



## SYMPOSIUM

# Ontogenetic Allometry in Shape and Flexibility Underlies Life History Patterns of Labrid Cleaning Behavior

Vikram B. Baliga<sup>1,\*</sup> and Rita S. Mehta<sup>\*</sup>

<sup>\*</sup>Department of Ecology and Evolutionary Biology, Long Marine Laboratory, University of California Santa Cruz, 100 Shaffer Road, Santa Cruz, CA 95060, USA

From the symposium “A Bigger Picture: Organismal Function at the Nexus of Development, Ecology, and Evolution” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2016 at Portland, Oregon.

<sup>1</sup>E-mail: vbaliga@ucsc.edu

**Synopsis** Body shape plays a crucial role in the movement of organisms. In the aquatic environment, the shape of the body, fins, and the underlying axial skeleton reflect the ability of organisms to propel and maneuver through water. Ontogenetic changes in body shape and flexibility of the axial skeleton may coincide with shifts in ecology (e.g., changes in habitat or feeding mode). We use the evolution of cleaning behavior in the Labridae (wrasses and parrotfishes) as a case study. Cleaner fishes are species that remove and consume ectoparasites from other organisms. In many cases, cleaning involves a high degree of maneuverability, as cleaners on the hunt for parasites may continuously dart around the body of their clients. In labrids, at least 58 species are known to clean. Over two-thirds of these species, however, clean predominately as juveniles, exhibiting an ontogenetic shift away from cleaning as they enter adulthood. Using a phylogenetic comparative framework, we examined features of the axial skeleton, overall body shape, and pectoral fin shape in 31 species of labrids spread across four major clades to assess how scaling patterns in these systems are associated with the ontogeny of cleaning behavior. We find that across wrasses, the ontogeny of body shape shows evolutionary concordance with the degree of flexibility across the vertebral column. A key driver of this relationship is that species that shift away from cleaning over ontogeny show stronger positive allometry for body depth and vertebral moment of inertia than other taxa. Species that clean throughout their life histories show a more elongate body and vertebral column, and tend to maintain the combination of these characteristics over ontogeny. Cleaning behavior in labrid fishes is thus an excellent model with which to investigate morphological patterns as they relate to evolution, development, and ecology.

## Introduction

Shape, the proportions and relative positions of parts, results from the interaction between genes, developmental processes, environmental variation, and evolutionary history (Klingenberg and Leamy 2001; Zelditch et al. 2008; Klingenberg 2010). Allometry describes the disproportionate change in shape as it relates to body size (Gould 1966). Allometric patterns can be described at different levels of taxonomic organizations: over an individual's lifetime (ontogenetic allometry), or across closely related taxa (evolutionary allometry). This emerging hierarchy provides an opportunity to understand how microevolutionary processes can guide

macroevolutionary patterns, an important question in evolutionary biology (Simpson 1944; Lande 1979; Eldredge et al. 2005).

While interspecific disparities in shape have been investigated in some vertebrate clades (Cherry et al. 1982; Klingenberg et al. 2003; Bergmann and Irschick 2010; Collar et al. 2013), it may be of little surprise that the most species-rich clade of vertebrates, actinopterygian fishes, has been the model for several recent studies focused on quantifying body shape (Collar et al. 2013; Claverie and Wainwright 2014), tracing the evolution of shape diversity (Agnese and Teugels 2005; Mehta et al. 2010; Collar et al. 2016), and understanding the developmental (Ward and Mehta 2010; Fleming et al. 2015) and ecological

(Aguirre 2009; Maxwell and Wilson 2013) bases for differences in shape. The adaptive significance of shape as it relates to performance is central to many functional studies. In fishes, body shape and fin diversity correspond to divergent locomotor strategies and physiology (Webb 1988; Lighthill and Blake 1990; Dickenson et al. 2000). In marine environments, fin shape can explain the ecological (Fulton and Bellwood 2005) and behavioral (Aguilar-Medrano et al. 2013) distribution of fish assemblages on and around coral reefs. Previous studies have emphasized that shape disparity across members of different fish clades is accompanied by morphological diversity in the axial skeleton (Lindsey 1975; Ward and Brainerd 2007). Across fishes, the axial skeleton varies in the number of individual vertebrae and vertebral aspect ratio along the precaudal and caudal regions of the axial skeleton (Ward and Brainerd 2007; Ward and Mehta 2014). Variation in vertebral number by changing the number of intervertebral joints (Brainerd and Patek 1998) and variation in vertebral aspect ratio by affecting moment of inertia of individual vertebrae and thereby body curvature (Porter et al. 2009) have the potential to affect the flexibility of fishes (Long 1992; Porter et al. 2009).

Since body shape affects locomotor behavior which is central to an organism's ecology, shape can strongly influence other functional systems such as feeding (Rice and Westneat 2005; Higham et al. 2007; Collar et al. 2008). Even more interesting is the fact that both body shape and feeding behavior vary over ontogeny. Therefore, a comparative evolutionary study focused on understanding the ontogeny and evolution of body shape can contribute to our understanding of feeding innovations. An integrated perspective on shape and feeding may be especially insightful for feeding specializations that are novel and restricted to particular life history stages. Here, we take the opportunity to examine the relationship between the ontogenetic scaling of body shape and the axial skeleton, and how these patterns relate to a specialized feeding mode, cleaning, in labrid fishes. The Labridae (wrasses, parrotfishes, and hogfishes) is a species-rich marine clade exhibiting tremendous diversity in morphology and trophic strategies showcasing functionally disparate trophic specializations such as corallivory, planktivory, and mucivory (Wainwright et al. 2004; Price et al. 2011). "Cleaning," otherwise known as ectoparasitivism, is a repeated dietary strategy that is linked to ontogeny.

Cleaner fishes remove and consume ectoparasites off other marine organisms. At least 58 labrid species

have been documented to clean, and the vast majority of these species do so predominately as juveniles or subadults (Coté 2000; Baliga and Law 2016). For simplicity, we term these "juvenile cleaners." Other labrid species clean throughout ontogeny, although ectoparasites do not comprise the majority of their diet (hereafter "facultative cleaners"). Among labrids only five species (all members of the *Labroides* genus) are considered obligate cleaners, attaining nearly all dietary items through cleaning (Coté 2000). While Baliga and Law (2016) found that cleaning likely evolved 26–30 separate times within the Labridae, the majority of these evolutionary events occur within five clades: (1) the Labrichthynes, (2) *Bodianus* and allies, (3) *Thalassoma* and allies, (4) New World *Halichoeres*, and (5) *Symphodus* and allies. Examining this dietary strategy within these clades offers us a rich system with which to understand the relationship between ontogenetic and evolutionary allometry and how they relate to trophic specialization. In particular, several species' ontogenetic transitions away from the juvenile state of cleaning toward a general macroinvertebrate diet in the adult form provides us with the opportunity to examine whether allometry of body shape, fins, or the axial skeleton corresponds with the ontogeny of this feeding strategy.

Client taxa (generally other fishes) often allow cleaners to remove ectoparasites that may occupy vulnerable, tightly-confined, and hard to reach places, such as the gills and the oral cavity (Grutter 1996; Coté 2000; Grutter 2010). Furthermore, some taxa, such as the obligate cleaner *Labroides dimidiatus*, often perform an oscillatory swimming "dance" to signal their presence to potential clientele (Randall 1958; Gorlick et al. 1978). Together, these observations/descriptions of cleaning lead us to predict that size, shape, and flexibility of the axial skeleton are important traits for cleaning and that cleaners may be evolutionarily constrained to exhibit a particular phenotype. Considering the varying degree in the reliance of ectoparasitivism as a resource across juvenile, facultative, and obligate cleaners, we hypothesize that the slope of ontogenetic trajectories for body shape and the axial skeleton will vary across cleaners. Using phylogenetic comparative methods, we test the hypothesis that ontogenetic trajectories for body shape and flexibility of the axial skeleton varies between the different cleaning strategies and those of non-cleaners for members of several labrid clades. Specifically, we predict that cleaners will exhibit a more elongate body and will exhibit vertebral characteristics that promote flexibility compared to non-cleaners. We also hypothesize that among cleaner fishes, the degree of allometry in body shape will

vary with respect to life history strategies for feeding. We predict that juvenile cleaners will exhibit the greatest ontogenetic allometric patterns in body shape as they transition away from cleaning into adulthood while obligate cleaners will maintain similar shapes and flexibility over ontogeny exhibiting isometric patterns. Finally, while facultative cleaners will exhibit allometric patterns in body shape and flexibility, their ability to clean opportunistically throughout their life, will result in allometric slopes that are less steep compared to juvenile cleaners.

## Methods

### Specimen collection and preparation

We collected 15–29 specimens for each of 31 species of wrasses, including members of *Halichoeres*, *Thalassoma*, *Symphodus*, and the Labrichthines (Fig. 1). Sample sizes within species are comparable to those used in other studies of evolutionary allometry (Klingenberg and Zimmermann 1992; Herrel and O'Reilly 2006). Specimens for each species ranged in size from around 40 mm standard length (SL) to the adult common or maximum SL reported for the species (Froese and Pauly 2016). We chose to use ~40 mm SL as a minimum size in order to safely ensure that specimens were in a post-recruitment phase (Leis et al. 2011). We acquired specimens from museum collections (California Academy of Sciences in San Francisco, CA; the LA County Museum of Natural History in Los Angeles, CA; the Smithsonian National Museum of Natural History in Suitland, MD; National Museum of Natural History in Paris, France), personal collections, and the aquarium trade (see Table S1 in the Supplementary Materials for more on specimen acquisition). Using information compiled by Coté (2000) and Baliga and Law (2016), we identified each species as belonging to one of the following dietary groups: non-cleaner, juvenile cleaner, facultative cleaner, or obligate cleaner (Fig. 1).

All specimens were initially fixed in 10% formalin and preserved in 60–70% ethanol. In addition to SL, we took six morphological measurements of body shape on ethanol-preserved specimens: maximum body depth, maximum body width, maximum tail depth, maximum tail width, pectoral fin length (leading edge), and pectoral fin width (Fig. 2A). We computed a pectoral fin elongation ratio (PFER) as the ratio of the length of the leading edge of the pectoral fin against maximum pectoral fin width. This method differs from those reported in previous studies e.g., pectoral fin aspect ratio (Wainwright et al. 2002); we did not seek to

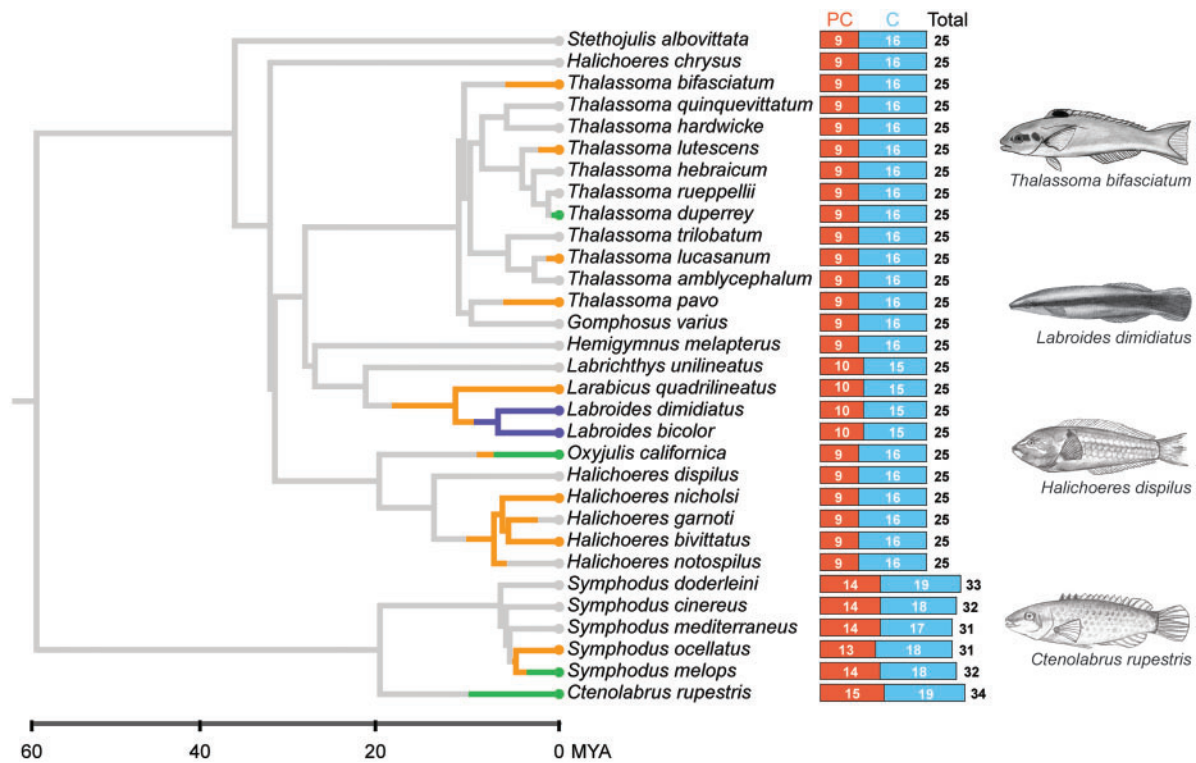
compute the total area of each fin as many of our specimens possessed fins with damaged or frayed edges. Our PFER measurement is comparable to that of pectoral fin aspect ratio: species with more elongate fins have higher values of each metric.

Following a modification of Dingerkus and Uhler (1977), we then cleared and double-stained all specimens for cartilage (using Alcian blue) and bone (using Alizarin red S). Using cleared and stained specimens, we counted the number of vertebrae in the precaudal and caudal regions, following (Ward and Brainerd 2007). We then measured additional traits of the axial skeleton: the length, width, and height of five precaudal and five caudal vertebrae (Fig. 2B). Vertebrae measured were located in the middle of each region.

### Scaling of body shape

Elongation ratio (ER) is a metric of body shape that is defined as the total length of the individual divided by the second largest major body axis (Ward and Azizi 2004). We adopted ER to compare the varying degrees of elongation across taxa. Our initial exploratory analyses of intraspecific body shape scaling patterns yielded striking patterns in the degree of elongation in cleaner taxa alone. We then decided to examine the components of ER and examine all 31 species using standardized major axis (SMA) regression (Warton et al. 2006) to assess how maximum body depth scaled within taxa. For each species' data, we performed a separate SMA regression of log-transformed maximum body depth against log-transformed SL. In this framework, species that show stronger negative allometry for maximum body depth exhibit a more elongate body over ontogeny, and vice versa.

To compare intraspecific scaling patterns among dietary groups, we employed multivariate generalized linear mixed models. Here maximum body depth was the response variable, SL was a covariate, dietary group was a fixed effect, and individuals within species were treated as random effects. To account for relationships between taxa (Hadfield and Nakagawa 2010), functions from the MCMCglmm package (Hadfield 2010) were used to fit our models. For the prior, we used an inverse-Gamma distribution with shape and scale parameters equal to 0.01 (Gelman 2006). The MCMC algorithm was run for 5 million generations, with a burn-in of 1000. Traces of the sampling were visualized in order to ensure stationarity had been attained. This approach allowed the estimation of intraspecific scaling patterns (i.e., the relationship between maximum body depth



**Fig. 1** Phylogenetic relationships between taxa in the present study, with dietary grouping and vertebral count data. The tree shows relationships between the 31 species and a single yet representative stochastic character map is superimposed on the phylogeny. Inference of both the tree and the trait history are taken from Baliga and Law (2016); stochastic character mapping was first performed on a labrid tree of 320 taxa and then pruned to the 31 species shown here. Colors on the phylogeny indicate dietary group membership: obligate cleaners are purple, facultative cleaners are green, juvenile cleaners are orange, and non-cleaners are gray. Vertebral counts indicate the number of precaudal (PC; red), cadual (C; blue), and total vertebrae (numbers in black, along the right edges) for each species. In our sampling, species did not show intraspecific variation in vertebral counts; counts for each region and for the total count were consistent across all specimens within each species. Drawings are of species that represent the different types of cleaner strategies and a non-cleaner.

and SL within each species) and patterns across closely-related taxa to be compared. In this approach, significant fixed effects indicated that a dietary group showed, on average, significantly different scaling patterns from those of the other groups.

### Scaling of the axial skeleton

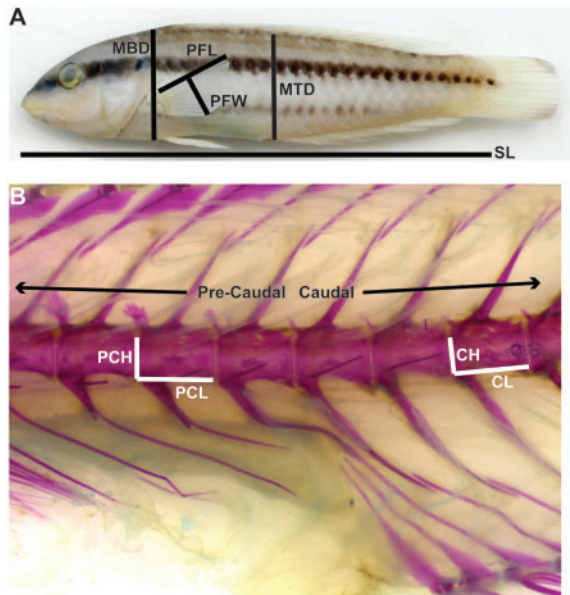
To examine whether cleaners exhibit greater flexibility than non-cleaners, we used moment of inertia ( $I_C$ ) as a proxy for flexibility. Moment of inertia, or second moment of area, has been shown to be a good predictor of body curvature in sharks (Porter et al. 2009). The moment of inertia of an object provides a measure of an object's resistance to changes in rotation around a central axis. Therefore, we modeled vertebrae as rods rotating around their center of mass:

$$I_C = \frac{1}{12} \rho \pi a b l^3,$$

where  $\rho$  is the density of the object,  $a$ ,  $b$  are the horizontal and vertical radii, respectively, and  $l$  is the length of the rod. The horizontal and vertical

radii were computed as half the magnitude of vertebral widths and heights, respectively. Using an engineering approach such as moment of inertia to model the behavior of vertebrae assumes isotropy and constant cross section. Similar to another study using this approach (Porter et al. 2009), both assumptions are violated with the vertebrae examined. Average  $I_C$  for each region of the axial skeleton was computed from data taken from five vertebrae from each region. Within our data set, nearly every species was found to have 25 total vertebrae; members of the genera *Symphodus* and *Ctenolabrus* were found to have 31–34 total vertebrae. Total vertebral counts were homogenous within each species. In order to craft a metric that could be used to compare trends within and across taxa that had subtle differences in vertebral regionalization, we calculated a weighted mean of  $I_C$  ( $WMI_C$ ) for each specimen:

$$WMI_C = \frac{(N_{PC})(I_{C-PC}) + (N_C)(I_{C-C})}{N_{Tot}},$$



**Fig. 2** Measurements performed on specimens in the study. **(A)** Photograph of *Halichoeres bivittatus* with linear measurements describing overall body shape were taken on ethanol-preserved specimens. Not shown are: maximum body width and maximum tail width (see “Methods” section). **(B)** Photograph of partial vertebral column of *Oxyjulis californica* with linear measurements of the axial skeleton taken on cleared and double-stained specimens. Not shown are: pre-caudal width and caudal width. CH: caudal vertebral height; CL: caudal vertebral length; MBD: maximum body depth; MTD: maximum tail depth; PCH: pre-caudal vertebral height; PCL: pre-caudal vertebral length; PFL: pectoral fin length; PFW: pectoral fin width; SL: standard length.

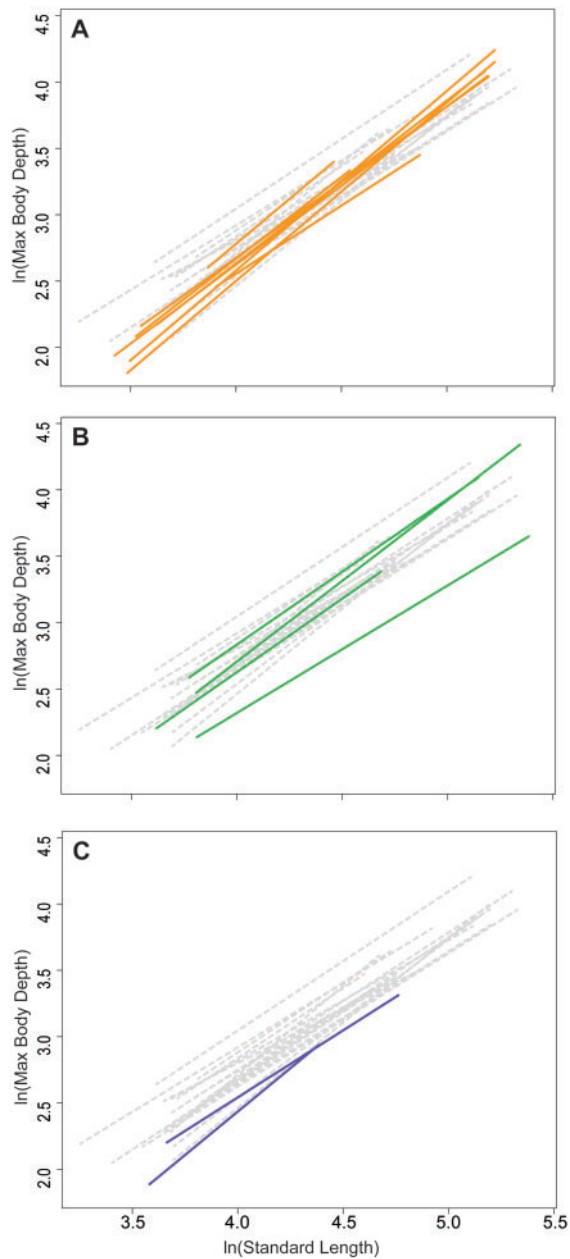
where  $N_{PC}$  is the number of precaudal vertebrae,  $I_{C-PC}$  is the mean moment of inertia for the precaudal region,  $N_C$  is the number of caudal vertebrae,  $I_{C-C}$  is the mean moment of inertia for the caudal region, and  $N_{Tot}$  is the total vertebral count. We then used SMA regressions to assess how  $WMI_C$  scales within taxa. For each species’ data, we performed a separate SMA regression of log-transformed  $WMI_C$  against log-transformed SL. We again employed multivariate generalized linear mixed models via MCMCglmm to compare intraspecific scaling patterns across dietary groups in a phylogenetically-informed manner. Here,  $WMI_C$  was the response variable, SL was a covariate, dietary group was a fixed effect, and individuals within species were treated as random effects. Calibration of the MCMC algorithm followed our procedure for the ln(maximum body depth) analysis. Significant fixed effects indicated that a particular dietary group exhibited significantly different scaling patterns from those of other groups.

### Co-variation of body and vertebral shape ontogenies

As body shape and the axial skeleton affect locomotion, we anticipate co-variation in these traits. We hypothesize that the extent of co-variation will depend on the degree of ectoparasitivism. To assess the extent to which the ontogenetic scaling of body shape relates to that of the vertebral column, we used phylogenetic canonical correlation analysis (PCCA; Revell and Harrison 2008). Canonical correlation analysis is a technique that, given two data sets, produces new sets of variables (orthogonal linear combinations of each parent data set) that maximize the correlations between the parent sets. Revell and Harrison (2008) provide methods to account for non-independence in data due to relatedness among taxa, generating new axes that maximize the evolutionary correlations between the parent sets. We performed PCCA using a data set that described the ontogeny of body shape and one which described the ontogeny of the vertebral column. First, to deduce each trait’s intraspecific scaling pattern, we performed a SMA regression of the log-transformed trait ( $y$ -axis) against log-transformed SL ( $x$ -axis), and extracted the regression slope. The body shape ontogeny data set comprised the SMA regression slopes of the following traits: maximum body depth, maximum body width, maximum tail depth, maximum tail width, pectoral fin length, and pectoral fin width. Similarly, the vertebral shape ontogeny data set contained the intraspecific scaling patterns of: precaudal vertebral width, precaudal vertebral length, caudal vertebral width, and caudal vertebral length. We found precaudal and caudal vertebrae to be nearly collinear with those of the vertebral widths. Therefore, the scaling patterns of precaudal and caudal vertebral heights were not included in this analysis because collinearity can produce numerical problems in parameter estimation (Slinker and Glantz 1985). We then used phylogenetic MANOVA with species’ canonical scores as the independent variable and dietary group as the dependent variable to test for significant differences in mean score, followed by post-hoc comparisons of dietary group differences.

### Results

Scaling relationships between log-transformed maximum body depth and log-transformed SL are shown in Fig. 3. Our MCMCglmm model indicated that juvenile cleaner taxa showed higher slopes than all other dietary groups, with the 95% credible interval falling outside and higher than zero (Table 1 (A)). No other groups showed significant differences in



**Fig. 3** Ontogenetic trends in body elongation in wrasses. SMA regressions of log-transformed maximum body depth vs. log-transformed SL were performed for each species (see Supplementary Table S2A, B). Each regression line represents data for a species. Regressions for non-cleaner species are shown in all three panels (dashed lines), and are contrasted with regressions for (A) juvenile cleaners, (B) facultative cleaners, and (C) obligate cleaners. Colors indicate dietary group membership: obligate cleaners are purple, facultative cleaners are green, juvenile cleaners are orange, and non-cleaners are gray. Juvenile cleaners showed higher slopes compared to other dietary groups.

mean slopes. Juvenile cleaners also show stronger positive allometry for  $\ln(WMI_C)$ ; only for the juvenile cleaner taxa did the 95% credible interval not contain zero (Table 1 (B)). Figure 4 shows the

scaling patterns of  $WMI_C$  across taxa within dietary groups.

Species-specific scaling patterns of all body, fin, and vertebral traits are documented in Supplementary Table S2A, B. A PCCA using these data resulted in four sets of canonical axes (Table 2), the first of which exhibited significant evolutionary correlation ( $r = 0.75$ ,  $\chi^2 = 38.94$ ,  $P < 0.01$ ). A phylogenetic MANOVA using scores from the PCCA showed significant differences in mean scores among the dietary groups. Post-hoc testing (Table 3) revealed that three pairs of groups showed significant differences: (1) non-cleaners and juvenile cleaners ( $t$ -value: 4.571,  $P < 0.001$ ); (2) juvenile cleaners vs. facultative cleaners ( $t$ -value:  $-3.617$ ,  $P < 0.001$ ); and (3) juvenile cleaners vs. obligate cleaners ( $t$ -value:  $-4.074$ ,  $P < 0.01$ ). Figure 5 depicts the relationship between the first canonical axes from the body and fin shape data set and the axial skeleton. Non-cleaners occupy the largest area across these two axes while the juvenile cleaners are clustered in their own mostly-non-overlapping region.

## Discussion

Overall, we found that there is concordance between the scaling of body shape, ontogenetic patterns of flexibility in the axial skeleton, and life history patterns of cleaning behavior across the species we sampled. The strongest patterns were found in the seven independent evolutions of juvenile cleaning. At small body sizes, juvenile cleaner species generally exhibit a smaller maximum body depth. These taxa then experience a greater increase in maximum depth as they reach adulthood and transition away from cleaning. Facultative cleaners, of which we examined four independent evolutions, follow this pattern to a lesser extent, while obligate cleaners maintain a consistently elongate form (small maximum body depth) throughout ontogeny. We also find a similar pattern when we examine the axial skeleton. Juvenile cleaners show stronger allometric changes along the vertebral column for (weighted) moment of inertia. Obligate cleaners (here, two sister taxa in *Labroides*) show some of the most elongate bodies and low moment of inertia throughout ontogeny. Facultative cleaners (who clean through ontogeny) show a more diverse pattern of shape, but are each fairly consistent over ontogeny. Non-cleaners show a wide diversity of allometric patterns for both body shape and vertebral characteristics, but as a group do not exhibit as strong allometry as the juvenile cleaner taxa. While the non-cleaner taxa sampled here may also exhibit

**Table 1** Summaries of fixed effects in multivariate generalized linear mixed models

	Posterior mean	95% CI	ESS	pMCMC
(A) Scaling patterns of ln(Max Body Depth) across dietary groups				
Intercept	<b>-1.726</b>	<b>-2.044 to -1.439</b>	<b>9998</b>	<b>&lt;0.001</b>
SL	<b>1.115</b>	<b>1.098 to 1.134</b>	<b>9998</b>	<b>&lt;0.001</b>
Juvenile cleaner	<b>0.144</b>	<b>0.059 to 0.228</b>	<b>9998</b>	<b>0.04</b>
Non-cleaner	-0.063	-0.175 to 0.047	9998	0.36
Obligate cleaner	-0.294	-0.573 to 0.021	10225	0.65
(B) Scaling patterns of ln(WMI <sub>C</sub> ) across dietary groups				
Intercept	<b>-24.705</b>	<b>-25.801 to -23.562</b>	<b>9855</b>	<b>&lt;0.001</b>
SL	<b>5.563</b>	<b>5.476 to 5.652</b>	<b>9998</b>	<b>&lt;0.001</b>
Juvenile cleaner	<b>0.157</b>	<b>0.099 to 0.256</b>	<b>9998</b>	<b>&lt;0.01</b>
Non-cleaner	0.034	-0.292 to 0.365	9998	0.83
Obligate cleaner	0.600	-0.499 to 1.536	9594	0.24

Notes: A multivariate generalized linear mixed model was fit to the (A) ln(Max Body Depth) and (B) ln(WMI<sub>C</sub>) data sets. In each case, SL was a covariate, dietary group was a fixed effect, and individuals within species were treated as random effects. Phylogenetic relationships between taxa were accounted for in the analyses following Hadfield and Nakagawa (2010). 95% CI: 95% credible interval; ESS: effective sample size; pMCMC: value for significance testing if the parameter is different from zero.

Bolding indicates parameter was significantly different from zero.

**Table 2** Coefficients, canonical correlations, and hypothesis testing from PCCA

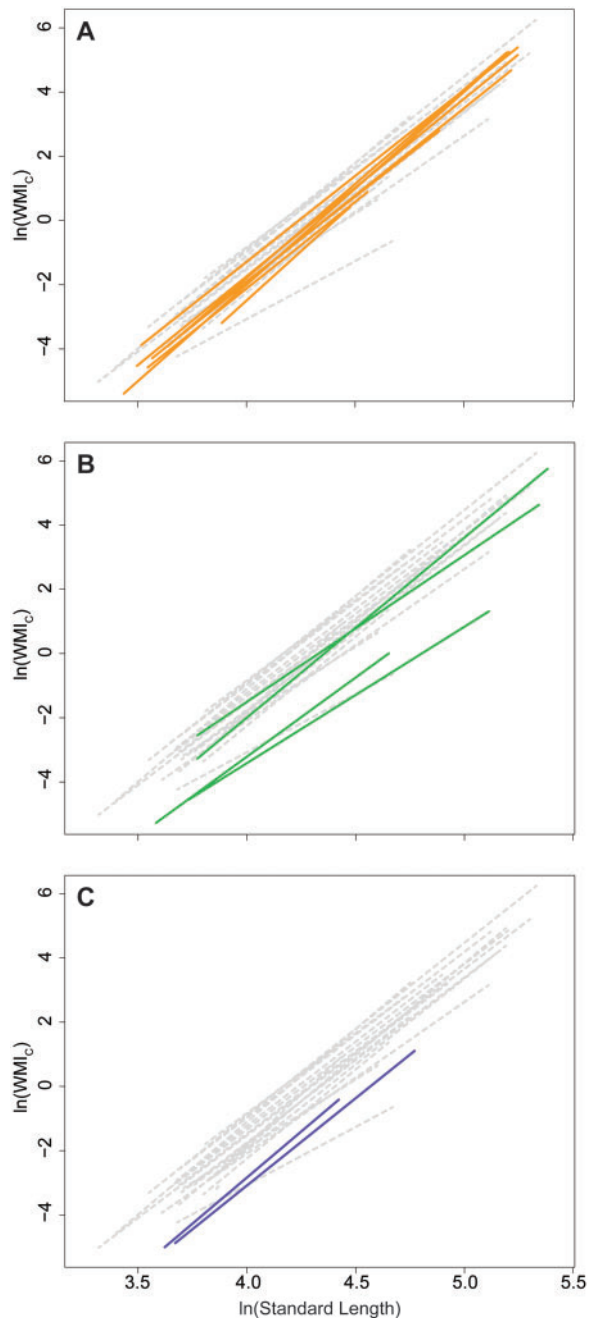
	CA1	CA2	CA3	CA4
Set1: MBD	-4.63	-3.65	-0.50	-2.71
Set1: MBW	-1.82	10.27	0.66	0.73
Set1: MTD	-2.42	-0.90	-2.77	-0.78
Set1: MTW	5.14	-4.99	-6.15	-2.35
Set1: PFAR	3.98	-0.33	4.07	-5.80
Set2: PCL	8.30	-8.57	-5.50	4.89
Set2: PCH	-13.31	5.19	-4.18	2.34
Set2: CL	11.97	11.84	-1.28	-9.66
Set2: CH	-5.51	-4.62	9.79	9.81
Canonical correlation	<b>0.75</b>	0.67	0.28	0.24
$\chi^2$	<b>38.94</b>	18.08	3.43	1.43
P-value	<b>&lt;0.01</b>	0.11	0.75	0.48

Notes: Canonical correlations,  $\chi^2$  values, and P-values correspond to hypothesis testing of significance for evolutionary correlations between canonical axes. Bolding indicates significant canonical correlation. MBD: maximum body depth; MBW: maximum body width; MTD: maximum tail depth; MTW: maximum tail width; PFAR: pectoral fin elongation ratio; PCL: precaudal vertebral length; PCH: precaudal vertebral height; CL: caudal vertebral length; CH: caudal vertebral height.

ontogenetic shifts in ecology (e.g., feeding on planktonic crustaceans to hard-shelled invertebrates over ontogeny as in *Hemigymnus melapterus* (Myers 1991)), the non-uniformity of allometric patterns among these taxa provides evidence that they have exhibited diverse responses to such ecological shifts.

### Elongation, flexibility, and ontogeny

Body elongation (and the evolutionarily concordant pattern of low moment of inertia) gives cleaners relatively lithe, flexible bodies when compared to non-cleaning congeners. These characteristics may enhance cleaning as these species (1) maneuver over the bodies of their clients, (2) enter tight, confined areas such as the gills or buccal cavity, or (3) 'dance' to present themselves as cleaners (*Labroides* only). Additionally, recent evidence suggests that body depth is a trait in fishes that generally increases in response to predation, either by plastic or adaptive processes (Manghagen and Heibo 2004; Eklöv and Johnson 2007; Frommen et al. 2011; Price et al. 2015). Fishes that engage in cleaning behavior generally experience diminished predation rates (Feder 1996; Darcy et al. 1974). Côté (2000) notes there have been few reported cases on predation of cleaners during cleaning sessions, despite the observation that cleaners may even enter the mouths of their clientele to forage for parasites. Predation of cleaners such as *Thalassoma noronhanum* has been observed to occur by potential client species, but outside of the context of cleaning stations (Francini-Filho et al. 2000). In contrast, closely-related non-cleaner species may be selected to have deeper bodies in order to minimize the effects of predation; since these taxa are not cleaning, they do not enjoy the respite from predation that cleaners do. Additionally, this hypothesis may aid our interpretation of the strong positive allometry in body depth among juvenile cleaner species. These taxa clean as juveniles, and while doing so



**Fig. 4** The scaling of axial shape. For each specimen, a weighted mean ( $WMI_C$ ) was calculated for the moment of inertia ( $I_C$ ) of vertebrae. Species-specific SMA regressions of log-transformed  $WMI_C$  vs. log-transformed SL describe intraspecific scaling patterns of this metric (see Supplementary Table S2A, B). Regressions for non-cleaner species are shown in all three panels (dashed lines), and are contrasted with regressions for (A) juvenile cleaners, (B) facultative cleaners, and (C) obligate cleaners. Colors indicate dietary group membership: obligate cleaners are purple, facultative cleaners are green, juvenile cleaners are orange, and non-cleaners are gray. Juvenile cleaners showed higher slopes compared to other dietary groups.

**Table 3** Summary of post-hoc tests following phylogenetic MANOVA

Group <sub>i</sub>	Group <sub>j</sub>	t-value	P-value
Non-cleaner	Juvenile cleaner	<b>4.571</b>	<b>&lt;0.001</b>
Non-cleaner	Facultative cleaner	-0.458	0.87
Non-cleaner	Obligate cleaner	-1.687	0.67
Juvenile cleaner	Facultative cleaner	<b>-3.617</b>	<b>&lt;0.001</b>
Juvenile cleaner	Obligate cleaner	<b>-4.074</b>	<b>&lt;0.01</b>
Facultative cleaner	Obligate cleaner	-1.162	0.88

Notes: A PCCA was run using data sets that summarized the intra-specific scaling patterns of body shape and of vertebral shape (see main text for more details). The scores from the PCCA were then used as the independent variables in phylogenetic MANOVA. This procedure indicated significant differences between the dietary groups' mean scores ( $F$ -ratio: 9.81,  $P < 0.001$ ). Listed above is information from post-hoc tests using adjustments following Benjamini and Hochberg (1995).

Bolding indicates significant differences between mean scores for a pair of groups.

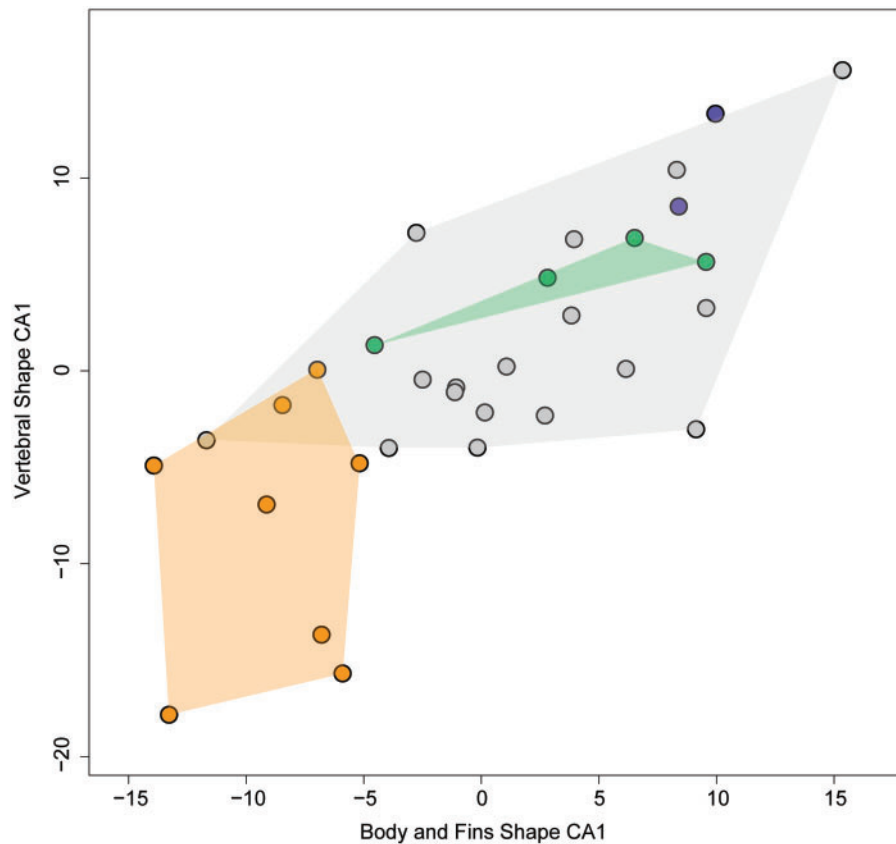
they may experience lower predation rates while exhibiting a slender body that enhances cleaning. As these species transition to other dietary strategies over ontogeny, they may face increased predation rates. Increasing body depth (through evolutionary allometry, plasticity, or both) could potentially mitigate some of the effects of this newfound predation.

### Understanding morphology and ontogeny through studies of macroevolution

Our study of 31 labrids provides additional evidence of the diversity of allometric patterns seen in nature. This adds to an already diverse body of work found across numerous taxonomic groups, such as reptiles (Herrel and O'Reilly 2006), mammals (Mitteroecker et al. 2004), fishes (Wainwright and Richard 1995), and insects (Klingenberg and Zimmermann 1992). In the present study, we found that across 10 traits related to body shape, fin shape, and vertebral shape, the majority of labrid species we sampled showed some form of allometry, be it positive or negative. Furthermore, every species showed allometry in at least three traits. Thus it is clear that phenotypic allometry is a general characteristic of the taxa sampled, highlighting the necessity of using phylogenetic comparative methods to understand the historical vs. adaptive bases of allometric patterns.

Additionally, the axial skeleton is an important source of morphological diversity supporting much of the obvious superficial changes in body shape. Variation in the number and distribution of vertebrae, as well as size of individual vertebrae in different regions of the axial skeleton affect important





**Fig. 5** Covariation between the ontogenetic scaling of body shape and that of the vertebral column. A PCCA was used to assess the extent to which body shape ontogeny co-varies with vertebral shape ontogeny. Separate data sets were first constructed for body shape and for vertebral shape. Each data set comprised slope values from species-specific SMA regressions of log-transformed traits against log-transformed SL. See main text for further details and Supplementary Table S2A for slope values. The first set of canonical axes is depicted here. Colors indicate dietary group membership: obligate cleaners are purple, facultative cleaners are green, juvenile cleaners are orange, and non-cleaners are gray.

behaviors. Brainerd and Patek (1998) provide evidence that a reduction in vertebral number (leading to a reduction in the number of intervertebral joints), particularly in Tetraodontiform fishes, impairs an organism's ability to bend its body, a crucial aspect of the C-start antipredator response. Changes in vertebral dimensions can also have influence on an organism's flexibility. For example, among vine-like arboreal snakes, increased lengths of vertebrae correspond to increases in the rigidity of the body, which may facilitate locomotion among tree branches (Johnson 1955). Although labrids lack extreme body shapes that arise in other fish clades (e.g., catfishes; Agnese and Teugels 2005), we see that even subtle changes in shape coincide with changes in the vertebral column, which can have large influence on how an organism moves. Although we did not quantify swimming or hovering performance in our labrid taxa, studies such as Porter et al. (2009) show that the second moment area of the centrum, and vertebral length and height

are strong predictors of maximum body curvature during swimming in sharks. Furthermore, other studies have clearly shown how an increase in vertebral number in a particular region of the body can affect burst performance behaviors (Swaine 1992; Ackerly and Ward 2015). In the present study, we find that subtle changes in the scaling of linear traits (e.g., vertebral width) can have large consequences on functional traits such as moment of inertia or second area moment, as lengths are cubed.

Body shape is often strongly integrated with other functional systems (Collar et al. 2008; Ward and Mehta 2010). Juvenile cleaner fishes show strong allometric patterns in cranial morphology (Baliga and Mehta 2014) and as we show in this study, body shape, indicating that the juvenile and adult forms may experience differing selection pressures. This lends credence to the notion that the observed phenotype of an organism at any one life history stage may be the result of specific selection pressures that operate within that life history stage. For instance,

tadpoles often possess elongate tails and streamlined bodies, enhancing undulatory aquatic locomotion, while metamorphosed adult frogs exhibit drastic developmental changes to appendages, girdles, and the axial skeleton, promoting new mechanisms of locomotion on land (Emerson 1979; Handrigan and Wassersug 2007). Among stichaeid fishes, the transition from juvenile carnivory to adult herbivory (that is often concomitant with shifts in habitat from tidepools to the rocky intertidal) is also accompanied by ontogenetic changes to gut length and enzymatic activity (German et al. 2004). Such studies point to the differential selection pressures an organism may face in different stages.

Klingenberg (2010) shows that while ontogenetic scaling has been traditionally thought to act as a constraint on morphology, the diversity of scaling trajectories and their correspondence with ecological patterns indicate that ontogenetic processes can themselves evolve via an adaptive basis. On a phylogeny, multiple evolutions of a character trait (whether behavioral, morphological, or physiological) provide powerful examples with which to understand what selective processes are facilitating repetition of a particular pattern. Here, we find that cleaning in the Labridae, presents a compelling case of the repeated evolution of a behavior coupled with differential scaling patterns associated with the ontogeny of the behavior.

Thus, as we inspect different levels of morphology to attempt to understand the trophic strategy of cleaning, we find that: (1) shape varies over ontogeny; (2) the axial skeleton is an important source of morphological diversity; axial variation can affect important behaviors related to turning and signaling clients; (3) body shape at any one life history stage may be tightly integrated with other functional systems such as the feeding apparatus; and (4) macroevolutionary studies can help conceptualize how evolutionary changes in ontogenetic scaling correspond to life history patterns. Here, we use cleaning as a case study to promote the value of integrating evolutionary patterns in development to provide a more comprehensive understanding of ecological shifts in nature. Cleaning in the Labrid system, is an example of how microevolutionary processes can affect macroevolutionary patterns of morphological diversity.

## Acknowledgments

We would like to thank P. Gignac and S. Santana, the organizers of the symposium within which this work was presented. We thank all of the other

presenters and their colleagues who contributed to making symposium a wonderful and captivating experience. S. A. Price provided valuable discussion on the interpretation of trends in body depth in fishes. We give special thanks to museum curators for providing specimen loans: J. T. Williams (Smithsonian), R. Feeny (LA County Museum), D. Catania (California Academy of Sciences), and G. Zora (Paris Museum). We thank M. E. Alfaro and an anonymous reviewer for help strengthening the article. Illustrations in Figure 1 by C.J. Law

## Funding

This work was supported by the Society for the Study of Evolution's Rosemary Grant Award for Graduate Student Research, awarded to V. B. Baliga. This work was also supported by funding through SICB by DCB and DVM. Funding for the symposium was provided by the National Science Foundation through their Meetings initiative, grant no. 1539880 awarded to Santana and Gignac.

## Supplementary data

Supplementary data available at *ICB* online.

## References

- Ackerly KL, Ward AB. 2015. Linking vertebral number to performance of aquatic escape responses in the axolotl (*Ambystoma mexicanum*). *Zoology* 118:394–402.
- Agnese JF, Teugels GG. 2005. Insight into the phylogeny of African Clariidae (Teleostei, Siluriformes): implications for their body shape evolution, biogeography, and taxonomy. *Mol Phy E* 36:546–53.
- Aguilar-Medrano R, Frederich B, Balart EF, de Luna E. 2013. Diversification of the pectoral fin shape in damselfishes (Perciformes, Pomacentridae) of the Eastern Pacific. *Zoomorphology* 132:197–213.
- Aguirre WE. 2009. Microgeographical diversification of three-spine stickleback: body shape-habitat correlations in a small, ecologically diverse Alaskan drainage. *Biol J Linn Soc* 98:139–51.
- Baliga VB, Law CJ. 2016. Cleaners among wrasses: phylogenetics and evolutionary patterns of cleaning behavior within Labridae. *Mol Phyl E* 94A:424–35.
- Baliga VB, Mehta RS. 2014. Scaling patterns inform ontogenetic transitions away from cleaning in *Thalassoma* wrasses. *J Exp Biol* 217:3597–606.
- Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc B* 57:289–300.
- Bergmann PJ, Irschick DJ. 2010. Alternate pathways of body shape evolution translate into common patterns of locomotor evolution in two clades of lizards. *Evolution* 64:1569–82.
- Brainerd EL, Patek SN. 1998. Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in Tetraodontiform fishes. *Copeia* 4:971–84.

- Cherry LM, Case SM, Kunkel JG, Wyles JS, Wilson AC. 1982. Body shape metrics and organismal evolution. *Evolution* 1:914–33.
- Claverie T, Wainwright PC. 2014. A morphospace for reef fishes: elongation is the dominant axis of body shape evolution. *PLoS One* 9:e112732.
- Collar DC, Quintero M, Buttler B, Ward AB, Mehta RS. 2016. Body shape transformation along a shared axis of anatomical evolution in labyrinth fishes (Anabantoidei). *Evolution* 70:555–67.
- Collar DC, Reynaga CM, Ward AB, Mehta RS. 2013. A revised metric for quantifying body shape in vertebrates. *Zoology* 116:246–57.
- Collar DC, Wainwright PC, Alfaro ME. 2008. Integrated diversification of locomotor and feeding in labrid fishes. *Biol Lett* 4:84–86.
- Coté IM. 2000. Evolution and ecology of cleaning symbioses in the sea. *Oceanogr Mar Biol Annu Rev* 38:311–55.
- Darcy GH, Maisel E, Ogden TC. 1974. Cleaning preferences of the gobies *Gobiosoma evelynae* and *G. prochilos* and the juvenile wrasse *Thalassoma bifasciatum*. *Copeia* 1974: 375–9.
- Dingerkus G, Uhler LD. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technol* 52:229–32.
- Dickinson MH, Farley CT, Full RJ, Koehl MAR, Kram R, Lehman S. 2000. How animals move: an integrative view. *Science* 288:100–6.
- Eldredge N, Thompson JN, Brakefield PM, Gavrilets S, Jablonski D, Jackson JBC, Lenski RE, Lieberman BS, McPeck MA, Miller W. 2005. The dynamics of evolutionary stasis. *Paleobiology* 31:133–45.
- Emerson SB. 1979. The ilio-sacral articulation in frogs: form and function. *Biol J Linn Soc* 11:153–68.
- Eklöv P, Johnson P. 2007. Pike predators induce morphological changes in young perch and roach. *J Fish Biol* 70:155–64.
- Feder HM. 1966. Cleaning symbiosis in the marine environment. In: Henry SM, editors. *Symbiosis*. New York: Academic Press. p. 327–80.
- Fleming A, Kishida MG, Kimmel CB, Keynes RJ. 2015. Building the backbone: the development and evolution of vertebral patterning. *Development* 142:1733–44.
- Francini-Filho RB, Moura RL, Sazima I. 2000. Cleaning by the wrasse, *Thalassoma noronhanum*, with two records of predation by its grouper client *Cephalopholis fulva*. *J Fish Biol* 56:802–9.
- Fulton CJ, Bellwood DR, Wainwright PC. 2005. Wave energy and swimming performance shape coral reef fish assemblages. *Proc Roy Soc Lond B* 272:827–32.
- Froese R, Pauly D, (eds.). 2016. FishBase. World Wide Web electronic publication. ([www.fishbase.org](http://www.fishbase.org)), version (1/2016).
- Frommen JG, Herder F, Engqvist L, Mehliis M, Bakker TC, Schwarzer J, Thünken T. 2011. Costly plastic morphological responses to predator specific odour cues in three-spined sticklebacks (*Gasterosteus aculeatus*). *Evol Ecol* 25:641–56.
- GarlandTJr., Dickerman AW, Janis CM, Jones JA. 1993. Phylogenetic analysis of covariance by computer simulation. *Sys Biol* 42:265–92.
- Gelman A. 2006. Prior distributions for variance parameters in hierarchical models. *Bayesian Anal* 1:515–33.
- German DP, Horn MH, Gawlicka A. 2004. Digestive enzyme activities in herbivorous and carnivorous pricklyback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. *Physiol Biochem Zoo* 77:789–804.
- Gorlick DL, Atkins PD, Losey GS. 1979. Cleaning stations as water holes, garbage dumps, and sites for the evolution of reciprocal altruism? *Am Nat* 112:341–53.
- Gould SJ. 1966. Allometry in size in ontogeny and phylogeny. *Biol Rev* 41:587–640.
- Grutter A. 1996. Parasite removal rates by the cleaner wrasse *Labroides dimidiatus*. *Mar Ecol Prog Ser* 130:61–70.
- Grutter AS. 2010. Cleaner fish. *Curr Biol* 20:547–49.
- Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J Stat Soft* 33:1–22.
- Hadfield JD, Nakagawa S. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. *J Evol Biol* 23:494–508.
- Handrigan GR, Wassersug RJ. 2007. The anuran Bauplan: a review of the adaptive, developmental, and genetic underpinnings of frog and tadpole morphology. *Biol Rev* 82:1–25.
- Herrel A, O'Reilly JC. 2006. Ontogenetic scaling of bite force in lizards and turtles. *Physiol Biochem Zool* 79:31–42.
- Higham TE, Hulsey CD, Řičan O, Carroll AM. 2007. Feeding with speed: prey capture evolution in cichlids. *J Evol Biol* 20:70–78.
- Johnson RJ. 1955. The adaptive and phylogenetic significance of vertebral form in snakes. *Evolution* 9:367–88.
- Klingenberg CP. 2010. Evolution and development of shape: integrating quantitative approaches. *Nat Rev Genet* 11:623–35.
- Klingenberg CP, Barluenga M, Meyer A. 2003. Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex. *Biol J Linn Soc* 80:397–408.
- Klingenberg CP, Leamy LJ. 2001. Quantitative genetics of geometric shape in the mouse mandible. *Evolution* 55:2342–52.
- Klingenberg CP, Zimmermann M. 1992. Static, ontogenetic, and evolutionary allometry: a multivariate comparison in nine species of water striders. *Am Nat* 140:601–20.
- Lande R. 1979. Quantitative genetic analysis of multivariate evolution applied to brain: body size allometry. *Evolution* 33:402–16.
- Leis JM, Hay AC, Gaither MR. 2011. Swimming ability and its rapid decrease at settlement in wrasse larvae (Teleostei: Labridae). *Mar Biol* 158:1239–46.
- Lighthill J, Blake R. 1990. Biofluidynamics of balistiform and gymnotiform locomotion. Part I. Biological background, and analyses by elongated-body theory. *J. Fluid Mech* 212:183–207.
- Lindsey CC. 1975. Pleomerism, widespread tendency among related fish species for vertebral number to be correlated with maximum body length. *J Fish Res B Can* 32:2453–69.
- Long JH. 1992. Stiffness and damping forces in the intervertebral joints of blue marlin (*Makaira nigricans*). *J Exp Biol* 162:131–55.

- Manghagen C, Heibo E. 2004. Growth in length and in body depth in young-of-the-year perch with different predation risk. *J Fish Biol* 64:612–24.
- Mehta RS, Ward AB, Alfaro ME, Wainwright PC. 2010. Elongation of the body in eels. *Integr Comp Biol* 50:1091–105.
- Mitteroecker P, Gunz P, Bernhard M, Shaefer K, Bookstein FL. 2004. Comparison of cranial ontogenetic trajectories among great apes and humans. *J Hum Evol* 46:679–98.
- Maxwell EE, Wilson LA. 2013. Regionalization of the axial skeleton in the ‘ambush predator’ guild—are there developmental rules underlying body shape evolution in ray-finned fishes? *BMC Evol Biol* 13:265.
- Myers RF. 1991. *Micronesian reef fishes*. Barrigada, Guam: Coral Graphics.
- Porter ME, Roque CM, Long JH. 2009. Turning maneuvers in sharks: predicting body curvature from axial morphology. *J Morphol* 270:954–65.
- Price SA, Friedman ST, Wainwright PC. 2015. How predation shaped fish: the impact of fin spines on body form evolution across teleosts. *Proc R Soc B* 282:1–9.
- Price SA, Holzman R, Near TJ, Wainwright PC. 2011. Coral reefs promote the evolution of morphological diversity and ecological novelty in labrid fishes. *Ecol Lett* 14:462–9.
- Randall JE. 1986. *Red Sea fishes*. London: Immel.
- Revell LJ, Harrison AS. 2008. PCCA: a program for phylogenetic canonical correlation analysis. *Bioinformatics* 24:1018–20.
- Rice AR, Westneat MW. 2005. Coordination of feeding, locomotor and visual systems in parrotfishes (Teleostei: Labridae). *J Exp Biol* 208:3503–18.
- Simpson GG. 1944. *Tempo and mode in evolution*. New York: Columbia University Press.
- Slinker BK, Glantz SA. 1985. Multiple regression for physiological data analysis: the problem of multicollinearity. *Am J Physiol* 249:R1–R12.
- Swain DP. 1992. The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. *Evolution* 46:987–97.
- Wainwright PC, Richard BA. 1995. Predicting patterns of prey use from morphology with fishes. *Environ Biol Fish* 44:97–113.
- Wainwright PC, Bellwood DR, Westneat MW. 2002. Ecomorphology of locomotion in labrid fishes. *Env Biol Fish* 65:47–62.
- Wainwright PC, Bellwood DR, Westneat MW, Grubich JR, Hoey AS. 2004. A functional morphospace for the skull of labrid fishes: patterns of diversity in a complex biomechanical system. *Biol J Linn* 82:1–25.
- Walker JA, Alfaro ME, Noble MM, Fulton CJ. 2013. Body fineness ratio as a predictor of maximum prolonged-swimming speed in coral reef fishes. *PloS One* 8:e75422.
- Ward AB, Azizi E. 2004. Convergent evolution of the head retraction escape response in elongate fishes and amphibians. *Zoology* 107:205–17.
- Ward AB, Brainerd EL. 2007. Evolution of axial patterning in elongate fishes. *Biol J Linn Soc* 90:97–116.
- Ward AB, Mehta RS. 2010. Axial elongation in fishes: using morphological approaches to elucidate developmental mechanisms in studying body shape. *Integr Comp Biol* 50:1106–19.
- Ward AB, Mehta RS. 2014. Differential occupation of axial morphospace. *Zoology* 117:70–76.
- Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. *Biol Rev* 81:259–91.
- Webb PW. 1988. Simple physical principles and vertebrate aquatic locomotion. *Am Zool* 28:709–25.
- Zelditch ML, Wood AR, Bonett RM, Swiderski DL. 2008. Modularity of the rodent mandible: integrating bones, muscles, and teeth. *Evol Dev* 10:756–68.