JOURNAL OF Evolutionary Biology

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Journal:	*Journal of Evolutionary Biology*
Manuscript ID	Draft
Manuscript Type:	Research Papers
Keywords:	encephalization, liver, brain, gonad, swim bladder, Evolutionary physiology, Life history evolution, reproductive biology, batrachoidiformes, allometry

Testing ontogenetic patterns of sexual size dimorphism against expectations of the expensive tissue hypothesis, an intra-specific example using Oyster Toadfish (*Opsanus tau*)

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Acknowledgements

We thank Michael Greco, Joseph Mathews, Christian Pruneda, Cameron Day, and Captain Mike Garvilla for collecting specimens during the Delaware Division of Fish and Wildlife trawl surveys, and S. Guthrie , W. Brooks, L. Roupe, Z. Creech for their help in processing fish used in this study.

Author Contributions

A.D. & R.W. conceived of the study. R.W. collected samples. A.D., K.Z., A.L., G.H., and L.L. collected data. R.M., A.D., and D.W. performed analyses. A.D., D.W., K.Z., R.M., T.I., A.L., and R.W. wrote the initial manuscript. All other authors contributed to the subsequent writing and development of the manuscript.

Funding

Trawl collections were funded by the U.S. Fish and Wildlife Service through the Federal Aid in Fisheries Restoration Act.

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Abstract

Trade-offs associated with sexual size dimorphism (SSD) are well documented across the Tree of Life. However, studies of SSD often do not incorporate ontogeny. This restrictive perspective limits our understanding of potential investment trade-offs between metabolically expensive structures under sexual selection and other morphological modules. Based on the expectations of the expensive tissue hypothesis (ETH), investment in one metabolically expensive structure should come at the direct cost of investment in another. Here we examine allometric trends in the ontogeny of oyster toadfish (*Opsanus tau*) to test whether investment in structures known to have been influenced by strong sexual selection conform to these expectations. Despite recovering clear changes in the ontogeny of a sexually selected trait between males and females, we find no evidence for predicted ontogenetic trade-offs with metabolically expensive organs. Our results are part of a growing body of work demonstrating that increased investment in one structure does not necessarily drive a wholesale loss of mass in one or more organs.

Introduction

Pronounced differences in ecology, life history, or morphology between males and females of the same species are common features of the vertebrate Tree of Life (Nottebohm & Arnold, 1976; Herler *et al.*, 2010; Barrett & Hough, 2012; Karp *et al.*, 2017; Lamb *et al.*, 2017). Sexual size dimorphism (SSD), the variation between sexes in aspects of size, is a particularly striking pattern that has commanded the attention of researchers since Darwin (Darwin, 1871; Scudder, 1876; Clutton-Brock *et al.*, 1977; Shine, 1978; Rohner *et al.*, 2016). The past several decades have yielded remarkable insights into the eco-evolutionary dynamics of SSD (Price, 1984; Legrand & Morse, 2000; Maan & Seehausen, 2011; Sonerud *et al.*, 2012), as well as the numerous evolutionary trade-offs associated with SSD (Gustafsson *et al.*, 1995; Simmons & Emlen, 2006; Dunn *et al.*, 2015). However, ontogeny is infrequently considered in studies of SSD (Glassman *et al.*, 1984; German, 2004; Hassell *et al.*, 2012; Holton *et al.*, 2016). This restrictive perspective precludes a broader understanding of how SSD shapes fundamental aspects of phenotypic evolution in vertebrates, in particular the investment in metabolically expensive organs.

The evolution of SSD requires selection to promote changes in some aspect of allometric growth (Bonduriansky, 2007). However, how modular these changes are remains unclear. Do such ontogenetic changes reflect trade-offs with other components of a given species' bauplan? This question is particularly relevant for SSD in metabolically or developmentaly costly organs, as organisms are faced with a finite energy budget that they can invest into different structures in order to accumulate biomass. This raises the question of not only how organisms have evolved the sometimes extreme differences in organ size observed today, but whether there are hidden costs to SSD. An often invoked answer to the generalized question of how organisms are able to

change biomass investment in metabolically expensive organs was first conceptualized by Aiello and Wheeler (1995) in the form of the expensive tissue hypothesis (ETH). This hypothesis specifically posited that investment in a major metabolically expensive organ, the brain, should come at a cost to one or more other organ systems. As costly traits characterized by SSD (such as gonads or ornaments) become expressed, expectations of the ETH suggest that energy budgets will be differentially balanced between sexes, thereby driving reduced investment in the brain or other structures for the sex under selection. While the ubiquity of trade-offs in life-history evolution provide intuitive appeal for the ETH, evidence supporting the expectations of this hypothesis has not been overwhelming.

Interspecific studies of metabolic trade-offs between organ systems have yielded mixed results for the ETH that include positive (Tsuboi *et al.*, 2015; Liao *et al.*, 2016; Sukhum *et al.*, 2016), contrary (Jones & MacLarnon, 2004; Bordes *et al.*, 2011), or a lack of support (Isler & van Schaik, 2006; Schillaci, 2006; Lemaître *et al.*, 2009; Navarrete *et al.*, 2011) for the ETH. Likewise, intraspecific studies have also yielded a mix positive support (Kotrschal *et al.*, 2013, 2015, 2016) and inconclusive/negative evidence (Warren & Iglesias, 2012). It is important to consider that the ETH was initially formulated with the intent of understanding size variation in the vertebrate brain (Aiello & Wheeler, 1995), and therefore work investigating trade-offs has been almost entirely focused on the potential costs associated with increased brain size. Whether the ETH provides a predictive framework for understanding the impact of SSD in gonads or other costly organs in devlopment remains unclear. Does SSD limit investment in the brain or other organs consistent with the expectations of the ETH?

Oyster toadfish (*Opsanus tau*) represent an exemplary species in which to investigate the impact of SSD on the ontogeny of metabolically costly traits. The physiology and life history of

this species has been consistently studied for over a century (Clapp, 1891; Tracy, 1926; Gray & Winn, 1961; Schwartz & Dutcher, 1963; Fine, 1975; Fine *et al.*, 1995; Fine & Waybright, 2015) and SSD has been well documented in one unusual metabolically expensive trait: the swim bladder. The swim bladders of oyster toadfish and their close relatives (Batrachoididae) are not primarily used for buoyancy, but instead serve as highly derived sound production organs that are unusual among teleost fishes (Fine, 1975). In oyster toadfish, both males and females use these organs for grunt based communication (Fine & Waybright, 2015), but male swim bladders emit a specific 'boat whistle'-like call to attract mates, resulting in a nearly two-fold increase in swim bladder size (Fine, 1975; Fine *et al.*, 1990). The demands of this call have driven a nearly 50% increase in the size and number of fibers in sonic muscles that surround the bladder, giving rise to one of the fastest twitching muscles found in vertebrates (Fine *et al.*, 1990). Although there is a clear metabolic cost associated with the development of these traits, the allocation of energy by male toadfish to tissue investment and the potential cost to the development of their brains or other organs remains unclear.

Here we analyze body, brain, liver, swim bladder, heart, and gonad mass collected from a population of oyster toadfish to test whether SSD drives ontogenetic trade-offs that support expectations of the ETH. We first quantify the allometric relationships of all organs to validate previous observations of SSD and test for differences in allometry between sexes. We then assess whether significant ontogenetic increases of organ masses with SSD are negatively correlated with the mass of other organs as anticipated by the predictions of the ETH. Results of our study provide a much needed ontogenetic investigation of SSD within the conceptual framework of the ETH, and provide a critical perspective on the need for further development of

theoretical expectations concerning the evolutionary relationship between sexual selection and organismal energy budgets.

Materials and Methods

Specimens for this study were collected by research trawl across several sites in the western Delaware Bay between August and November 2016 (Greco 2017) (Supplemental Figure 1). For each fish, standard and total length were recorded in millimeters (mm), and wet body mass, liver mass, heart mass, swim bladder mass, brain mass, and eviscerated body mass were recorded in milligrams (mg). Thirty six males ranging from 91 to 262 mm and 19 females, ranging from 120 to 237 mm in total length were examined (Supplemental Materials Table 1).

To quantify allometric relationships between eviscerated body mass and each organ mass for males and females, we used analysis of covariance (ANCOVA). Prior to analysis, organ and eviscerated body masses were $\log_{10}$ transformed. A linear model was fit with tissue mass as the dependent variable and eviscerated body mass, sex, and sex*eviscerated body mass as independent variables. Body mass was treated as a covariate and sex difference was assessed by determining whether separate regression lines fit the data better than a single regression line. Sex difference was tested using an F test with 2 degrees of freedom (df) in the numerator, one df for a sex difference in slope and the other df for a sex difference in intercept. An alpha level of 0.05 was used to infer a sex difference in body mass allometry for each organ. When an allometric difference between sexes was inferred, the result for a test for difference in slope was reported.

To evaluate ETH for male swim bladder as a hypothetically expensive tissue, we estimated partial correlation coefficients between swim bladder and each of four candidate tissues (gonad, heart, brain, liver). As applied here, partial correlation measures linear association between swim bladder and a candidate tissue while controlling for the influence of

body mass (see supplemental materials). When controlling for a single variable, in this case body mass, the partial correlation coefficient can be obtained from three correlation coefficients, swim bladder – body mass, target organ – body mass and swim bladder – target organ (see supplemental materials). Again, all tissue masses were $\log_{10}$ transformed prior to computing correlations and 95% confidence intervals for partial correlation coefficients were based on a t distribution with n-3 degrees of freedom. A negative partial correlation coefficient for a target organ supports an ETH interpretation for swim bladder. Results obtained through partial correlation were additionally compared to multivariate and univariate regression (see supplemental materials).

Results

We found significant allometric differences between males and females for swimbladder mass ($F_{2,56}$= 13.38, $p < 0.0001$), gonad mass ($F_{2,55}$= 42.02, $p < 0.0001$) and liver mass ($F_{2,56}$= 12.13, $p < 0.0001$). No significant difference between sexes was found for heart mass ($F_{2,56}$= 2.902, p= 0.063) or brain mass ($F_{2,56}$= 1.924, p= 0.155). ANCOVA results supported a sex difference in slope for the swim bladder and body mass relationship ($F_{1,56}$ = 20.06, $P < 0.001$), providing strong evidence for a difference in allometric growth of the swim bladder between males and females. Visualizations of the allometric trajectory for swim bladder mass show large males possess larger swim bladders than females of comparable body mass (Figure 1). Females appear to diverge from males in the slope of the regression of gonad on body mass (Figure 1), however, the 1 df test for a sex difference in slope did not achieve statistical significance ($F_{1,56}$= 3.762, P = 0.058). Likewise, no strong support was found for an allometric difference between sexes in liver mass ($F_{1,56}$= 0.978, p= 0.327; Figure 1).

Point estimates and associated 95% confidence intervals for partial correlation coefficients between swim bladder and four organs (gonad, heart, brain, liver) are plotted in Figure 2. Three partial correlation coefficients point estimates are negative and one is positive. However, all confidence intervals include zero (Figure 2), thereby providing no strong evidence for a negative linear association between swim bladder and any of the four internal organs individually examined. The sum of heart, brain and liver mass was also evaluated for a negative association with swim bladder and exhibited the same result as individual organs. Collectively, all of our results provide no support for a negative correlation between swim bladder mass and the mass of any other candidate organ, and thereby do not support the expectations of the ETH.

Discussion

Swim bladder SSD has been well documented in oyster toadfish (Fine, 1975; Fine *et al.*, 1990). Fine (1975) proposed that the size differences in the toadfish swimbladder are the result of different growth trajectories between males and females. Our findings support this hypothesis (**Figure 1**), suggesting that a change in allometric slope underlies these changes (**Figure 1**). Despite finding clear evidence of a significant change in the allometric slope of swim bladder growth between toadfish sexes, our analyses did not recover any evidence of a coordinated trade-off in the ontogeny of another organ (**Figure 2**). These results run contrary to the expectations of the ETH, but it is unlikely that this lack of coordinated change is the result of swim bladder ontogeny not being metabolically expensive in this species. Male toadfish swim bladder mass has previously been found to be highly correlated with both sonic muscle size and the number of fibers (Fine, 1975), making swim bladder mass a good proxy for the heavy energetic cost associated with the development of the male toadfish acoustic repertoire. It is certainly possible that a trade-off between a tissue or life-history trait and swim bladder mass may exist and was

simply not examined here. However, assuming a hypothesis is true until evidence is found to validate it while ignoring negative results is well outside of the principles of evidence based science (Sober, 2008). Given that we find no significant negative change in brain, liver, or heart allometric trajectories, our study adds to the growing number of studies that have failed to recover support for the ETH in traits where the expectation of positive evidence is likely (reviewed in Warren and Iglesias 2012).

Changes in allometric slope are thought to be rare within species, and the evolvability of allometric slopes has repeatedly been hypothesized to be low (Voje *et al.*, 2014). In a survey of allometric data spanning insects to primates, Voje et al. (2014) found only two examples of allometric slope differences below the species level. Although our findings support the oyster toadfish swimbladder to represent an additional case of an evolvable allometric slope (**Figure 1**), care must be taken to not dismiss a hypothesis of low allometric slope evolvability between sexes. Other species of Batrachoidiformes, such as members of the genus *Porichthys* (Midshipman) have been found to be sexually dimorphic in swim bladder size (Mohr *et al.*, 2017). *Porichthys* and *Opsanus* share common ancestry at least 30 million years ago (Near *et al.*, 2013), suggesting that the changes in ontogenetic slope found in our study may have deep evolutionary origins that vastly exceed the origin of this species. Future work placing the ontogenetic trends of the Batrachoidiform swim bladder evolution into a phylogenetic framework as well as work that assesses the devlopmental mechanisms of ontotgenetic change between sexes of multiple species both represent exciting frontiers that are necessary to evaluate the origin of this unusual organ and related musculature.

The decoupling of ontogenetic changes between the swim bladder and other organs found in our study additionally raises the possibility that the ontogeny of the swim bladder and related

191 musculature may represent a morphological module. Modularity, the degree of separation of one
192 axis of phenotype from other organismal parts, is a fundamental principle of biological
193 organization (Esteve-Altava, 2017). However, the expectations of the ETH suggest that the
194 modular organization of biological forms can impact the development of the brain as a
195 consequence of resource allocation constraints. We found no clear relationship between brain
196 mass and the mass of any other organ. This raises the question of what extent we expect
197 increased investment of one module directly having a negative impact on the ontogenetic
198 trajectory of another.

199 Studies of resource allocation trade-offs have suggested tissue proximity to be a potential
200 predictor of changes in investment (Emlen, 2001). Although organisms must use a finite energy
201 budget to accumulate body mass, the ubiquity of modularity in organismal systems ranging from
202 mammals to fishes (Larouche *et al.*, 2015; Esteve-Altava, 2017) without obvious trade-offs
203 between adjacent tissues (reviewed in Warren and Iglesias 2012) suggests that simple economic
204 predictions between morphological modules may not have much explanatory power for
205 understanding the evolution of most ontogenetic pathways without a more detailed perspective of
206 lineage specific energy budgets and life history. While investment trade-offs between
207 morphological modules have provided evidence for the expectations of the ETH in few animal
208 lineages (Emlen, 2001; Moczek & Nijhout, 2004; Liao *et al.*, 2016), the large number of studies
209 that have failed to recover support in other lineages suggests that the broad expectations of the
210 ETH are far from a universal rule (reviewed in Warren and Iglesias 2012).

211 While increases in the energetic cost of one morphological module do not often lead to
212 negative coordinated changes in another, this does not preclude the possibility that some modules
213 are in fact faced with a possible deficit in energy as sexual selection emphasizes trait investment

(Moczek & Nijhout, 2004). However, without a detailed understanding of the ecology and energy requirements of a species, it is not clear to what extent organisms can offset deficits through changes in behavior or feeding ecology. Such subtle changes may in part explain the strong evidence for the ETH in experimental laboratory settings with tight controls (Kotrschal *et al.*, 2013, 2015, 2016), despite limited support from wild populations such as the fishes in this study. Further, an organism is comprised of a suite of morphological modules that collectively use an energy budget to invest mass into their respective structures. Numerous subtle increases or decreases to energy consumption across any number of modules can therefore offset the cost of strong sexual selection to a select module. Such a readjustment of energy budgets is reminiscent of many-to-one mapping of form to function, where numerous phenotypic solutions give rise to the same functional properties of a trait (Wainwright *et al.*, 2005). As such, the repeated lack of evidence for the ETH may not result from a lack of trade-offs, but rather from subtle and complex adjustments of ontogenetic investment between numerous morphological modules across an entire organism that cannot be detected through mass based approaches alone. Future studies assessing the behavior, feeding ecology, and energy costs of different morphological modules will be needed to determine if any of these hypotheses explain the lack of trade-offs between the male toadfish swimbladder and other organs.

Conclusion

Determining the impact of energetic trade-offs in the ontogenetic pathways that give rise to the diversity of phenotypes we observe today is a fundamental axis of evolutionary biology. While the ETH held promise of a general evolutionary principle, evidence for direct trade-offs between the brain and other metabolically expensive organs has been limited to few clades. In

contrast, numerous studies, including this study, have reported negative evidence over a broad spectrum of clades. While a one-to-one mapping of brain investment increase to trait reduction does appear to exist in some species (Tsuboi *et al.*, 2015, 2016; Kotrschal et al. 2013), these examples are few in number. Refinement of the ETH as well as the formulation of new metabolic investment hypotheses are warranted and needed to broaden our perspective on energetic trade-offs. Such hypotheses are critical if we are to develop new insights into the role of sexual selection in shaping other aspects of organismal form.

Competing Interests

The authors declare no competing financial interests.

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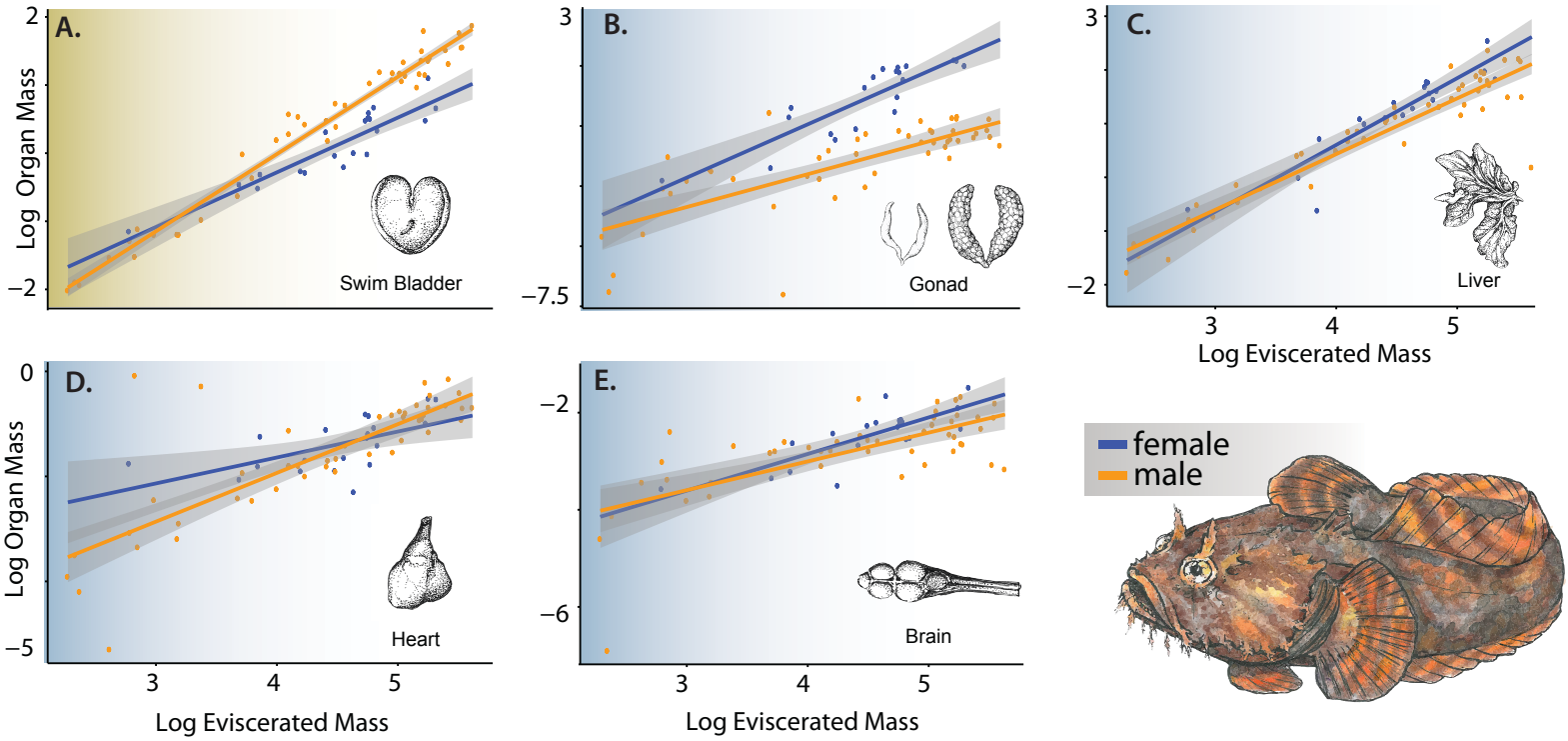
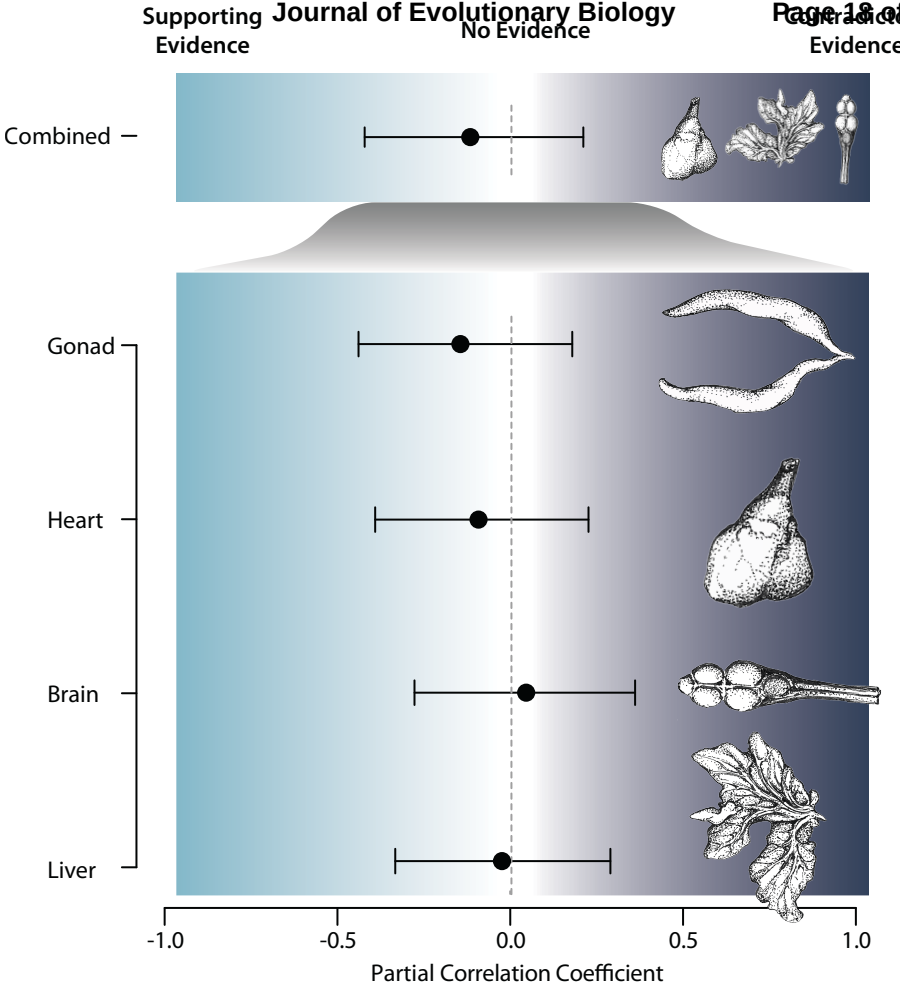
Figure Legends

Figure 1: Patterns of SSD in oyster toadfish for (A) Swim Bladder, (B) Gonad, (C) Liver, (D) Heart, and (E) Brain masses. Females are depicted in blue, males are depicted in orange. Light shading of the plot indicates significant evidence for SSD based on an ANCOVA.

Figure 2: Partial regression coefficient estimates for linear relationships between mass of swim bladder and other putative metabolically expensive tissues. A 95% confidence interval for a negative correlation coefficient that excludes zero would support the expectation of the ETH (light shading), while a confidence interval for a positive coefficient that excludes zero would provide contrary evidence (dark shading). Partial correlation intervals that include 0 provide no evidence for or against the expectations of the ETH.


A.
B.
C.
D.
E.
Log Organ Mass
Log Eviscerated Mass
Swim Bladder
Gonad
Liver
Heart
Brain
female
male
2
−2
3
−7.5
0
−5
−6
4
5


Supporting Evidence
No Evidence
Contradictory Evidence
Combined
Gonad
Heart
Brain
Liver
-1.0
-0.5
0.0
0.5
1.0
Partial Correlation Coefficient

Supplemental materials

Methods

Partial correlation

Partial correlation measures linear association between two variables while controlling for one or more other variables, and has been suggested as a useful measure of effect size in biological studies (Nakagawa and Cuthill 2007). Correlation is an intuitively attractive measure because it is unit-less, lies in a closed interval [-1,1] and is symmetric in x and y. The partial correlation coefficient derives from a partial regression coefficient that, in this application, estimates the slope for tissue effect on swim bladder adjusted for body mass in a multiple regression model. The partial regression coefficient can be standardized resulting in a standardized partial regression coefficient, which is closely related to the partial correlation coefficient. In this study, there is a single covariate, body mass, to be controlled in assessing the linear relationship between swim bladder mass and a candidate tissue mass. The partial correlation is numerically equivalent to the correlation between residuals from separately regressing swim bladder on body mass and candidate tissue on body mass. Residuals from the same individual are treated as paired data in computing Pearson's product moment correlation. Alternatively, when there is a single covariate to be controlled the partial correlation coefficient can be computed explicitly as a function of three zero-order correlation coefficients using the following expression

$$r_{xy.z} = (r_{xy} - r_{xz}\, r_{yz})/(\sqrt{(1 - r_{xz}^2)(1 - r_{yz}^2)})$$

Here x represents swim bladder, y represents candidate tissue and z represents body mass.

Multivariate and Univariate Regression

In addition to the above approaches, estimates of the correlation between swim bladder residuals and residuals of each of the other tissues (i.e. brain, liver, gonad and heart) after accounting for body mass were obtained by fitting a multivariate linear regression model. In this model, the dependent variable is a vector of log-transformed organ masses, $[\text{swim bladder, brain, liver, gonad, heart}]^T$, and the independent variable is a log-transformed body mass. Partial correlation coefficients between elements of the multivariate dependent variable, which include partial correlations between swim bladder and each of the other tissues, can be obtained by computing correlation coefficients from the variance-covariance matrix of residuals from this model. When swim bladder and other tissue masses contain no missing values, correlations obtained from this approach are the same as those obtained from the above described procedures. Analyses were also repeated using univariate regression as this approach is commonly used for studies of this type (e.g., Berner 2011). All analyses were conducted in R.

Results

Multivariate and Univariate Regression

Results of a multivariate regression correspond to results obtained through partial correlation analysis. We recover a mixture of small positive and negative partial correlation coefficients (Supplemental Figure 2), however, no significant effect was detected between swim bladder mass and other masses from other candidate organs. Likewise, univariate regression yielded regression coefficients whose estimates overlap with zero (Supplemental Figure 3), and identical correlations to the multivariate results when data was standardized to include no missing values. Plots of the residual variation of organ mass on swim bladder mass following a regression on body mass demonstrating the lack of trend expected when no strong relationship is present (Supplemental Figure 4).

Supplemental Tables

	Eviscerated Mass (g)	Gonad Mass (g)	Liver Mass (g)	Brain Mass (g)	Swim Bladder Mass (g)	Heart Mass (g)	Total Length (mm)	Standard length (mm)
	Mean/SD/n	Mean/SD/n	Mean/SD/n	Mean/SD/n	Mean/SD/n	Mean/SD/n	Mean/SD/n	Mean/SD/n
Male	112.10/87.22/41	0.47/0.46/40	3.35/2.96/41	0.08/0.05/39	2.27/1.94/41	0.31/0.26/41	182.96/54.72/46	157.25/48.64/46
Female	98.69/46.92/19	5.83/5.02/19	3.89/1.97/19	0.10/0.05/19	1.18/0.51/19	0.26/0.13/19	180.82/29.49/23	154.78/24.75/23

Supplemental Table 1: Summary statistics of morphological data. SD=Standard deviation, n=sample size, g=grams.

Supplemental Figure Legends

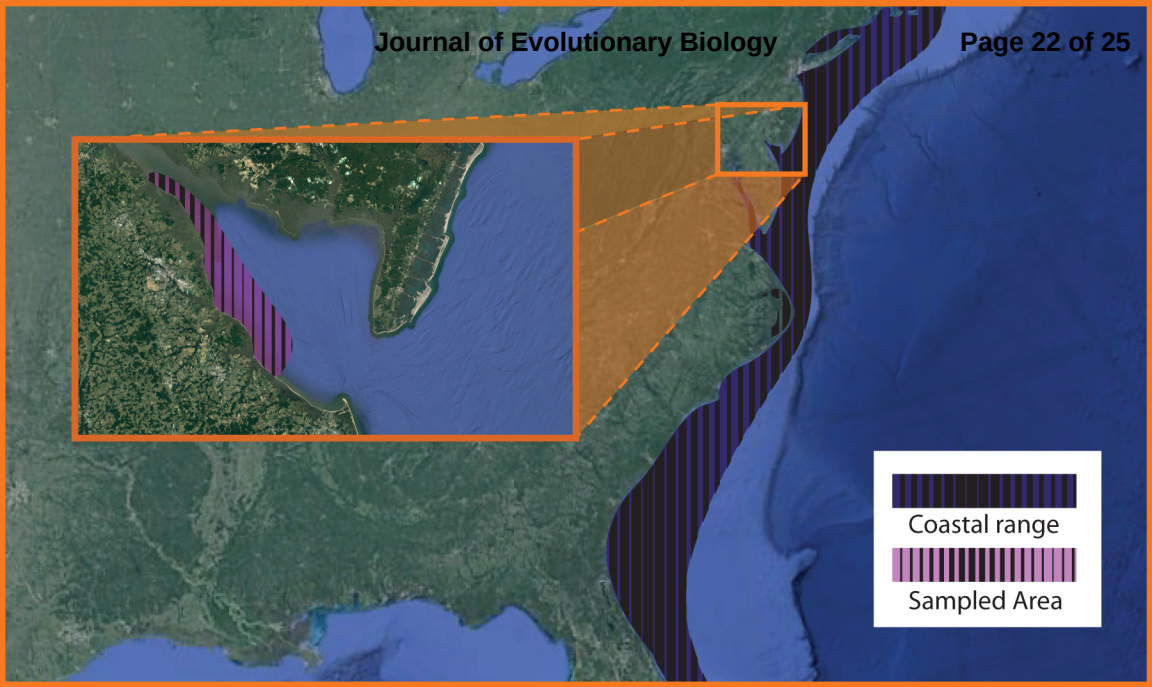
Supplemental Figure 1. Partial Geographical range of oyster toadfish in Eastern North America and location of area sampled (Google Maps, 2017).

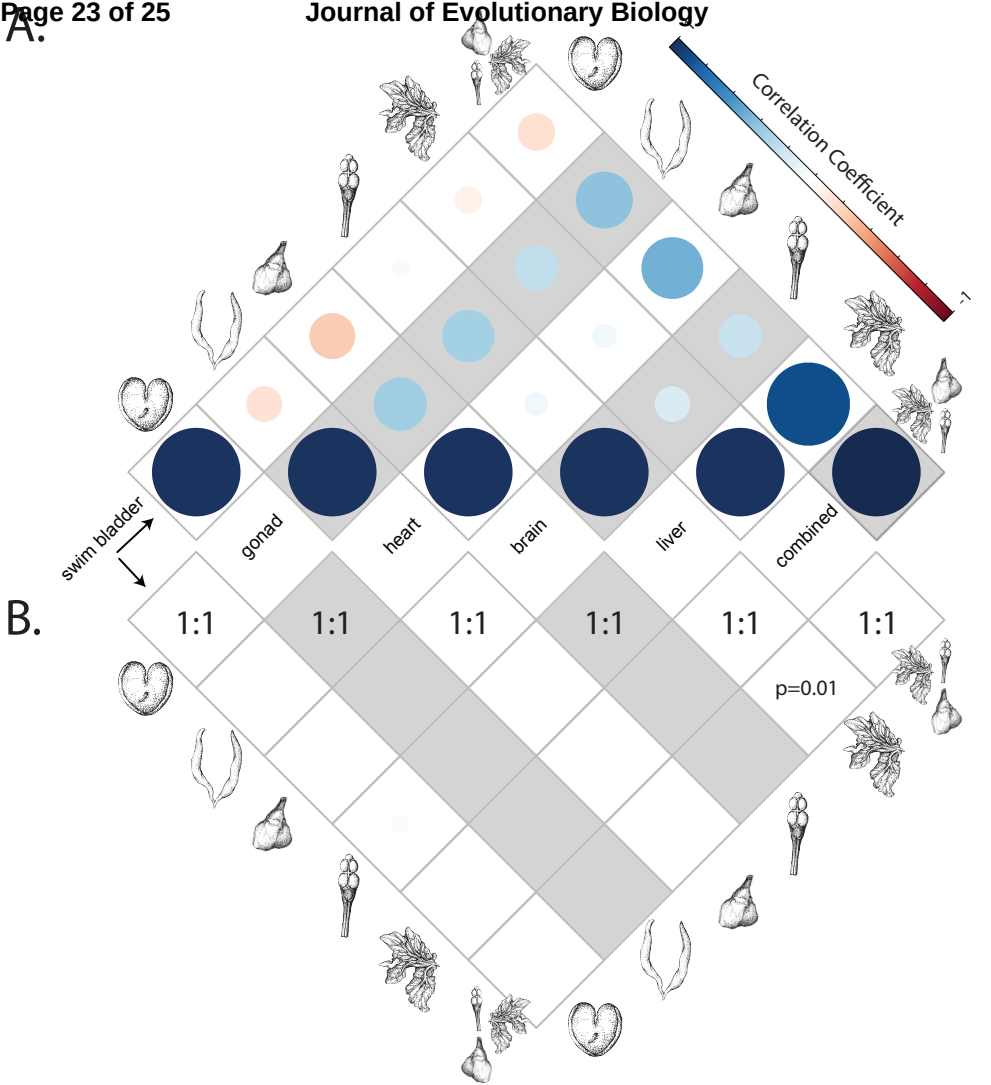
Supplemental Figure 2. Summary of multivariate regression. A. Correlation coefficients estimated for all comparisons. Circle sizes and color indicate strength of correlation indicated in the legend. Strong negative (red) correlations would support the expectations

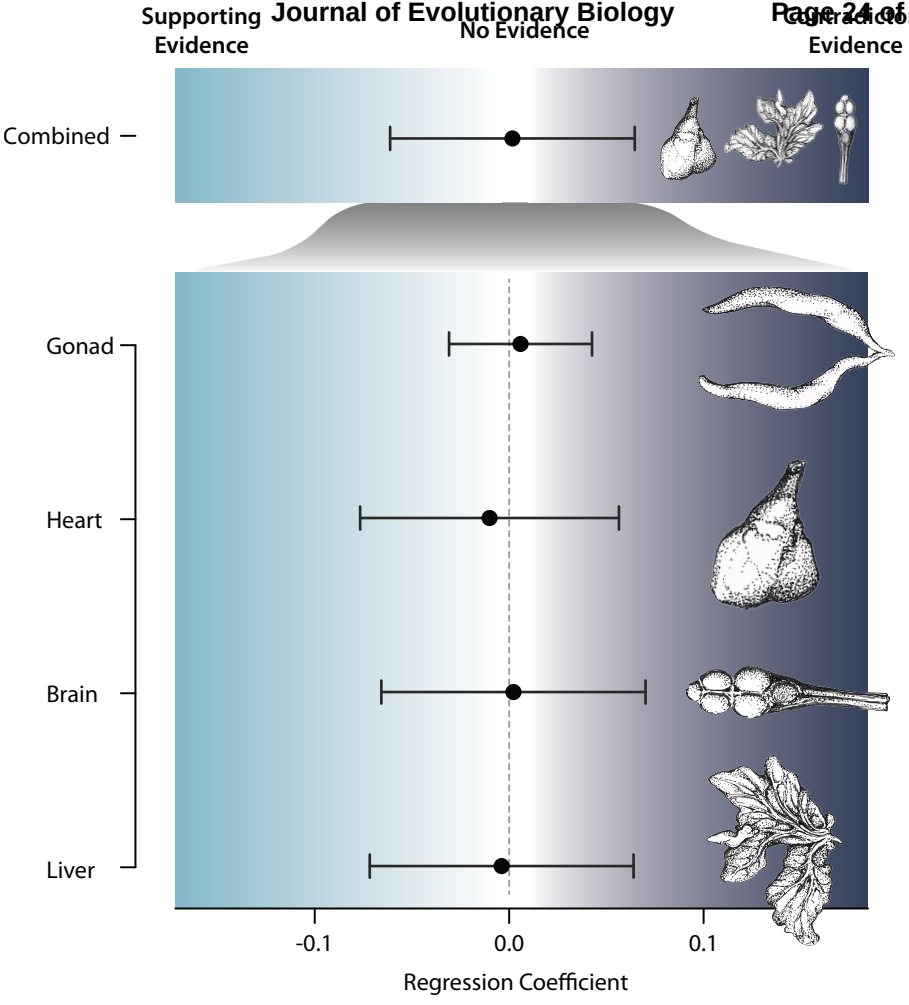
of the ETH. B. Significance tests of each correlation. Diagonal of matrix indicated by 1:1. Only p values less 0.05 are shown.

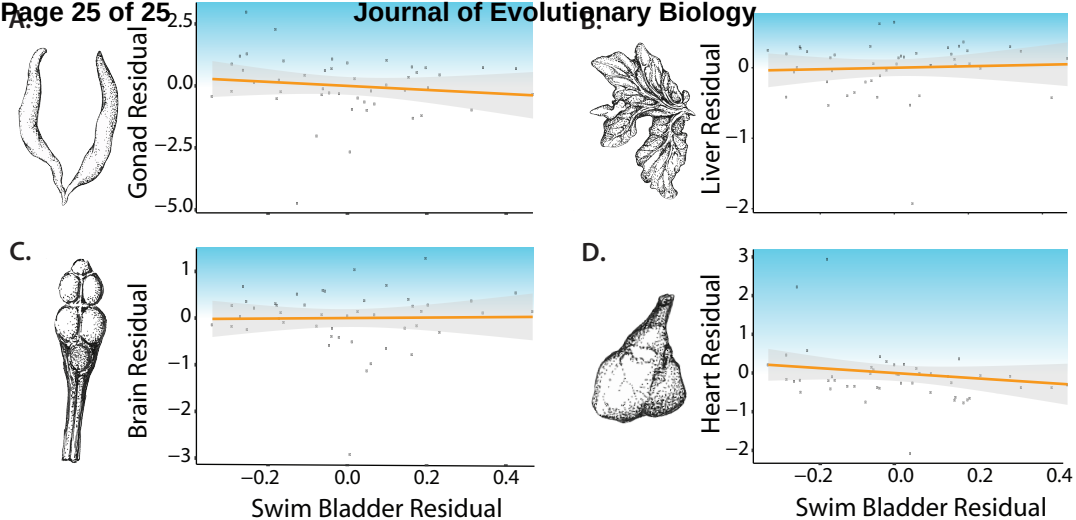
Supplemental Figure 3. Regression coefficients for relationship between mass of swim bladder and other putative metabolically expensive tissues. Negative regression coefficient estimates with 95% confidence intervals that exclude zero would support the expectations of the ETH (light shading), while positive regression coefficient estimates with 95% confidence coefficient intervals that exclude zero would provide contrary evidence (dark shading). confidence intervals that overlap with 0 provide no evidence for or against the expectations of the ETH.

Supplemental Figure 4. Residuals from log-log regression of organ mass versus body mass plotted against residuals from log-log regression of swim bladder mass versus body mass. Colored gradients in each plot depict the area above the origin.


Coastal range
Sampled Area


A.
Correlation Coefficient
1
-1
swim bladder
gonad
heart
brain
liver
combined
B.
1:1
1:1
1:1
1:1
1:1
1:1
p=0.01


Supporting Evidence
No Evidence
Contradictory Evidence
Combined
Gonad
Heart
Brain
Liver
-0.1
0.0
0.1
Regression Coefficient


A.
Gonad Residual
2.5
0.0
−2.5
−5.0
B.
Liver Residual
0
−1
−2
C.
Brain Residual
1
0
−1
−2
−3
−0.2
0.0
0.2
0.4
Swim Bladder Residual
D.
Heart Residual
3
2
1
0
−1
−2
−0.2
0.0
0.2
0.4
Swim Bladder Residual