative brain size, an individual might increase the size of a different organ or the time spent on other energetic activities, such as reproduction and locomotion. In cases where there is no clear correlation between relative brain size and metabolic rate, or between relative brain size and the sizes of other organs, we suggest this reflects individual variation in the allocation of energy to different organs and functions.

Since a trend between metabolic rate and relative brain size is only found within *B. brachyistius*, this pattern might be specific to species with low encephalization. Increasing relative brain size in a smaller brain results in a larger proportional increase in brain tissue than increasing relative brain size in a medium- or large-brained species. This larger proportional increase may result in a stronger relationship between metabolic rate and relative brain size in *B. brachyistius*. Although a large brain confers cognitive advantages [Reader and Laland, 2002; Sol et al., 2005; Burns and Rodd, 2008; Kotrschal et al., 2013; Benson-Amram et al., 2016], small brains potentially allow for more plastic phenotypes and a wider variety of suitable habitats due to a more generalist approach, which may be more advantageous in low-oxygen environments [Crispo and Chapman, 2010].

The intraspecific relationships between hypoxia tolerance and relative brain size may vary from the interspecific relationship because individuals within a species have a wide range of hypoxia tolerance due to developmental differences [Elliott, 1948; Chapman and Hulen, 2001]. Intraspecific correlations between hypoxia tolerance and relative brain size may only be evident after controlling for environmental variation throughout each specimen's lifespan. Further, our results suggest that increasing brain size does not negatively affect hypoxia tolerance within species. In fact, we found the opposite, both *B. niger* and *B. brachyistius* had a positive relationship between hypoxia tolerance and relative brain size, although this was only significant in our smallest-brained species, *B. brachyistius*. Because fish were restricted to a tube during the course of hypoxia experiments, it seems unlikely that this correlation is due to certain behavioral adaptations that a large brain might facilitate, such as behavioral flexibility in a complex environment [Sol, 2009] or assessing environment to overcome resource scarcity [van Woerden et al., 2011]. Instead, this correlation between relative brain size and hypoxia tolerance is more likely due to indirect effects, such as both traits being related to some other trait. One possible example is fish health. A healthier fish may have both an increased brain size and a higher hypoxia tolerance.

A limitation of this study is the small number of species and individuals used. Including more individuals and a wider variety of species in an intraspecific analysis would provide more robust results and increase comparative power. However, it is important to note that power analyses suggest we have enough statistical power to detect a relationship if the relationship is of similar strength to the interspecific relationship we had found previously. Observing current species distributions and oxygen quality in the aquatic environments these fish occupy in Africa would provide further insight into the ecological constraints on brain size evolution and the behavioral adaptations these particular species use to escape hypoxia. Future studies of mormyrid brain size evolution could also benefit from analyzing brain size differences across different populations of the same species [Gonda et al., 2009], as this would be a more direct measure of the potential ecological and selective pressures currently associated with brain size evolution. It is also important to note that, within species, brain size is developmentally plastic and can be dependent on environmental conditions, such as oxygen concentration during embryogenesis [Eifert et al., 2015]. Although it is

possible that individuals could have been raised in lower oxygen conditions, this was not accounted for in the current study, but could be a relevant avenue of future research for examining the strength of selective pressure acting on brain size evolution in the wild.

In summary, we find the intraspecific relationships between relative brain size and relative organ size, metabolic rate, and hypoxia tolerance are largely absent compared to the strong correlations demonstrated across species. Therefore, the observed interspecific correlations are likely the result of species-specific co-adaptations between evolutionary changes in brain size and organismal energetics that reflect macroevolutionary patterns. Overall, this study provided the unique opportunity to examine the metabolic costs of encephalization between species with varying degrees of brain size and, thus, permitted a more in-depth look at the relationships between brain size, metabolic costs, and energetic trade-offs.

Statement of Ethics

All procedures were in accordance with the guidelines established by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee at Washington University in St. Louis.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization and methodology, K.V.S. and B.A.C.; investigation and formal analyses, K.V.S. and M.K.F.; writing K.V.S. (original draft) and , B.A.C. and K.V.S. (review and editing).

References

Aiello L, Wheeler P. The Expensive-Tissue Hypothesis: The Brain and the Digestive System in Human and Primate Evolution. Curr Anthropol. 1995;36(2):199–221.

Armstrong E. Relative brain size and metabolism in mammals. Science. 1983 Jun;220(4603):1302–4.

Benson-Amram S, Dantzer B, Stricker G, Swanson EM, Holekamp KE. Brain size predicts problem-solving ability in mammalian carnivores. Proc Natl Acad Sci USA. 2016 Mar;113(9):2532–7.

Boddy AM, McGowen MR, Sherwood CC, Grossman LI, Goodman M, Wildman DE. Comparative analysis of encephalization in mammals reveals relaxed constraints on anthropoid primate and cetacean brain scaling. J Evol Biol. 2012 May;25(5):981–94.

Burns JG, Rodd H. Hastiness, brain size and predation regime affect the performance of wild guppies in a spatial memory task. Anim Behav. 2008;76(3):911–22.

Carlson B, Arnegard E (2011): Neural innovations and the diversification of African weakly electric fishes. Commun Integr Biol. 2011 Nov 1;4(6):720–5.

Champely S. Package 'pwr'. R package. 2018.

Chapman LJ, Chapman CA. Hypoxia Tolerance of the Mormyrid Petrocephalus catostoma: Implications for Persistence in Swamp Refugia. Copeia. 1998;3:762-8.

Chapman LJ, Chapman CA, Nordlie FG, Rosenberger AE. Physiological refugia: swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. Comp Biochem Physiol A Mol Integr Physiol. 2002;133:421-37.

Chapman LJ, Hulen KG. Implications of hypoxia for the brain size and gill morphometry of mormyrid fishes. J Zool (Lond). 2001;254(4):461–72.

Crispo E, Chapman LJ. Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. J Evol Biol. 2010; 23:2091-103.

Eifert C, Farnworth M, Schulz-Mirbach T, Riesch R, Bierbach D, Klaus S, et al. Brain size variation in extremophile fish: local adaptation versus phenotypic plasticity. J Zool (Lond). 2015;295(2):143–53.

Elliott KAC (1948): Metabolism of brain tissue slices and suspensions from various mammals. J Neurophysiol. 1948 Nov;11(6):473–84.

Emde G, h. Finding food: senses involved in foraging for insect larvae in the electric fish Gnathonemus petersii. J Exp Biol. 1998 Apr;201(Pt 7):969–80.

Fonseca-Azevedo K, Herculano-Houzel S. Metabolic constraint imposes tradeoff between body size and number of brain neurons in human evolution. Proc Natl Acad Sci USA. 2012 Nov;109(45):18571–6.

Gallagher D, Belmonte D, Deurenberg P, Wang Z, Krasnow N, Pi-Sunyer FX, et al (1998): Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass. Am J Physiol. 1998 Aug;275(2):E249–58.

Gonda A, Herczeg G, Merilä J. Adaptive brain size divergence in nine-spined sticklebacks (Pungitius pungitius)? J Evol Biol. 2009 Aug;22(8):1721–6.

Hauber ME, Bierbach D, Linsenmair KE. A description of teleost fish diversity in floodplain pools ('Whedos') and the Middle-Niger at Malanville (north-eastern Benin). J Appl Ichthyology. 2011;27(4):1095–9.

Isler K, van Schaik C. Costs of encephalization: the energy trade-off hypothesis tested on birds. J Hum Evol. 2006 Sep;51(3):228–43.

Isler K, van Schaik CP. Metabolic costs of brain size evolution. Biol Lett. 2006 Dec;2(4):557–60.

Isler K, van Schaik CP. The Expensive Brain: a framework for explaining evolutionary changes in brain size. J Hum Evol. 2009 Oct;57(4):392–400.

Kotrschal A, Rogell B, Bundsen A, Svensson B, Zajitschek S, Brännström I, et al. Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. Curr Biol. 2013 Jan;23(2):168–71.

Martin RD. Relative brain size and basal metabolic rate in terrestrial vertebrates. Nature. 1981 Sep;293(5827):57–60.

Moritz T, Linsenmair KE. The air-breathing behavior of Brevimyrus niger (Osteoglossomorpha, Mormyridae). J Fish Biol. 2007;71(1):279–83.

Nilsson G. Brain and body oxygen requirements of Gnathonemus petersii, a fish with an exceptionally large brain. J Exp Biol. 1996;199(Pt 3):603–7.

Pontzer H, Brown MH, Raichlen DA, Dunsworth H, Hare B, Walker K, et al. Metabolic acceleration and the evolution of human brain size and life history. Nature. 2016 May;533(7603):390–2.
R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2012.
Reader SM, Laland KN. Social intelligence, innovation, and enhanced brain size in primates. Proc Natl Acad Sci USA. 2002 Apr;99(7):4436–41.
Sol D, Duncan RP, Blackburn TM, Cassey P, Lefebvre L. Big brains, enhanced cognition, and response of birds to novel environments. Proc Natl Acad Sci USA. 2005 Apr;102(15):5460–5.
Sol D. Revisiting the cognitive buffer hypothesis for the evolution of large brains. Biol Lett. 2009 Feb;5(1):130–3.
Striedter GF. Principles of Brain Evolution. Sunderland (MA): Sinauer Associates, Inc.; 2005.
Sukhum KV, Freiler MK, Wang R, Carlson BA. (2016): The costs of a big brain: extreme encephalization results in higher energetic demand and reduced hypoxia tolerance in weakly electric African fishes. Proc Biol Sci. 2016 Dec;283(1845):20162157.
Talling J. The Chemical Composition of African Lake Waters. Int Rev Hydrobiol. 1965;3:421-3.
van Woerden JT, Willems EP, van Schaik CP, Isler K. Large brains buffer energetic effects of seasonal habitats in catarrhine primates. Evolution. 2012 Jan;66(1):191–9.
Wong RY, Hopkins CD. Electrical and behavioral courtship displays in the mormyrid fish Brienomyrus brachyistius. J Exp Biol. 2007 Jul;210(Pt 13):2244–52.