

Ontogenetic shape changes in Pomacentridae (Teleostei, Perciformes) and their relationships with feeding strategies: a geometric morphometric approach

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The present study explores the shape changes of cranial structures directly involved in food capturing during growth after reef settlement in two species of Pomacentridae (*Dascyllus aruanus* and *Pomacentrus pavo*). Landmark-based geometric morphometrics were used to study allometric patterns and related shape changes in four skeletal units: neurocranium, suspensorium and opercle, mandible and premaxilla. At settlement, the larvae of both species have a relatively similar morphology, especially with respect to the mandible. Their shapes suggest a feeding mode defined as ram/suction-feeding. Ontogenetic shape changes show a shift to a suction feeding mode of prey capture. The main transformations involved are an increase in height of the suspensorium and the opercle, an elevation of the supraoccipital crest, a relative shortening of the mandible, and a lengthening of the ascending process of the premaxilla. Shape changes of the mandible in the two studied species also reflect an increase of biting capacities. The high disparity between adult shape results from differences in the rate and in the length of ontogenetic trajectories, from divergence of the ontogenetic trajectories (neurocranium, mandible, and premaxilla) and parallel shifts of the trajectories in the size-shape space (suspensorium and opercle). In an evolutionary context, allometric heterochronies during ontogeny of different skeletal unit of the head may be considered as a basis for the explanation of the diversity of damselfishes. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, 95, 92–105.

ADDITIONAL KEYWORDS: Allometry – damselfishes – development – feeding apparatus – growth – heterochrony – ontogeny – reef fishes – skull.

INTRODUCTION

Damselfishes (Pomacentridae), similar to most coral-reef fishes, possess a complex life cycle involving a pelagic dispersion period of larvae and a demersal juvenile and adult life period that is reef associated (Leis & McCormick, 2002). This transition of habitat is a crucial phase leading to behavioural changes and morphological modifications (McCormick, Makey & Dufour, 2002; Frédérick, Parmentier & Vandewalle 2006).

Ontogenetic shifts in diet are well known in reef fishes (McCormick, 1998; Lukoschek & McCormick,

2001) and some studies reveal diet changes during the growth of damselfishes (Ciardelli, 1967; Nakamura *et al.*, 2003). In Pomacentridae, larvae are usually zooplanktivorous (Leis & McCormick, 2002) whereas juveniles and adults may have varied diets in zooplanktivorous, herbivorous, and omnivorous species (Allen, 1991; Kuo & Shao, 1991). Due to a common feeding habit, larvae can be expected to be more similar than would be the case for the adults. Recently, variations in buccal morphology between larvae and adults were highlighted in five species of pomacentrids and were related to the differences in their trophic ecology (Frédérick *et al.*, 2006). However, to date, very little is known about the ontogenetic changes related to the trophic apparatus morphology during the settlement phase of the Pomacentridae

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and all other reef fishes, nor about the possible heterochronic patterns underlying it. To our knowledge, no quantitative analysis aiming to characterize this part of the development exists.

Allometry is the pattern of covariation among several morphological traits or between measures of size and shape (Klingenberg, 1998) and can be used to summarize the developmental history of growing parts of an animal (Weston, 2003). By comparing how allometric growth differs between species, it is possible to reveal differences in their pathways of development that promote the morphological differentiation of species (Weston, 2003). In the damselfish, shifts of habitat and diet during their growth indicate the need to investigate the implications of allometries underlying the diversification processes of this group and in the preservation or improvement in efficiency of mechanical functions during and after the reef settlement.

If oral morphology appears to determine the fundamental trophic niche (Wainwright & Richard, 1995), dietary shifts could be associated with morphological modifications of the feeding apparatus (Liem, 1991, 1993; Wainwright, 1991). Moreover, in some fish species, ontogenetic changes in diet could also be related to changes in feeding behaviour or feeding mode (Schmidt & Holbrook, 1984; Eggold & Motta, 1992). These behavioural changes in feeding mode may be influenced by food availability and/or by mouth and head morphology because certain modes may be inefficient when used with a suboptimal morphology (Liem, 1993).

The primary objective of the present study was to characterize the ontogenetic shape changes of cranial structures directly involved in food capturing (neurocranium, suspensorium and opercle, mandible, premaxilla) in two closely-related species of Pomacentridae with different diets at the adult stage: *Pomacentrus pavo* Bloch, 1787 and *Dascyllus aruanus* Linnaeus, 1758 (Fig. 1). The two species are omnivorous, although *P. pavo* predominantly feeds on filamentous algae whereas *D. aruanus* feeds mainly on small elusive preys (Allen, 1991). In the study, landmark-based geometric morphometrics (Bookstein, 1991; Rohlf & Marcus, 1993; Marcus *et al.*, 1996; Adams, Rohlf & Slice, 2004) were used to compare allometric patterns and define the potential developmental changes that lead to morphological differences between pomacentrid species. This method, which allows description and statistical analysis of form, aims to answer the following questions:

1. Are the shapes of settling larvae already species-specific?
2. At settlement, are the shape differences smaller between larvae than between the adults?

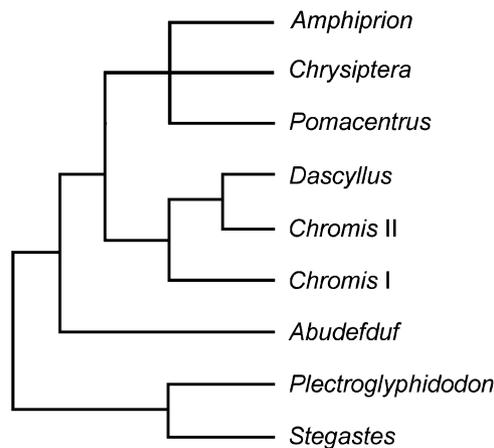


Figure 1. Phylogenetic relationships among the most speciose genera of Pomacentridae according to Quenouille, Bermingham & Planes (2004).

3. If isometry is rejected, do the two species share a common allometric pattern for each skeletal structure?
4. What kind of shape changes occurs in the two species during ontogeny?
5. Is the amount of changes undergone during ontogeny equal for the two species?
6. Do the two species have the same rate of ontogenetic shape changes?

MATERIAL AND METHODS

SAMPLING

Pomacentrus pavo ($N = 63$; standard length, SL: 13–63 mm) was collected in Moorea (Society Islands, French Polynesia) in July and August 1998 and *D. aruanus* ($N = 25$; SL: 7–39 mm) in Toliara (Madagascar) in June 2004. The samples represent a complete ontogenetic series from larvae (=larvae settling reef) (7 mm SL in *D. aruanus* and 13 mm SL in *P. pavo*; Wellington & Victor, 1989) to adult specimens. The two collected species originates from two different geographical locations but *D. aruanus* and *P. pavo* are both Indo-West Pacific species having the same habitat in Moorea and Toliara: *Dascyllus aruanus* lives always in association with branched corals and *P. pavo* inhabits isolated patch reefs in sandy areas of lagoon reefs (Allen, 1991).

Juvenile and adult specimens of *P. pavo* were collected after exposure to dissolved rotenone powder or to a solution of quinaldine. Larvae (SL = 13 mm) were caught with a net at night when they arrived on the reef crest (1.5 m wide \times 0.75 m height \times 5 m in length, 1-mm mesh net) similar to one used by Dufour, Riclet & Lo-Yat (1996). Samples were preserved in 10%

neutralized and buffered formalin during 10 days, then transferred to 70% alcohol. All *D. aruanus* specimens were caught and preserved in the same manner as *P. pavo* adults.

All specimens were cleared and stained with alizarin red S (Taylor & Van Dyke, 1985) to reveal the osseous skeleton.

GEOMETRIC MORPHOMETRICS

The ontogenetic shape changes of each bony element involved were studied using landmark-based geometric morphometric methods (Bookstein, 1991, 1996; Rohlf & Marcus, 1993).

Each skeletal unit (in lateral view) was analysed separately. Ten homologous landmarks were defined on the neurocranium, 12 on the unit 'suspensorium and opercle', 11 on the mandible and six on the premaxilla (Fig. 2, Table 1). Those were chosen according to their accuracy of digitization and homologization, as well as to represent the unit and its parts

as good as possible. The amount of methodological measurement errors was reduced at different levels: (1) each structure is positioned in glass pearls so to stabilize them in a comparable lateral plane and (2) each structure is sufficiently laterally flattened, so the projection of three-dimensional landmarks into a two-dimensional plane involves a low dimensionality reduction error. Landmarks were localized on 63 specimens in *P. pavo* and 25 specimens in *D. aruanus* using a Leica M10 binocular microscope coupled to a camera lucida. The coordinates of landmarks were digitized using TpsDig, version 1.39.

Landmark configurations of each of the four structures of all specimens were superimposed using generalized procrustes analysis (Rohlf & Slice, 1990) to remove nonshape variation (scale, orientation, translation) and to obtain the consensus configuration (average) of landmarks for each skeletal unit. Partial warp scores (PW) including both uniform and non-uniform components were calculated and used as descriptors of variation in shape (Bookstein, 1991; Rohlf, 1993).

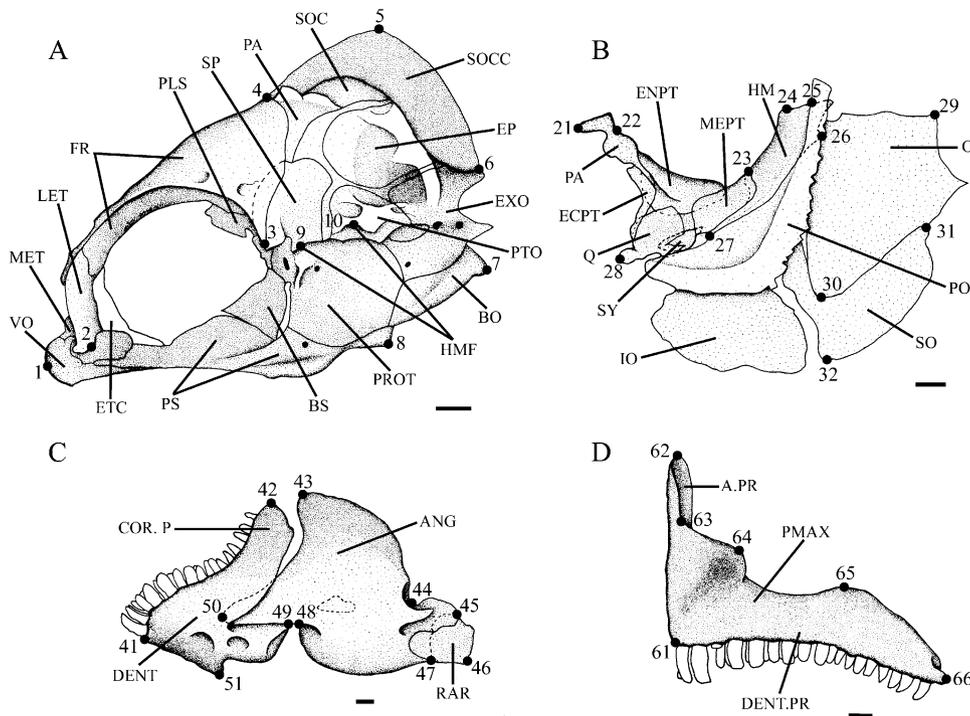


Figure 2. *Pomacentrus pavo*; adult. Localization of the different landmarks (LM) on the neurocranium (A); the suspensorium and the opercle (B); the mandible (C); the premaxilla (D). Scale bars = 1 mm. ANG, articulo-angular; A.PR, ascending process of the premaxilla; BO, basioccipital; BS, basisphenoid; COR.PR, coronoid process; DENT, dentary; DENT.PR, dentigerous process; ECPT, ectopterygoid; ENPT, entopterygoid; EP, epiotic; ETC, ethmoid cartilage; EXO, exoccipital; FR, frontal; HM, hyomandibular; HMF, hyomandibular fossa; IO, interoperculum; LET, lateral ethmoid; MEPT, metapterygoid; MET, mesethmoid; O, operculum; PA, parietal; PAL, palatine; PLS, pleurospenoid; PMAX, premaxilla; PO, preoperculum; PROT, prootic; PS, parasphenoid; PTO, pterotic; Q, quadrate; RAR, retroarticular; SO, subopercle; SOC, supraoccipital; SOCC, supraoccipital crest; SP, sphenotic; SY, symplectic; VO, vomer.

Table 1. Descriptions of landmarks

Element	Landmarks	Descriptions
Neurocranium (A)	1	Former end of the vomer
	2	Zone of articulation of the palatine on the lateral ethmoid
	3	Postero-ventral end of the frontal
	4	Anterior-most point of the supraoccipital crest
	5	Top of the supraoccipital crest
	6	Postero-dorsal end of the exoccipital
	7	Posterior end of the basioccipital
	8	Articulation of the upper pharyngeal jaws
	9	Anterior articulation fossa of the hyomandibular on the sphenotic
	10	Posterior articulation fossa of the hyomandibular on the pterotic
Suspensorium and opercle (B)	21	Anterior-most point of the maxillar process of the palatin
	22	Articulation of the palatin on lateral ethmoid
	23	Dorsal limit between the metapterygoid and the hyomandibular
	24	Anterior articulation condyle of the hyomandibular on the sphenotic
	25	Posterior articulation condyle of the hyomandibular on the pterotic
	26	Opercular condyle of the hyomandibular
	27	Antero-ventral end of the hyomandibular
	28	Articulation process of the quadrate
	29	Postero-dorsal end of the opercle
	30	Ventral end of the opercle
	31	Posterior intersection between the opercle and the subopercle
	32	Ventral end of the subopercle
Lower jaw (C)	41	Rostral tip of the dentary
	42	Dorsal tip of the coronoid process of the dentary
	43	Dorsal tip of the articular process
	44	Articulation fossa of articulo-angular with the quadrate
	45	Dorsal tip of the retroarticular
	46	Posterior end of the retroarticular
	47	Anterior-most point of the retroarticular
	48	Ventral start of anterior process of the articulo-angular
	49	Posterior end of the dentary
	50	Anterior-most point of the articulo-angular
51	Ventral-most point of the dentary	
Premaxilla (D)	61	Anterior-most point of the dentigerous process
	62	Tip of the ascending process
	63	Ventral-most point of the interprocess edge
	64	Dorsal point of the area which receives the maxillary process
	65	Crest of the dentigerous process
	66	Posterior end of the dentigerous process

To estimate the variation in repeated measures of one specimen, landmarks configurations were recorded ten times on different days in the same fish. This variance of shape was compared with the variance of the total dataset using the Procrustes distances (PD) about the mean (see below). According to the skeletal unit, the error variance accounts for 0.20–3.22% of the total shape variation.

The allometric patterns of shape variation were calculated by multivariate regressions of PW scores on log-transformed centroid size (CS) (Monteiro, 1999;

Zelditch, Sheets & Fink, 2000, 2004; Mitteroecker, Gunz & Bookstein, 2005) by TpsRegr, version 1.28. CS (Bookstein, 1991) was used because this is the only measure of size uncorrelated to shape in the absence of allometry (Bookstein, 1991). The CS was log-transformed (log-CS) because the allometric relationships are better described by a linear model that takes into account the progressive decrease of the rate of shape change during growth (e.g. comparison of rate of shape changes with plots of PD versus CS). The fit of the regression models was evaluated by the

explained variance of the model and by Goodall's *F*-test.

The interpolation function 'thin plate spline' (TPS) (Bookstein, 1991) was used to compute the deformation grid with least bending energy between reference and target landmark configurations. The TPS allows illustrating the shape changes occurring during ontogeny. Multivariate regression models of shape on size for each unit were used to provide graphical illustrations of ontogenetic allometries in the two species. Thus, shape changes between larvae and adult were depicted as vectors of landmark displacements using Regress6k (IMP software).

The samples of settling larvae and adults are limited. Consequently, for the tests that compare shape variation between larvae at settlement and between adults, a standardized regression residual analysis was used (Zelditch, Sheets & Fink, 2003a; Bastir & Rosas, 2004). From the separate multivariate regression of shape on log-CS for each species, the non-allometric residual fraction is standardized by Standard6 (IMP software). 'Standardized' data sets of settling larvae and adults, which are the predicted shapes of the entire population at these stages, are generated and can be then analysed (for detailed explanations on this procedure, see Zelditch *et al.*, 2003a; Bastir & Rosas, 2004). To compare shapes at the two stages, pairwise *F*-tests were performed in TwoGroup6h (IMP software). The statistical significance of the differences is tested by a resampling-based *F*-test. For each skeletal unit, the amount of the overall shape differences between species was estimated using the PD, which is a proper metric for shape dissimilarity in the Kendall shape space (Bookstein, 1996). This distance was used as an univariate measure of shape difference, but needs to be considered as an overall measure of multivariate shape components (i.e. partial warps). PD between the average shapes at the two stages (settling larvae and adults) are given by TwoGroup6h. Confidence limits are placed on this measure by a bootstrapping procedure.

Comparisons among the ontogeny of shape were performed by a multivariate analysis of covariance (MANCOVA) using the whole ontogeny dataset of each species, testing the null hypothesis of homogeneity of linear allometric models. In this test, uniform components and non-uniform PW scores are considered as variates, log-CS as covariate and species are the grouping factor. This statistical analysis was performed using TpsRegr. When the allometric models differed for the two species, the differences were analysed by comparing the angle between the species-specific multivariate regression vectors using VecCompare6 (IMP software). An elaborate explanation of this approach is provided

elsewhere (Zelditch *et al.* 2000, 2003a, 2004). Briefly, in the context of the study of ontogenetic allometry, a within-species vector is composed of all regression coefficients of the shape variables (PW) and the log-transformed CS. The range of angles between such vectors within each species is calculated using a bootstrapping procedure ($N = 400$). This range was then compared with the angle between the vectors of both species. If the between-species angle exceeds the 95% range of the bootstrapped within-species angles, the between-species angle is considered as significantly different, and thus the ontogenetic allometries are different.

The rate of change in the overall shape for each skeletal unit in the two species was estimated using the PD. Dynamics of shape changes are visualized by plots of PD between each specimen and the average shape of the smallest larvae in the datasets for both species, on their CS. They were also regressed on log-transformed CS and the rates of divergence away from the average larval shape for each species were compared using the slope of the regressions using Regress6k (IMP software) (Zelditch *et al.*, 2003a, 2004). The average shape of larvae was calculated based on the larvae of 7, 9, and 10 mm SL in *D. aruanus* and the ten larvae of 13 mm SL in *P. pavo* using also Regress6k.

To compare the amount of shape changes over ontogeny in the two species, the length of their ontogenetic trajectories of shape, which is a function of the rate of shape change and the duration of ontogeny (Zelditch *et al.*, 2003a), is used. These lengths were calculated by the PD between the average shape at settlement and at the adult stage. Confidence intervals are placed on these lengths by a bootstrapping procedure.

The TPS morphometric software (TpsDig and TpsRegr) were written by F. J. Rohlf and are freely available at: <http://life.bio.sunysb.edu/morph/>. Regress6k, Standard6, VecCompare, and TwoGroup6h originating from the IMP geometric morphometric software package were created by H. D. Sheets and are also freely available at: <http://www2.canisius.edu/~sheets/morphsoft.html>.

JAW LEVER MECHANICS

The exploration of variations of the lower jaw-lever mechanics during ontogeny in both species was performed using landmarks (LM) 41, 43, 44, and 46 on the mandible, which allows an effective comparison of the efficiency (kinetic versus force efficiency) of jaw closing and opening systems (Wainwright & Richard, 1995). The distance from LM 44 to LM 46 corresponds to the jaw opening in-lever. The in-lever for jaw closing is the distance between LM 44 and LM 43

Table 2. Fit of regressions of shape versus log-values of centroid size for each structural unit

	<i>Dascyllus aruanus</i>			<i>Pomacentrus pavo</i>		
	% Explained variance	Goodall's <i>F</i> -test		% Explained variance	Goodall's <i>F</i> -test	
		<i>F</i> -value	<i>P</i>		<i>F</i> -value	<i>P</i>
Neurocranium	65.7	49.8898	0.0000	61.2	94.7799	0.0000
Suspensorium and opercle	42.3	19.8770	0.0000	43.7	48.2325	0.0000
Mandible	80	104.6715	0.0000	79.5	233.6178	0.0000
Premaxillary	42.6	20.1333	0.0000	35.1	33.5832	0.0000

(the point of insertion of the bundle A2 of the adductor mandibulae muscle on the articulo-angular) and the jaw closing out-lever is the distance between LM 44 and LM 41 (Fig. 2). Dimensionless ratios were calculated for the jaw opening and closing systems:

Jaw opening lever ratio (JO) = opening in-lever (LM 44 – 46)/closing out-lever (LM 41 – 44);
 Jaw closing lever ratio (JC) = closing in-lever (LM 43 – 46)/closing out-lever (LM 41 – 44).

RESULTS

TESTING ALLOMETRY

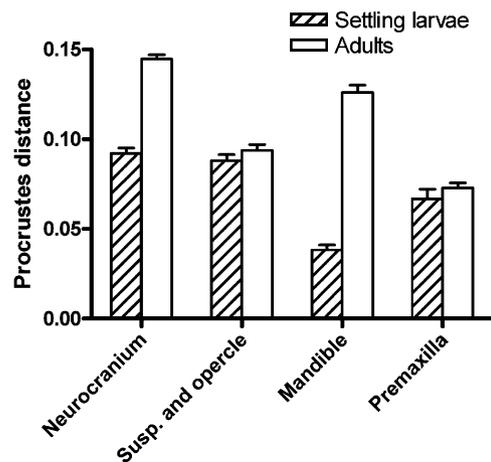
The null hypothesis of isometric growth is rejected. Each skeletal unit in the two species is highly significantly allometric (Table 2). These results are well supported by the Goodall test (all $P < 0.05$), which means that there is a linear relation between log-CS and shape variables during the whole ontogenetic dataset. The significant regression models in shape space account for > 61% and > 79% in the neurocranium and the mandible, respectively. The lower percentage of variance explained in the unit 'suspensorium and opercle' and the premaxillary bone (42–44%, Table 2) indicates that some variability in shape is possibly due to factors other than size or that the ontogeny could be nonlinear.

COMPARISON OF THE LARVAL SHAPES AT SETTLEMENT

For each skeletal structure, pairwise *F*-tests reveal statistically significant differences between the two species at settlement ($P < 0.0025$). Thus, larval shapes are already species-specific. However, the dissimilarity between *D. aruanus* and *P. pavo* varies among the different structure (Fig. 3). For example, PDs between the mean shapes are the lowest for the mandible and the highest for the neurocranium (Fig. 3), demonstrating that the larvae are more similar in mandible shape than in that of the neurocranium at settlement.

Table 3. Tests for common linear allometric models for the shape and size relationship in *Pomacentrus pavo* and *Dascyllus aruanus* using whole ontogeny dataset: results of multivariate analysis of covariance using TpsRegr

	λ_{WILKS}	<i>F</i>	<i>P</i>
Neurocranium	0.322105	9.339	7.290×10^{-12}
Suspensorium and opercle	0.420787	4.818	4.392×10^{-7}
Mandible	0.212610	14.197	1.304×10^{-16}
Premaxillary	0.647107	5.590	1.183×10^{-5}

**Figure 3.** Differences in units of Procrustes distance between mean larval shapes and between adult shapes.

COMPARISON AMONG ALLOMETRIC MODELS

Tests for common allometric models performed by a MANCOVA using TpsRegr are highly significant, showing that the same linear model cannot be employed for the description of the ontogenetic allometries of each structure in both *D. aruanus* and *P. pavo* (Table 3). The differences in the models of the two species could be attributed to their different rate in shape changes or to their different ontogenetic trajectories of shape. Three possibilities could induce

differences in the ontogenetic trajectories: (1) the intercept of the linear regression is different between the two species but the slope is equal; (2) the slope is different and, thus, the ontogenetic trajectories diverge; or (3) species differ in both intercept and slope.

The analysis of the angles between multivariate regression vectors of ontogenetic allometries within- and between-species showed that, for the neurocranium, the angle between *D. aruanus* and *P. pavo* is 25.9° and thus higher than the ranges of the within-species angles (14.9° for *D. aruanus* and 14.3° for *P. pavo*) (Table 4). The two species therefore differ significantly in their trajectories of shape changes. The same is true with respect to the mandible and premaxillary bone (Table 4). Test results for the unit 'suspensorium and opercle', however, show that the angles between multivariate regression vectors are not significantly different (Table 4). Consequently, due to our sample sizes, the null hypothesis of an identical direction to the ontogenetic vectors cannot be rejected.

COMPARISON OF THE ADULT SHAPES

Comparisons of adult shapes support the results of allometric models. Pairwise *F*-tests reveal statistically significant differences of shapes between the two species at the adult stages ($P < 0.0025$). As shown in

Table 4. Tests for common slopes for the shape and size relationship

	Between species	<i>Dascyllus aruanus</i>	<i>Pomacentrus pavo</i>
Neurocranium	25.9	14.9	14.3
Suspensorium and opercle	19.2	27.8	20.7
Mandible	21	9.5	8.4
Premaxillary	36.1	24	24

Results of bootstrapping procedure ($N = 400$) comparing the multivariate regression vectors using VecCompare6. Angles are in decimal degree.

Table 5. Results of the regressions between Procrustes distance and log-values of centroid size for each skeletal unit

	<i>Dascyllus aruanus</i>			<i>Pomacentrus pavo</i>		
	Rate	SE	R^2	Rate	SE	R^2
Neurocranium	0.112	0.007	0.957	0.108	0.005	0.944
Suspensorium and opercle	0.097	0.007	0.939	0.066	0.003	0.924
Mandible	0.282	0.015	0.967	0.280	0.008	0.977
Premaxillary	0.077	0.010	0.826	0.055	0.006	0.744

SE, standard deviation in slope; R^2 , square of the correlation coefficient.

Figure 3, the PDs between the average shapes of adults are clearly higher than the PDs between larvae for the neurocranium, the mandible and, to a lesser extent, the premaxilla and the 'suspensorium and opercle' unit. Thus, the dissimilarity between the two species increases during growth. Figure 3 also shows that the premaxilla is the structure that is least divergent between the two species at the adult stage (PDs for the neurocranium, the mandible, and the 'suspensorium and opercle' unit are higher than that for the premaxilla).

DYNAMICS OF SHAPE CHANGES

Dynamics of shape changes during ontogeny for each skeletal structure, expressed as the relation between PD and CS, are given in Figure 4. All trajectories are shown to be asymptotic, decreasing during growth. In the case of the neurocranium and the 'suspensorium and opercle' unit, this is not so clear in *D. aruanus* (Fig. 4A, B): variation in shape is more constant during the whole size range or the plateau is not reached in the size range studied. In *P. pavo*, a decrease in the rate of ontogenetic shape changes in neurocranium and unit 'suspensorium and opercle' starts for CS values between 7 and 9 and 9–11, respectively (Fig. 4A, B). These both ranges of centroid size correspond to an SL in the range 25–30 mm. In both species, larger shape modifications occur at a range of CS of 2–4 for the mandible and 1–2.5 for the premaxilla which corresponds to an SL in the range 10–25 mm.

The rates of shape changes are similar for the neurocranium and the mandible in the two species (Table 5). *Dascyllus aruanus* shows higher rate values than *P. pavo* for the unit 'suspensorium and opercle' ($0.097 \pm 0.007 > 0.066 \pm 0.003$) and the premaxillary bone ($0.077 \pm 0.010 > 0.055 \pm 0.006$).

The PDs between the average shapes at settlement and at the adult stage show that the ontogenetic trajectory of each skeletal structure is always longer in *D. aruanus* than in *P. pavo* (Fig. 5). Although the adult body size of *D. aruanus* (SL = 39 mm SL) is

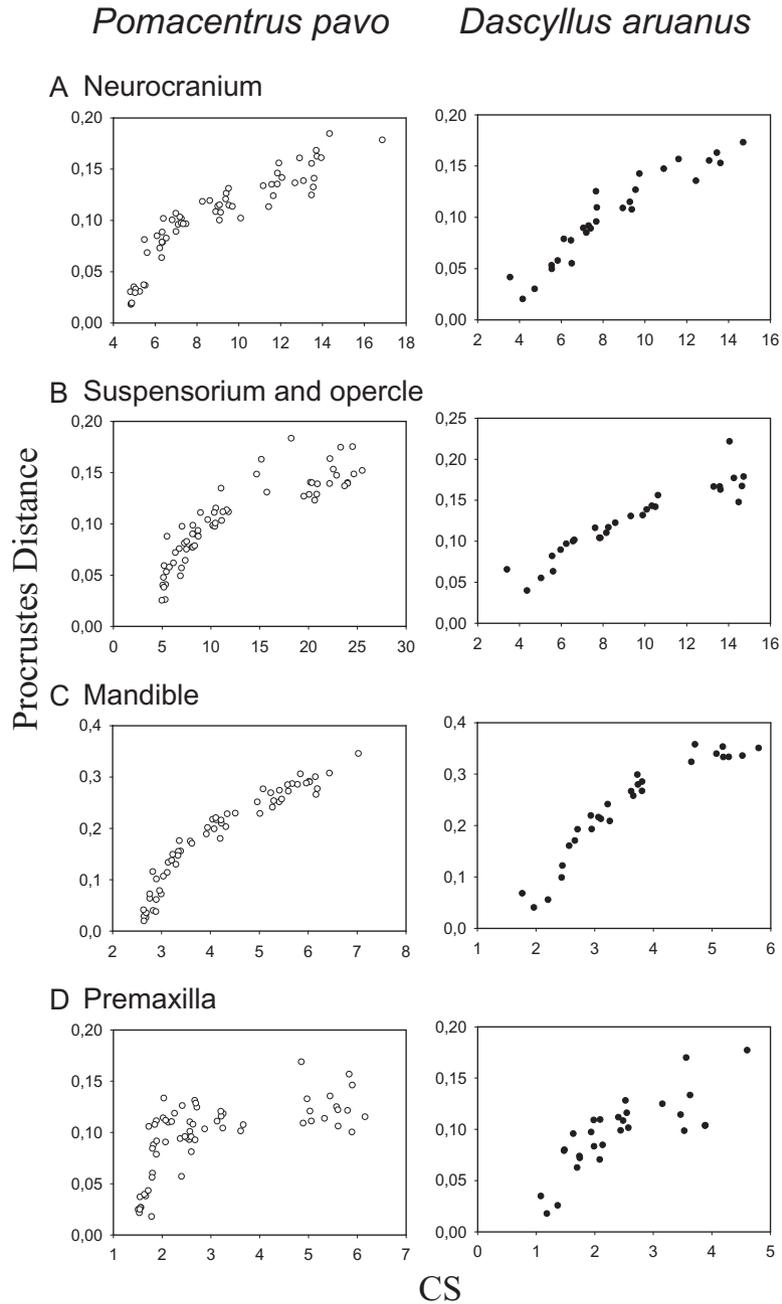


Figure 4. Plots of Procrustes distance between each specimen and the shape of larvae on centroid size (CS) in *Dascyllus aruanus* and *Pomacentrus pavo* for the neurocranium (A); the suspensorium and the opercle (B); the mandible (C); the premaxilla (D).

smaller than that of *P. pavo* (SL = 63 mm SL), the former undergoes more shape changes for each skeletal unit during the ontogeny.

DESCRIPTION OF ALLOMETRIES

According to the regression model of ontogenetic trajectories, the larva of *P. pavo* present a neurocranium that is longer and thus proportionally less

higher than that of *D. aruanus*. This trend is reinforced during growth (Fig. 6A). Globally, in the two species, ontogenetic shape changes of the neurocranium mainly comprise: (1) a relative shortening of the orbital region (LM 1, 2, and 8) and (2) a relative increase in height and an elongation of the supraoccipital crest (LM 4, 5, and 6). The supraoccipital crest lengthens more in *P. pavo* during growth (LM 4).

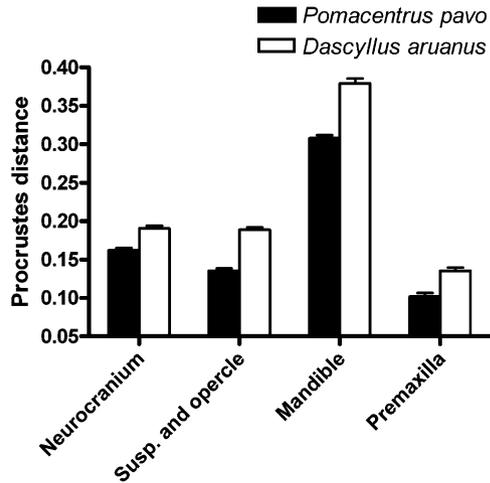


Figure 5. Lengths of ontogenetic trajectories in units of Procrustes distance for each skeletal units.

Both species present a relative increase in height of the suspensorium and the opercle and thus this unit is proportionally shorter along the antero–posterior axis (Fig. 6B). In both species, the ontogenetic shape changes in the suspensoria and opercles are: (1) a shortening and a downward bending of the maxillary process of the palatine (subsequently, the ethmo-palatine articulation comes to sit at the level of the mandibular joint) (LM 21 and 22); (2) a general extending of the hyomandibula along its length axis (LM 23, 24, 25, and 27); and (3) an increase in height of the opercle (LM 30 and 32). In the two species, the extending hyomandibula coupled with a shortening of the central part of the suspensorium implies a forward displacement of the quadrate-mandible articulation (LM 28).

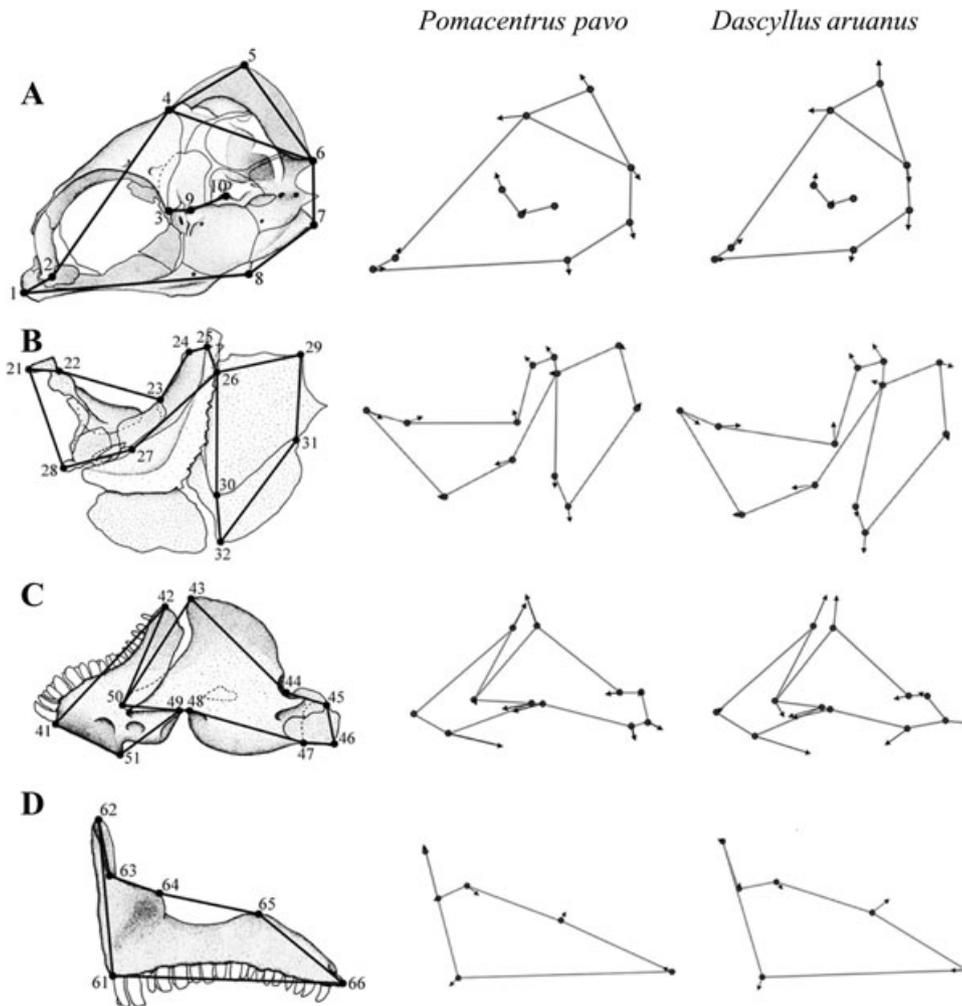


Figure 6. Ontogenetic shape variations of the two species calculated by multivariate regressions of shape on log-transformed size for the neurocranium (A); the suspensorium and the opercle (B); the mandible (C); the premaxilla (D). Ontogenetic transformations are depicted as vectors of landmark displacements. Dots are LM-positions for the larvae and tips of vectors are LM-positions for the adult.

Both species present a relative increase in height of the mandible during growth, especially at the level of the coronoid process (LM 42, 43, 48, and 49). Thus, the mandible appears proportionally shorter in the adults (Fig. 6C). The space between the dentary and the articulo-angular at the coronoid process is reduced (LM 42 and 43). The symphyseal part of the dentary lengthens (LM 41 and 51) and the central part of the articulo-angular enlarges and thus becomes greater compared with the dentary (LM 48, 49 and 50). *Dascyllus aruanus* shows a retroarticular process that is rostrocaudally more extended than in *P. pavo*. This difference is elaborated during growth (LM 46 and 47).

Regression models reveal a relative lengthening of the ascending process of the premaxilla (LM 1 and 2) during growth in the two species (Fig. 6D). A relative shortening and heightening of the dentigerous process (LM 65, 64 and 61) occurs in *D. aruanus*.

Figure 7 shows an increase of the JO and JC during growth in *D. aruanus* and *P. pavo*. The asymptotic trajectories are relatively similar to that of shape changes. However, the JO is always higher in *D. aruanus* during post-settlement ontogeny (Fig. 7A).

DISCUSSION

Because of a major change in their ecology during ontogeny (Leis & McCormick, 2002), Pomacentridae

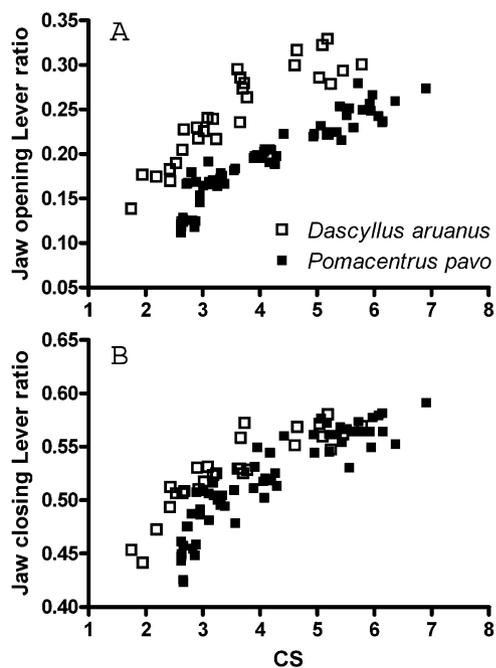


Figure 7. Ontogenetic changes of the jaw opening (A) and closing (B) lever ratios in *Pomacentrus pavo* and *Dascyllus aruanus*. CS, centroid size.

comprises an interesting taxon in which to study morphological changes and their ecomorphological relationships. In *D. aruanus* and *P. pavo*, the substantial shape changes observed during growth involve each of the studied skeletal units (neurocranium, suspensorium and opercle, mandible, premaxillary). After a pelagic and oceanic larval phase, modifications of the structures could be expected to reflect possible adaptations to their new demersal life on the reef.

Early ontogeny (just after settlement) is characterized by very rapid shape changes, followed by a decrease in the rate of these changes until they reach an apparently stable stage (Fig. 4). This observation agrees with that on sparid fishes of temperate regions, which show similar size-related shape changes during the early juvenile stages (Loy *et al.*, 2001). These sparids also have a life cycle with a transition from pelagic larvae to necto-benthic juvenile and adult stages associated with the reef environment. The similar pattern suggests the need for a rapid morphological shift during a transition of habitats. On the other hand, a very rapid rate of shape changes early in ontogeny is also characteristic of mammals (Zelditch *et al.*, 2003b). Consequently, a shift of habitat or function might not solely explain such a general pattern. In the present study, the rates of shape changes are calculated relative to size and it is plausible that size changes slowly whereas shape changes more quickly. However, rapid growth was recently observed in new recruits of *Pomacentrus amboinensis* Bleeker, 1868 during the third weeks of their benthic life (Gagliano & McCormick, 2007). After 30 days, *P. amboinensis* shows a mean standard length of 20 mm SL (Gagliano & McCormick, 2007). The present study clearly shows important shape modifications occurring at a size in the range 10–20 mm SL in the two species. Ecologically, a rapid allometric growth after settlement could enhance the success of the transition to the benthic environment.

The larvae of *D. aruanus* (7–9 mm SL) and *P. pavo* (13–15 mm SL) already show species-specific shapes. However, they share a more similar morphology at settlement than at the adult stage, especially for the mandible (Fig. 3). Two major factors could explain a closer similarity among settling larvae than among adults. Being phylogenetically closely related, damselfishes' ontogeny could be expected to have an underlying 'common' shape during their younger stages, with the major shape modifications arising subsequently and resulting in the adult shape (Liem, 1991; Zelditch *et al.*, 2003a). To maximize survival in each environment, these modifications should have appeared at the end of the larval stage at the moment of the colonization of the reef (Alberch *et al.*, 1979). Moreover, because the larvae live in the pelagic zone

of the ocean feeding on zooplankton (Leis & McCormick, 2002), the similarity of their shape could be related to a common environment and a very similar diet, essentially comprising the naupliar through to adult stages of copepods (Hunter, 1981).

The proportions of size-related shape change after reef settlement in *D. aruanus* and *P. pavo* vary from 42% ('suspensorium and opercle' unit) to 80% (mandible) according to the skeletal units of the head (Table 2). Why the degree of allometry is so variable between the different structures is unclear. Other factors such as external conditions (e.g. food supply) and internal conditions (e.g. physiology, sex, behaviour) could also explain some shape variations and should be investigated. However, these proportions (42–80%) suggest that allometry is an important aspect characterizing post-settlement ontogeny in damselfishes. The results of the present study show that shape variations between the two studied species are larger at the adult stage than at the settling larval stage, and this is for each skeletal unit (Fig. 3). The interspecific divergence continues after reef settlement and ontogenetic allometries could be considered as an important source of the known morphological diversification in pomacentrids. In a broad sense, all evolutionary changes or all dissociation of size, shape, and age during ontogeny could be referred as heterochronic processes (Klingenberg, 1998; Webster & Zelditch, 2005). Having no information on chronological age of the two studied species, size (CS) was used as a proxy of developmental age (Adriaens & Verraes, 2002). Thus, the term 'allometric heterochrony' should be more appropriate in our discussion (for a discussion on terminology, see Klingenberg, 1998) although, in the present study, the start and the end of the ontogenetic trajectories are clearly defined in an ecological time permitting effective comparison of ontogenetic changes between the two species: the start refers to the reef settlement and the end is the adult stage.

From the settling larvae to adult stage, the magnitude of shape modifications in each skeletal unit is always higher in *D. aruanus* (Fig. 5) revealing that, after the oceanic and pelagic larval stage, the length of its ontogenetic trajectories is longer than in *P. pavo*. This difference may be related to a variation in timing during ontogeny: (1) each species settles on the reef at a different developmental stage or (2) the two species settle at the same developmental age but the duration of shape transformations after the reef colonization varies between species. According to Wellington & Victor (1989), *P. pavo* and *D. aruanus* have a different pelagic larval stage duration: 20–27 days and 16–24 days, respectively. This difference in timing for the colonization of the reef could partially explain the variability in length of their ontogenetic trajectories. The rates of ontogenetic shape changes also vary

among both species (Table 5, Fig. 4). In *P. pavo* and *D. aruanus*, the developmental rate appears to be rather similar for the neurocranium and the mandible. On the other hand, *D. aruanus* shows faster shape changes for the premaxilla and unit 'suspensorium and opercle'.

The model of ontogenetic allometries of each structure is different for the two species (Table 3). The differences involve not only the rates and the timing, but also the trajectories of shape change (Table 4). Rather than progressing along the same ontogenetic trajectories at different rates, the two species follow different ontogenetic trajectories. For the neurocranium, the mandible, and the premaxilla, the differences in adult shape (Fig. 3) result from divergent ontogenetic trajectories (Table 4), indicating a stronger dissimilarity between the two species during the post-settlement phase. On the other hand, the allometric trajectories for the 'suspensorium and opercle' unit in *P. pavo* and *D. aruanus* point in the same direction (test for common slopes by comparing the multivariate regression vectors; Table 4). As the two species are dissimilar at each stage (settling larvae and adults) (Fig. 3), the ontogenetic trajectories of the 'suspensorium and opercle' unit are parallel in the size-shape space showing a case of parallel transposition (same slope but different intercept for the model of each species) where a dissociation had occurred in an earlier period to reef settlement. In our comparison of allometric patterns of *D. aruanus* and *P. pavo*, we reveal that, depending on the cephalic skeletal unit, the morphological differentiation between the two species are related to: (1) differences in the rate of ontogenetic shape changes; (2) differences in onset and offset timing; (3) changes of the direction of the ontogenetic trajectories; and (4) parallel shifts of the ontogenetic trajectories.

In fishes, prey capture can be realized according to variations of three main strategies: (1) inertial suction, during which prey is sucked into the buccal cavity by a stationary predator; (2) ram feeding in which the predator, moving with an open mouth, overtakes its prey; and (3) biting, in which the teeth of upper and lower oral jaws are applied to the prey (Lauder, 1980; Liem, 1993). In species that mainly practice suction feeding, the prey-capture apparatus is modelled as a truncated cone (Liem, 1993). This can be gradually expanded and compressed by the action of muscles in order to produce a water flow into the buccal and opercular cavities (Alexander, 1967; Lauder, 1980; Vandewalle & Chardon, 1981). The presence of such a cone, and the improvement of its performance, is related to various characteristics such as upper jaw protrusion facilitated by a long ascending process of the premaxilla (Gosline, 1987), relatively short mandibles, and high suspensoria and opercles (Liem, 1993). In ram feeders, the buccopharyngeal cavity has

a more cylindrical shape. The characteristics enhancing the efficiency of ram feeding should be long mandibles and shallow suspensory apparatus (Liem, 1993; Wainwright & Richard, 1995). The majority of biters (e.g. grazer, scraper) usually combine biting movements with sequences of suction and/or ram feeding allowing the manipulation and the passage of food into the buccopharyngeal cavity. These species may combine the designs that enhance suction and biting (i.e. a high supraoccipital crest for improved neurocranial elevation, a deep and narrow suspensory apparatus for efficient lateral expansions of the buccal cavity, and relatively short jaws with beneficial in- and output lever ratios for fast jaw opening and powerful biting; Liem, 1993; Wainwright & Richard, 1995). However, as shown recently, features relying on skeletal elements only cannot always explain differences in feeding performances (Van Wassenbergh *et al.*, 2005a).

The morphological modifications observed in *D. aruanus* and *P. pavo* during development appear to fit more the model for an improved suction feeding system. These main changes include: (1) an increase in height and length of the suspensorium and the opercle; (2) an elevation of the supraoccipital crest; (3) a forward displacement of the mandibular articulation with respect to the neurocranium; (4) a mandible becoming higher (thus it appear proportionally shorter in the adult); and (5) a lengthening of the ascending process of the premaxillary bone (Fig. 6). These modifications have also been observed during ontogeny in *Amphiprion frenatus* Brevoort, 1856 (Dilling, 1989) and other suction feeders such as flatfishes (Wagemans & Vandewalle, 2001). The shortening of the mandible observed during ontogeny in *D. aruanus* and *P. pavo* implies the formation of a small mouth, which could facilitate suction and is also beneficial for a powerful biting (Liem, 1993).

For both lever ratios, there is a change from a mechanism with a high velocity transmitting efficiency to a system that has better force transmission at the expense of speed (Fig. 7) (Wainwright & Richard, 1995). These changes confirm the same differences observed between settling larvae and adults in four other pomacentrids species (Frédérich *et al.*, 2006). The JO is always higher in *D. aruanus* during post-settlement ontogeny (Fig. 7A) and may be related to some difference in suction abilities between the two species. However, it would be premature to link this to a higher decrease in suction performance in *D. aruanus* (i.e. due to the lever ratio indicating an increased reduction in mouth opening speed) because its kinematics are largely dependent on the kinematic efficiency and speed of the input systems (i.e. opercular and hyoid four bar system) (Adriaens, Aerts & Verraes, 2001; Van Wassenbergh *et al.*, 2005b).

The appearance of incisiform teeth in *P. pavo* during ontogeny (Frédérich *et al.*, 2006) coupled to the increasing of JC (Fig. 7B) could also be considered in relation to the acquisition of the capacity to graze algae or other fixed organisms (Allen, 1991). The ontogenetic heightening of the coronoid process reflects this as well, as the subsequent improved input lever condition and thus increased moment of rotation of the forces exerted by the jaw adductors will generate a higher biting force (Fig. 7B) (but see also Van Wassenbergh *et al.*, 2005a). The symphyseal part of the dentary is longer in the two adult species (Fig. 6C). This lengthening, which allows a strong joint between the two mandibles, could also be considered as an adaptation of forceful biting.

The ontogenetic shape transformations in the head in *D. aruanus* and *P. pavo* could thus be considered to reflect a switch in prey-capture tactics as also observed in *A. frenatus* (Liem, 1991). The larvae can be considered as suction/ram feeders that change towards a optimized suction-feeding during later ontogeny. Moreover, mandible shapes in the two studied species probably reflect a second mode of feeding (i.e. biting).

In *P. pavo* and *D. aruanus*, analysis of the functional units of the skull by ontogenetic shape changes shows that these units (neurocranium, 'suspensorium and opercle', mandible and premaxilla) are already species-specific at settlement. The present study reveals that post-settlement ontogeny follows significantly different patterns in *D. aruanus* and *P. pavo*, both at a qualitative and quantitative level. The interspecific divergence at the adult stage results from differences in the rate of ontogenetic shapes changes, differences of timing, divergences of the ontogenetic trajectories, or lateral shifts of the trajectories in the size-shape space. From a functional point of view, the transformations observed in the two species during reef settlement involve modifications that may enhance suction-feeding and/or algae scraping.

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