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NOTE

Large Brains, Small Guts: The Expensive Tissue Hypothesis Supported within Anurans

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ABSTRACT: Brain size differs substantially among species, and several hypotheses have been proposed to explain the evolution of brain size. Because the brain is among the most energetically expensive organs in the vertebrate body, trade-offs have been hypothesized to exert constraints on brain size evolution. Prominently, the expensive tissue hypothesis (ETH) proposes that reducing the size of another expensive organ, such as the gut, should compensate for the cost of a large brain. But energetic constraints may also drive covariation between the brain and other costly traits—such as body maintenance, locomotion, or reproduction—as formulated in the energy trade-off hypothesis. To date, these hypotheses have mainly been tested in homeothermic animals and within the ectothermic animals, primarily in fishes. Here, we undertake a comparative test of the interplay between energetic limitations and brain size evolution within amphibians. After controlling for phylogenetic relationships and body size, we find a negative correlation between brain mass and the length of the digestive tract within 30 species of anurans. We further find that the evolution of large brain size is accompanied by an increase in female reproductive investment into egg size. Our results suggest that the evolution of brain size follows general patterns across vertebrate clades.

Keywords: anurans, brain size evolution, expensive tissue hypothesis, trade-off, phylogenetic comparative methods.

Introduction

Brain size varies dramatically between vertebrate species, and uncovering the factors underlying the evolution of brain size is in the focus of much current research (Huber et al. 1997; Abbott et al. 1999; Safi and Dechmann 2005; Striedter 2005; Pollen et al. 2007; Gonda et al. 2009; Kotrschal et al. 2016; Pontzer et al. 2016). Large brains confer cognitive benefits (Kotrschal et al. 2013, 2014; Benson-Amram et al. 2016), but

the high amount of energy associated with maintaining brain tissue (Mink et al. 1981) should impose serious constraints on the evolution of brain size (Striedter 2005). Investigating the cost side of brain size evolution has produced several hypotheses; the direct metabolic constraints hypothesis, for example, predicts a positive association between basal metabolic rate and brain size because brain tissue is so metabolically active (Martin 1981). This was supported in mammals (Armstrong 1983) but not in birds (Isler and van Schaik 2006a). Nevertheless, metabolism is still regarded as a strong candidate to have driven encephalization, because metabolic changes at the base of the primate tree might have enabled the evolution of comparatively larger brains in primates compared with other mammal groups (Pontzer et al. 2014). However, the fact that this is not a general pattern has led to the development of the expensive tissue hypothesis (ETH; Aiello and Wheeler 1995). The ETH was based on the observation that although the human brain is three times larger than that of the chimpanzee, the basal metabolic rate per unit of body mass is very similar in both species. This was hypothesized to be driven by the decreased energy consumption of the much smaller human gut (Aiello and Wheeler 1995; Aiello et al. 2001; Aiello and Wells 2002), and it was argued that a smaller gut is able to support a larger brain only with a concurring (likely cognitively demanding) switch to higher-quality food. Since then, the ETH has been extended to other costly traits besides the gut, then termed the “energy trade-off hypothesis” (Isler and van Schaik 2006a). It predicts that in order to relocate more energy to the brain, an organism must reduce the costs of maintenance of other tissues and/or reduce the costs of reproduction and development (Isler and van Schaik 2006a, 2006b; Isler and van Schaik 2009; Navarrete et al. 2011; Liu et al. 2014). Uncovering functional trade-offs is difficult, but according to Agrawal et al. (2010), a negative genetic correlation between two traits—which are both reasonably assumed to be likely under positive direc-

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tional selection—can be interpreted as a trade-off. Evidence of selection or a shared limiting resource can strengthen this interpretation (Roff et al. 2002).

Since these hypotheses were developed, primarily comparative studies have tested their predictions. While some studies have found a negative association between brain and gut size, supporting the ETH (Kaufman et al. 2003; Tsuboi et al. 2015), or negative associations between the brain and other costly tissues (Isler and van Schaik 2006a; Pitnick et al. 2006), other studies have not found such patterns (Lemaître et al. 2009; Barrickman and Lin 2010; Navarrete et al. 2011). Most research on the cost of brain size evolution has focused on homeothermic animals (e.g., Aiello and Wheeler 1995; Jones and MacLarnon 2004; Isler and van Schaik 2006a; Pitnick et al. 2006; Navarrete et al. 2011); only recently have ectothermic vertebrates also been investigated (Kotrschal et al. 2013; Tsuboi et al. 2015). Ectothermic animals should be especially suitable to investigate the energetic constraints underlying brain size evolution because for them the cost of maintaining brain tissue should be relatively higher than for homeothermic animals. First, in terms of metabolic oxygen consumption of the central nervous system, ectotherms' brain tissue is as costly as that of homeotherms (Mink et al. 1981), but they show a 10-fold lower whole-body metabolic rate (White et al. 2006). Second, their brain metabolism is less responsive to ambient temperature than that of their bodies (Heath 1988). This may be the reason that—even though developed to explain brain size variation in primates—the strongest evidence in favor of the ETH has been found in fishes. For instance, a comparative analysis in Lake Tanganyika cichlids showed a negative association between brain size and gut length (Tsuboi et al. 2015), while artificial selection on brain size in the guppy (*Poecilia reticulata*) produced larger-brained fish with a reduced gut mass (Kotrschal et al. 2013). To determine whether this apparent brain versus gut trade-off is a general pattern in ectothermic vertebrates, it needs to be investigated in amphibians, the second major ectothermic class of vertebrates aside from the fishes. Anurans (frogs and toads) show remarkable variation in brain size and gut length (Liao et al. 2015). They therefore provide an appealing opportunity to test the ETH. Their diverse levels of reproductive investment (Fei et al. 2010) further allow investigating whether energetic requirements of encephalization may lead to covariation between brain size and reproductive investment.

Here we provide the first test of the relationship between the brain and other organs across anurans. On the basis of the ETH, we predict that a larger brain should accompany a smaller digestive tract. The heart, lungs, liver, and kidneys have been recognized as metabolically costly in previous studies, but no general patterns of covariation between the brain and those other organs have been reported (Aiello

and Wheeler 1995; Barrickman and Lin 2010; Navarrete et al. 2011; Warren and Iglesias 2012). It is therefore difficult to predict whether and how they may covary with brain size. For muscle tissue, we might predict an inverse relationship with brain mass because locomotion and brain tissue may be traded off against each other, similar to birds (Isler and van Schaik 2006a), where the mass of the main flight muscle and the brain are negatively correlated. For female reproductive investment, however, we predict—in line with what was previously found in tests of the energy trade-off hypothesis (Tsuboi et al. 2015)—that larger-brained anuran species produce larger eggs to provide the energy necessary for a larger brain but smaller clutches to compensate for the energetic requirements of large eggs. While male bats appear to trade off reproductive investment (testes mass) with investment in brain size (Pitnick et al. 2006), across all mammals (Lemaître et al. 2009) and within anurans, this does not seem to be the case (Zeng et al. 2016). Besides testes mass, sperm size determines male reproductive investment within anurans (Byrne et al. 2003; Zeng et al. 2014). We therefore also tested for a relationship between sperm size and brain size.

Methods

Data Collection

We collected a total of 251 adult male individuals from 30 species of anurans during the breeding seasons 2007–2013 at the Hengduan Mountains in China (table A1; tables A1–A3 available online). All animals were transferred to the laboratory, kept in individual rectangular tanks (0.5 m × 0.4 m × 0.4 m), and fed daily for 1 week. After starving them overnight, they were anesthetized with benzocaine and euthanized by double pithing (Jin et al. 2016). This method damaged the structural integrity of the brain stem; however, whole brain mass was not affected by this method (Jiang et al. 2015). All specimens were preserved in 4% phosphate-buffered formalin for tissue fixation. After 2 weeks to 2 months of preservation, body size (snout-vent length [SVL]) was measured to the nearest 0.01 mm with calipers, and body mass was recorded to the nearest 0.1 mg with an electronic balance. Brains were dissected out and weighed (to the nearest 0.1 mg) with an electronic balance. The number of days samples spent in the buffered formalin before dissection does not affect brain weight when correcting for body weight (Liao et al. 2015). All visceral organs (i.e., digestive tract, heart, lungs, kidneys, liver, spleen) and limb muscles (as an indicator of energetic organ; Isler and van Schaik 2006a) were separated, cleaned, and immediately weighed to the nearest 0.1 mg with an electronic balance. We measured the length of the digestive tract to the nearest 0.01 mm with calipers. Data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.k17j5> (Liao

et al. 2016). Additionally, we used published measures of brain size, clutch size, and egg size from 43 anuran species (Fei et al. 2010; Liao et al. 2015) and sperm size from another 36 species (Zeng et al. 2014; table A2).

Statistical Analyses

All statistical analyses were performed in the R statistical environment (R Development Core Team 2011). For our comparative analysis, we used the phylogeny of Pyron and Wiens (2011) to reconstruct a phylogenetic tree for the 30 study species we collected ourselves and the 36 and 43 species we obtained from the literature (figs. A1–A3, available online). Because information on branch lengths was not available for all species, they were first arbitrarily set to one following Pagel (1992). Computer simulations have shown that Felsenstein's (1985) method yields acceptable type I error rates under Brownian motion but inflates rates under other models (Diaz-Uriarte and Garland 1996). However, rescaling branch lengths decreases the rates of type I error, and the maximum type I error rate never exceeds twice the nominal P value of .05.

To account for the evolutionary relationships among species, we performed phylogenetically controlled generalized least squares (PGLS) regression analyses using log-transformed data in the APE package (R Development Core Team 2011) in the R software package (V.2.13.1). All variables were log transformed before analysis. The PGLS regression estimates a phylogenetic scaling parameter λ using the maximum-likelihood method. The parameter λ estimates the effect of phylogenetic signal on the relationship between brain mass and other visceral organs analyzed ($\lambda = 0$ indicating no phylogenetic signal, $\lambda = 1$ indicating strong phylogenetic signal). We found strong phylogenetic signals for all traits examined in our study (λ : brain mass = 0.400, digestive tract = 0.528, heart = 0.424, lungs = 0.529, liver = 0.493, kidneys = 0.508, spleen = 0.377, limb muscles = 0.517; sperm length = 0.370, clutch size = 0.411, egg size = 0.423), showing that our models require phylogenetic corrections. To test for relationships between brain mass and the visceral organs, we used brain mass as the response variable, visceral organs as independent variables, and SVL as a covariate in a series of phylogenetically controlled linear models. We analyzed the effects of interactions between SVL and the mass of the various tissues on brain mass. Seven separate multiple regression models (one for each organ) were constructed to test for the relationship between brain and organ mass. We also tested for relationships among the other visceral organs and SVL as a covariate. In three further models, we tested the relationships between brain size and three aspects of reproductive investment (sperm length, clutch size, and egg size) and SVL as a covariate. We also tested the potential influence of breeding mode (terrestrial vs. aquatic) on brain and egg

size, using a PGLS treating brain or egg size as the response variable, breeding mode as an independent variable, and SVL as a covariate. All analyses were performed by using type III sums of squares.

Results

In support of the ETH, we found a significant negative correlation between digestive tract length and brain mass when controlling for body size (fig. 1; brain mass: slope = -0.597 , $t = -3.355$, $P = .002$; body size: slope = 2.542 , $t = 8.948$, $P < .001$). Regarding the relationship between brain size and aspects of reproductive investment, we found that after controlling for the significant effect of body size (slope = 1.686 , $t = 7.328$, $P < .001$), egg size was positively correlated with the mass of the brain (fig. 2; slope = 0.475 , $t = 1.998$, $P = .049$). There was no significant relationship between the size of the brain and clutch size (slope = 0.027 , $t = 0.437$, $P = .664$) and sperm length (slope = -0.105 , $t = -0.495$, $P = .624$), despite a significant body size effect (both: slope > 1.53 , $P < .001$). Breeding mode was not associated with brain or egg size (brain: $t = -0.579$, $P = .566$; egg: $t = 0.660$, $P = .513$; fig. 2).

None of the other organs (heart, lungs, liver, kidneys, spleen, limb muscles) showed any significant correlations with brain size (all: slope > 1.668 ; table 1), after controlling for the effect of body size ($t = 2.223$, $P < .035$). The multivariate analysis showed the same results (digestive tract length: slope = -0.575 , $t = -2.782$, $P = .012$; other organs: slope > 0.322 , $t < 1.766$, $P > .093$). Brain size was not correlated with the sum of all the other organs after remov-

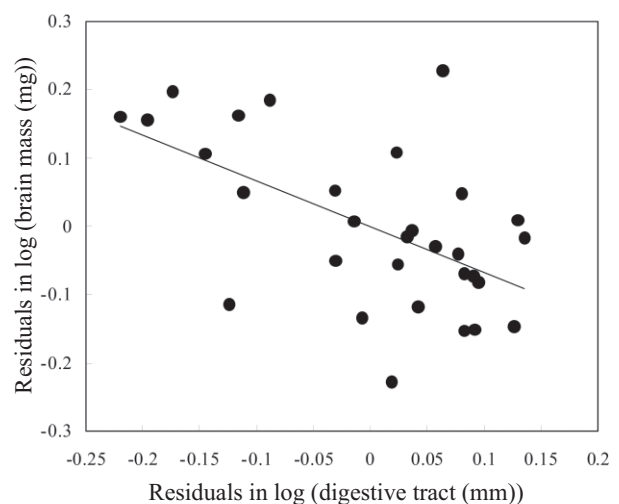


Figure 1: Correlation between residual brain mass and residual digestive tract in 30 species of anurans, controlling for body size. Phylogenetically controlled generalized least squares: slope = 3.65 , $t = 5.85$, $P < .001$.

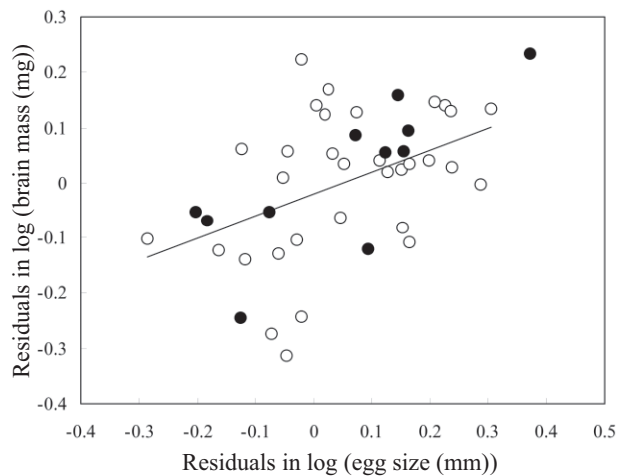


Figure 2: Correlation between residual brain mass and residual egg size in 43 species of anurans (filled circles, terrestrial breeders; open circles, aquatic breeders), controlling for body size. Phylogenetically controlled generalized least squares: slope = 0.475, $t = 1.998$, $P = .049$.

ing the body size effect (slope = -0.108 , $r = 0.238$, $t = -1.683$, $P = .104$). We did not find significant interactions between the covariate SVL and the mass of the various tissues on brain size (all $P > .09$). We found positive correlations between heart, kidneys, digestive tract, and limb muscles (fig. 3) but not after controlling for the effect of body size (fig. 3; table A3).

Discussion

Our results demonstrate a negative association between brain size and digestive tract size within anurans, which supports the ETH. Furthermore, the evolution of a larger brain is accompanied by an increase in female reproductive investment into egg size. Our study therefore not only pinpoints some of the energetic constraints underlying interspecific patterns of brain size diversification within amphibians but also adds information supporting the conclusion that the ETH is widely applicable across ectothermic vertebrates. The energy trade-off hypothesis is not supported by our results because brain size and the mass of all other measured organs were not related.

Although the data set on which the ETH is based stems from humans and other primates (Aiello and Wheeler 1995), the most convincing evidence in favor of the ETH comes from ectothermic animals, such as the elephant nose fish *Gnathonemus petersii* (Kaufman et al. 2003), 73 species of Lake Tanganyika cichlids (Tsuboi et al. 2015), the Omei wood frog *Rana omeimontis* (Jin et al. 2015), and the guppy (Kotrschal et al. 2013). In homeothermic animals, negative relationships between the brain and the gut seem rather ab-

sent (birds: Isler and van Schaik 2006a; bats: Jones and MacLarnon 2004; primates: Barrickman and Lin 2010; mammals: Navarrete et al. 2011). The ETH might therefore explain cases of encephalization in specific lineages of homeotherms but overall cannot be considered valid in these groups (Aiello et al. 2001). For instance, the ETH was originally developed as a specific explanation for the extraordinarily large brains of several anthropoid primates and humans (Aiello and Wheeler 1995). In those animals, brain mass corresponds to 1%–2% of total body mass (Striedter 2005). Potentially, the paucity of previous studies on the ETH in smaller-brained species—such as most ectotherms—can be explained by the potential misconception of them having brains too small for energy-restricting trade-offs to play crucial roles in their evolution. After all, in Lake Tanganyika cichlids, brain mass on average corresponds to only 0.07% of body mass. In the frogs and toads used here, brain mass on average corresponds to 0.3% of body mass. However, the robust demonstrations of negative associations between brain and gut in this and other recent studies both across species (Tsuboi et al. 2015) and within single species (Kotrschal et al. 2013; Jin et al. 2015) suggest that energetic constraints play an important role in vertebrate, especially ectotherms' brain evolution. Two additional non-mutually exclusive reasons may explain why negative associations between gut and brain mass seem prevalent in some and yet absent in other vertebrate groups. First, allocation strategies may differ among vertebrate clades. In birds, for instance, Isler and van Schaik (2006a) have found a trade-off between brain size and pectoral muscle mass but no relationship between brain and gut size. The authors argued that the proportionally high investment in flight ability (pectoral muscle mass) underlies this energetic trade-off in birds. Second, different vertebrate clades or even species may show more variation in total energy budget than in energy allocation. If variation in overall energy budget is larger than variation in energy allocation, two traits constrained

Table 1: Regressions of brain mass on other organ masses

Hypothesis tested and parameter	r	Slope	t	P
Expensive tissue:				
Digestive tract	.504	-.5975	-3.3551	.0024
Energy trade-off:				
Heart	.689	-.1292	-1.1009	.2806
Lungs	.747	.0617	.6185	.5414
Kidneys	.812	.0733	.4555	.6524
Liver	.695	-.2095	-1.998	.0558
Spleen	.632	-.0332	-.6258	.5367
Limb muscles	.815	.0433	.2203	.8273

Note: Data were controlled for body size. Sample size: 251 individuals, 30 species.

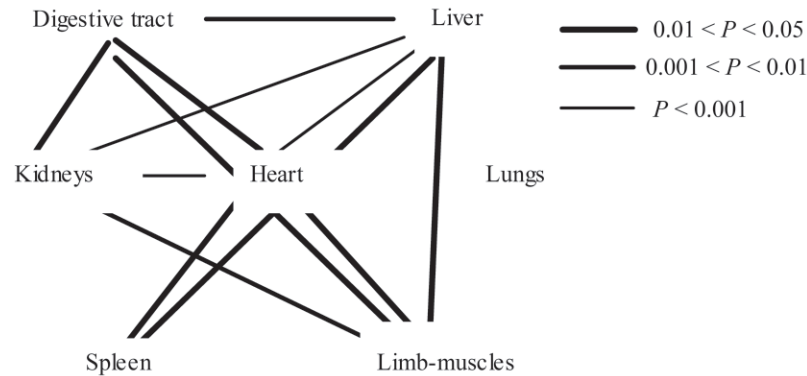


Figure 3: Correlations between visceral organs' masses in anurans. The analysis is based on a sample of 30 species and controls for phylogenetic relationships and body size. Statistical details are listed in table A3, available online.

by an energetic trade-off will not show negative correlation overall (Houle 1991; Agrawal et al. 2010).

An inverse relationship between one very costly organ (the brain) and the very organ that procures energy (the gut), as formulated in the ETH, is counterintuitive at first. Potentially, this relationship may materialize if an evolutionary increase in brain size enables a potentially cognitively driven shift toward a more nutritious diet. This is well documented in primates, where the smallest-brained species with the largest guts feed on plant matter, while the larger-brained species are more omnivorous; humans, with the largest brain and smallest gut, even outsourced part of its gut function to cooking pots (Aiello and Wheeler 1995). Some studies have argued to find support for the ETH in primates by using diet quality as a proxy of gut morphology (Fish and Lockwood 2003) and showing a positive correlation between brain size and gut. In lemurs and lorises, however, diet quality does not correlate with brain size (Allen and Key 2012). Often animals with smaller guts show higher frequencies of carnivory than those with longer guts (e.g., snakes: Secor 2001; across amphibians: Naya and Bozinovic 2004; within one species of toad: Naya et al. 2009). And even though a more carnivorous diet is not necessarily associated with larger brains (Gonzalez-Voyer et al. 2009), the better cognitive abilities that larger brains confer may be necessary to exploit more nutritious food sources. Within the anuran species used here, the potential for dietary divergence may be limited because all are insectivorous as adults. However, they may feed on insects of different nutritive value, which may demand varying levels of cognitive ability to catch; whether this is the case and, if so, whether it relates to their brain anatomy are currently unknown.

Although negative associations between two traits—such as gut versus brain size—are commonly considered as evidence for a trade-off between those two traits (e.g., Aiello and Wheeler 1995; Tsuboi et al. 2015; Kotrschal et al. 2016),

this is not necessarily so. Hard evidence for a trade-off requires demonstration of a genetic correlation between two traits and evidence that both are under positive directional selection (Roff et al. 2002; Agrawal et al. 2010). Via artificial selection on brain size and survival assays in guppies, this was established for gut and brain size in fishes (Kotrschal et al. 2013, 2015). Accepting such a scenario also within anurans seems parsimonious but needs to be substantiated in upcoming experiments, scrutinizing both the evidence for a genetic correlation between brain and gut size and whether they are under positive directional selection. The fact that a negative association between gut and brain size is also apparent within one frog species (Jin et al. 2015) corroborates our interpretation of the negative association between brain and gut size as a trade-off.

Previous studies have shown that the energetic requirements of encephalization can be met through a change in energy allocation in reproductive investment (Isler and van Schaik 2009, 2014). For instance, species with larger brains produce larger neonates in mammals (Isler and van Schaik 2009) and larger egg size in cichlids (Tsuboi et al. 2015). Our findings of a positive association between egg and brain size in anurans are congruent. Larger eggs contain more yolk and other energy reserves (Berven 1982); those reserves can increase growth rate, starvation resistance, and overall survival (Cummins 1986; Merilä et al. 2000; Altwegg and Reyer 2003; Wells 2007). It is becoming evident that large eggs are commonly found in species with relatively larger brains. Increasing propagule size may therefore be a general requirement of encephalization in vertebrates. Moreover, egg size evolution is intricately linked to the evolution of reproductive modes. The evolution of large eggs in anurans, for instance, is associated with terrestrial breeding; aquatic breeders tend to lay large clutches with small eggs, whereas terrestrial breeders tend to lay fewer but larger eggs (Duellman and Trueb 1986). Potentially, such differences

in breeding habitat may also select for differences in brain anatomy and thereby confound the relationship between egg and brain size within anurans. In our data set, this is unlikely because neither brain size nor egg size differed between aquatic and terrestrial breeders.

It seems surprising that we found larger eggs in larger-brained species but no difference in clutch size; those results appear to violate the classic “few but large versus many but small” trade-off in offspring production (Duarte and Alcaraz 1989), and they are inconsistent with the finding that guppies artificially selected for larger brains produce fewer offspring (Kotrschal et al. 2013). We suggest that larger-brained anurans may afford a generally increased reproductive investment because of their hypothesized better ability to exploit food resources of higher quality via better cognitive abilities. Non-mutually exclusive, they may also be able to save energy by reducing another unmeasured trait, such as the number of clutches per season (Wells 2007). However, the lack of negative association between egg size and clutch size may simply be due to lack of statistical power and reduced variability within the limited set of species used; after all, such a trade-off is apparent when a greater number of species is considered (Gomez-Mestre et al. 2012). Within male anurans, the fact that we did not find a relationship between brain size and sperm size suggests that postcopulatory sexual selection (sperm competition) does not promote a larger brain (see also Zeng et al. 2016); even though failure to detect a significant effect should not be used as evidence against such a relationship, our results add to the growing body of evidence suggesting that male reproductive investment and brain size are unrelated across vertebrates (Lemaitre et al. 2009; Kotrschal et al. 2013).

Is there any evidence for further trade-offs between the brain and other organs? In motion, muscle tissue consumes the most energy, and even when at rest, it requires a considerable proportion of energy. Muscle tissue was therefore included in the ETH (Aiello and Wheeler 1995) and indeed was found to be negatively associated with brain size in birds (Isler and van Schaik 2006a). Considering terrestrial movement, the efficient form of bipedal locomotion that arose alongside the transition from australopithecines to early *Homo* should have led to reductions in energy expenditure (Pontzer et al. 2010). Anurans predominantly use their large hind limbs for movement; the mass of those muscles may therefore be a proxy for the costs of locomotion. Also, within the anurans, species/individuals with better locomotive capability should have better ability to (1) search for mates, (2) move to areas where the diet is of better quality, and (3) avoid predators (Duellman and Trueb 1986). In all of those aspects, better cognitive abilities (hence larger brains) should be an alternative strategy to higher muscle mass because better cognitive abilities should (1) increase reproductive fitness because of better social acuity (Dunbar

1998), (2) facilitate the location and exploitation of food sources of better quality (Lefebvre et al. 1997), and (3) help to evade predators (Kotrschal et al. 2015). However, our data do not support those assumptions, because we found no evidence for a trade-off between brain size and limb muscle mass. Also, and in line with Navarrete et al. (2011), we did not find any negative correlations between brain mass and heart, liver, lungs, spleen, or kidney mass. This can be explained by the inference that energetic costs of one expensive organ may not necessarily directly affect another expensive organ but rather may distribute its effects on the investment in several other organs (Lemaitre et al. 2009). A similar reasoning may be applied to explain why we did not find any negative correlations among the organs besides the brain.

Acknowledgments

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Group spawning in *Polypedates megacephalus*, Beihai, Guangxi Zhuang Autonomous Region. Photo credit: Wen Bo Liao.