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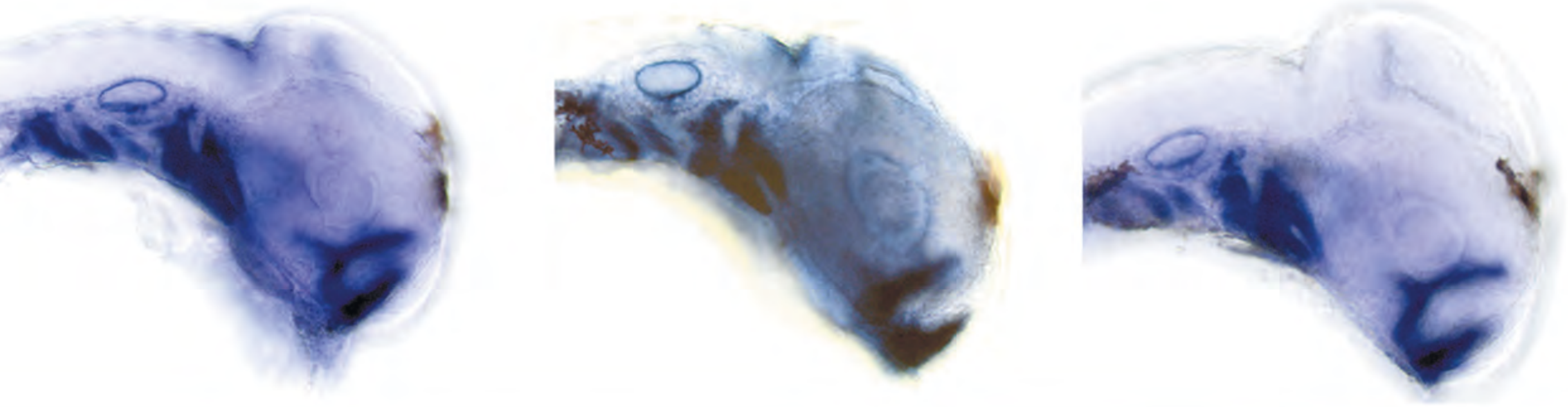

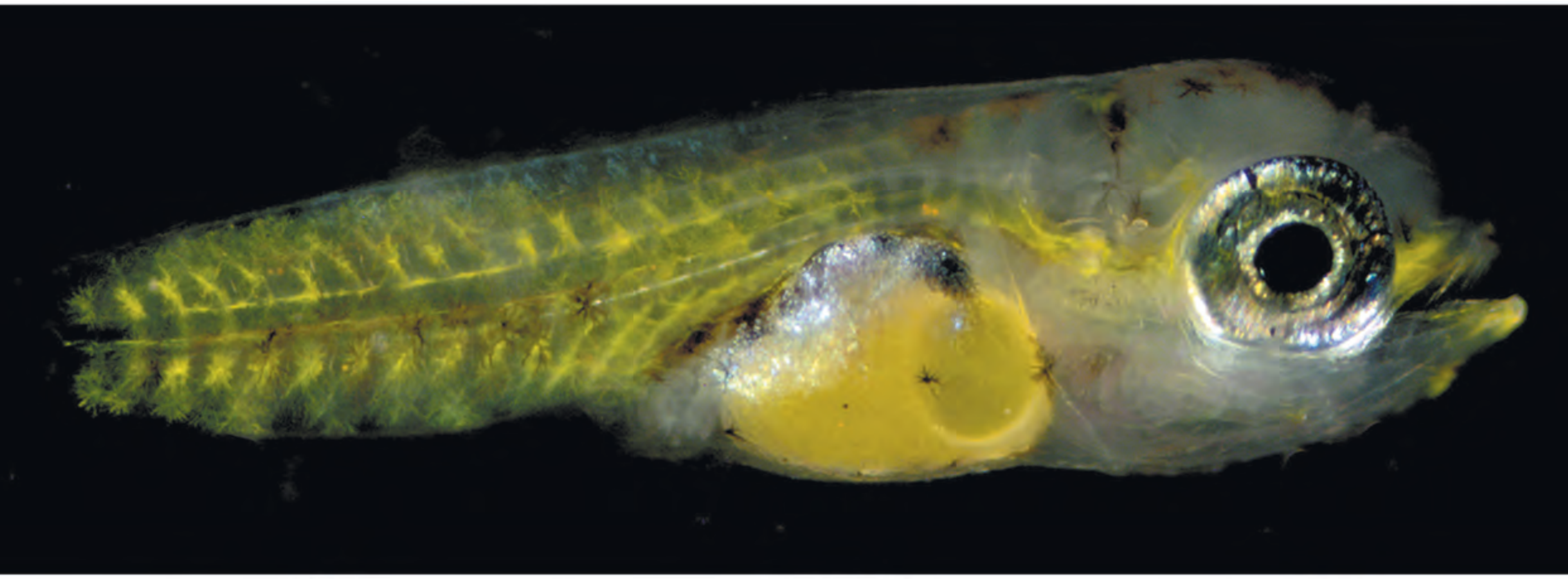

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RESEARCH

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Functional morphogenesis from embryos to adults: Late development shapes trophic niche in coral reef damselfishes

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Abstract

The damselfishes are one of the dominant coral reef fish lineages. Their ecological diversification has involved repeated transitions between pelagic feeding using fast bites and benthic feeding using forceful bites. A highly-integrative approach that combined gene expression assays, shape analyses, and high-speed video analyses was used to examine the development of trophic morphology in embryonic, larval, juvenile, and adult damselfishes. The anatomical characters that distinguish pelagic-feeding and benthic-feeding species do not appear until after larval development. Neither patterns of embryonic jaw morphogenesis, larval skull shapes nor larval bite mechanics significantly distinguished damselfishes from different adult trophic guilds. Analyses of skull shape and feeding performance identified two important transitions in the trophic development of a single species (the orange clownfish; *Amphiprion percula*): (a) a pronounced transformation in feeding mechanics during metamorphosis; and (b) more protracted cranial remodeling over the course of juvenile development. The results of this study indicate that changes in postlarval morphogenesis have played an important role in damselfish evolution. This is likely to be true for other fish lineages, particularly if they consist of marine species, the majority of which have planktonic larvae with different functional requirements for feeding in comparison to their adult forms.

KEYWORDS

adaptive radiation, metamorphosis, skull

1 | INTRODUCTION

Evolutionary changes in the functional morphology of feeding have been intensively studied in a large number of fish groups (e.g., Albertson, Streelman, & Kocher, 2003; Collar, O'Meara, Wainwright, & Near, 2009; Cooper et al., 2010; Ferry-Graham, Wainwright, & Bellwood, 2001; Konow, Bellwood, Wainwright, & Kerr, 2008; Mehta & Wainwright, 2007; Streelman, Alfaro, Westneat, Bellwood, & Karl, 2002; Westneat, 1994). The evolution of a lineage is shaped by chance, ecological opportunity and the characteristics of its development (Losos, 2010; Shubin, Tabin, & Carroll, 1997; Travisano, Mongold, Bennett, & Lenski, 1995). Of these three elements, only aspects of the developmental organization are intrinsic to any species or clade. Although patterns of trophic evolution have been described in detail for many fishes, we know little about the developmental features or ontogenetic changes that have shaped these patterns.

The damselfishes (Pomacentridae; Actinopterygii) are one of the dominant coral reef fish families (Bellwood & Hughes, 2001) and the evolution of their feeding biomechanics has been the subject of extensive study (Aguilar-Medrano, Frederich, De Luna, & Balart, 2011; Cooper, 2009; Cooper & Westneat, 2009; Cooper, Carter, Conith, Rice, & Westneat, 2017; Frederich & Vandewalle, 2011; Frederich, Cooper, & Aguilar-Medrano, 2016; Frederich, Olivier, Litsios, Alfaro, & Parmentier, 2014; Frederich, Pilet, Parmentier, & Vandewalle, 2008; Frédérich, Sorenson, Santini, Slater, & Alfaro, 2013; Damien Olivier, Gajdzik, Parmentier, & Frédérich, 2017; Damien Olivier, Parmentier, & Frédérich, 2016). Their trophic evolution has involved rapid and repeated transitions along an ecomorphological axis that has hard-biting, benthic-feeding species at one end and fast-biting, pelagic-feeding species at another (Cooper & Westneat, 2009; Cooper et al., 2017; Frederich & Vandewalle, 2011; Frederich et al., 2016). The transition along this axis involves changes to the linked cranial lever systems employed during feeding (e.g., those in the jaws, suspensorium, and hyoid apparatus; Cooper & Westneat, 2009; Cooper et al., 2017).

Rapid jaw movements allow pelagic-feeding damselfishes to capture evasive zooplankton such as copepods, while benthic-feeding damselfishes predominantly use high bite forces to remove algae from hard substrates and to prey upon benthic invertebrates (Cooper & Westneat, 2009; Cooper et al., 2017; Frederich et al., 2016; Olivier, Frederich, Spanopoulos-Zarco, Balart, & Parmentier, 2014; Damien Olivier et al., 2017; Damien Olivier et al., 2016). In regard to lever mechanics, force and speed are inverse to one another. Bones act as levers rotated around fulcra (joints) by muscles and a given musculoskeletal configuration can multiply either force or speed but cannot do both (Westneat, 2004). The adult skull morphologies and bite mechanics of pelagic-feeding damselfishes are significantly different from those that feed extensively from the benthos (Cooper & Westneat, 2009; Cooper et al., 2017). The developmental stage during which these differences arise is unknown.

The goal of this study was to determine whether the cranial characters that allow adult damselfishes to specialize on either benthic or pelagic food sources appear during early development (embryonic and/or larval stages) or after metamorphosis, when damselfish larvae undergo rapid and extensive cranial remodeling (McCormick, Makey, & Dufour, 2002). As with most marine fish species, larval damselfishes consume zooplankton regardless of their adult diet (Bernardi, Longo, & Quiros, 2017; Carassou, Le Borgne, & Ponton, 2009; Jackson & Lenz, 2016; Sampey, McKinnon, Meekan, & McCormick, 2007). This suggests that those aspects of cranial morphogenesis that facilitate adult trophic specialization may not arise before the larva-to-juvenile transition, although this view has been recently challenged based on developmental data from wrasses (Labridae; Kolker, Meiri, & Holzman, 2019).

Damselfish reproductive strategies facilitate the performance of comparative developmental studies. Pomacentrid eggs are attached to hard substrates and guarded by males until hatching (Allen, 1991; Itzkowitz, Draud, Triefenbach, & Haley, 2001; Picciulin, Verginella, Spoto, & Ferrero, 2004). Their territorial behavior is conspicuous and promotes the location and collection of embryos (Gumm, van Breukelen, Draud, & Itzkowitz, 2010; Haley & Muller, 2002; Saunders, Harvey, & Kendrick, 2013). Many species will readily deposit eggs in artificial structures that can promote sampling from the wild (Knapp, 1993; Petersen, 1995; Sasal, 2006). Damselfishes from the genera *Amphiprion* and *Premnas* (i.e., the Anemonefishes or Clownfishes; Subfamily Pomacentrinae, Tribe Amphiprionini; Cooper & Santini, 2016) will also readily breed in aquaria (Allen, 1991; Anil, Santhosh, Prasad, & George, 2012; Arbuatti, Amendola, Pantaleo, De Angelis, & Robbe, 2011; Dhaneesh, Kumar, Swagat, & Balasubramanian, 2012; Madhu, Madhu, Mathew, & Retheesh, 2011).

A highly integrative approach was used to investigate the development of cranial functional morphology in a large number of damselfish species. A combination of gene expression labeling, shape analyses, analyses of biomechanical performance, and phylogenetic-comparative analyses was used to examine trophic morphology in embryonic, larval, juvenile, and adult damselfishes. Gene expression labeling was used to visualize the anatomy of embryonic jaw precursors (Figure 1). Both geometric-morphometric analyses and phylogenetic-comparative analyses were used to compare the head shapes of embryos and larvae from species that occupy different adult trophic niches. Shape analyses and kinematic analyses were used to test for developmental transitions in the functional morphology of feeding using the larvae, juveniles, and adults of a single damselfish species: the orange clownfish (*Amphiprion percula*).

The following predictions were tested: (a) the shapes of the first jaw precursors to develop are not significantly different in the embryos of damselfishes from different

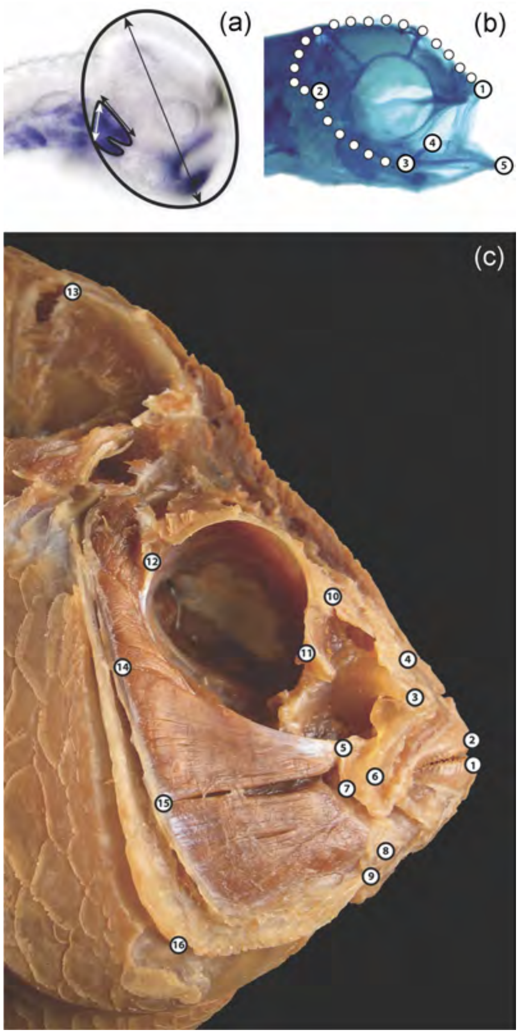

FIGURE 1 Landmarks and measurements used in analyses of shape and larval bite MA. (a) Labeled expression of *dlx2a* in a pharyngula-stage embryo of *Pomacentrus moluccensis* showing the measurements used to determine PA1 height (white arrowed line), PA1 length (short, black arrowed line) and head length (long, black arrowed line). The outlines shown were used to compute the 2-dimensional area of the head and the 2-dimensional area of PA1. See Figure S2 for representative images of embryos from all species examined. (b) Cleared-and-stained head of a hatchling *Amphiprion frenatus* larva showing the LM (numbered) and Semi-LM (unnumbered) used to measure head shape and larval bite MA: LM 1 (anterior tip of neurocranium); LM 2 (posterio-ventral junction of the hyosymplectic cartilage with the parachordal cartilage); LM 3 (joint between Meckel's cartilage and the palatoquadrate cartilage; i.e., the lower jaw joint); LM 4 (insertion of adductor mandibulae); LM 5 (anterior tip of Meckel's cartilage). Distance between LM 3 and LM 4 = mouth closing in-lever length. Distance between LM 3 and LM 5 = mouth closing out-lever length. (c) Landmarks used in analyses of head shape in adult damselfishes and juveniles of *Amphiprion percula* (shown on a specimen of the Garibaldi, *Hypsypops rubicundus*): (1) tip of the anterior-most tooth on the dentary; (2) tip of the anterior-most tooth on the premaxilla; (3) maxillary–palatine joint; (4) posterior tip of the ascending process of the premaxilla; (5) insertion of the *pars malaris* division of the adductor mandibulae on the maxilla; (6) maxillary–articular joint; (7) insertion of the *pars rictalis* division of the adductor mandibulae on the primordial process of the articular; (8) articular-quadrate joint; (9) insertion of the interopercular ligament on the articular; (10) joint between the nasal bone and the neurocranium; (11) ventral tip of the preorbital process; (12) most posterio-ventral point of the eye socket; (13) dorsal-most tip of the supraoccipital crest on the neurocranium; (14) most dorsal point on the origin of the *pars malaris* division of the adductor mandibulae on the preopercular; (15) most dorsal point on the origin of the *pars rictalis* division of the adductor mandibulae on the preopercular; (16) posterio-ventral corner of the preopercular. Muscle nomenclature after Datovo and Vari (2013). LM, landmarks; MA, mechanical advantage; PA1, 1st pharyngeal arch [Color figure can be viewed at wileyonlinelibrary.com]

adult trophic guilds; (b) larval head shapes are not significantly different in damselfishes from different adult trophic guilds; (c) larval bite mechanics are not significantly different in damselfishes from different adult trophic guilds; (d) cranial movements during feeding are significantly different in premetamorphic and postmetamorphic *A. percula*; and (e) the juvenile and adult head shapes of *A. percula* are significantly different.

2 | MATERIALS AND METHODS

2.1 | Specimens

The embryos and hatchling larvae of nine damselfish species were collected in the lagoon adjacent to the One Tree Island Research Station on the Southern Great Barrier Reef in Queensland, Australia: *Abudefduf bengalensis*, *Abudefduf sexfasciatus*, *Amblyglyphidodon curacao*, *Dascyllus aruanus*, *Dischistodus prosopotaenia*, *Pomacentrus amboinensis*, *Pomacentrus bankanensis*, *Pomacentrus moluccensis*, and *Pomacentrus philippinus*. See the Supporting Material and Figure S1 for details of collecting methods.

Mated pairs of *A. percula* were also bred in saltwater aquaria ranging in volume from 40 to 100 L under standard conditions at Washington State University (salinity 32 g/L, 28°C, 14/10 light/dark cycle, twice daily feedings of commercial marine fish food) in accordance with WSU animal care Protocol 04287. The offspring of these specimens were used in the kinematic and morphometric studies described below. Specimens from a wide range of developmental stages were also either cleared and stained using standard methods (Potthoff, 1984) or dissected to observe the development of their trophic morphology. The upper and lower jaws from a developmental series of postmetamorphic *A. percula* were removed and photographed. The age range sampled extended from newly metamorphosed juveniles collected at 21 days posthatch (dph) to 2-year-old adult females. See the Supporting Material for details of *A. percula* rearing methods.

Captive-bred, newly hatched larvae from two additional *Amphiprion* species (*Amphiprion frenatus* and *Amphiprion peridaeraion*) were purchased directly from Oceans, Reef, and Aquariums (Ft. Pierce, FL). Preserved adult specimens from 55 damselfish species (Table S1), including the 12 species from which embryos and larvae were collected and representing all damselfish genera, were obtained from The Field Museum (Chicago, IL).

2.2 | Pharyngeal arch visualization and testing for differences in early jaw morphogenesis

During early embryonic development migrating neural crest cells pattern the pharyngeal arches (Depew, Simpson, Morasso, & Rubenstein, 2005; Minoux & Rijli, 2010; Schilling et al., 1996). The terms “pharyngeal arch” and “branchial arch” are synonymous when referring to embryos (Minoux & Rijli, 2010), but embryonic pharyngeal arches and adult gill arches (which are also sometimes referred to as branchial arches) are not identical. The 1st pharyngeal arch (PA1) of pharyngula-stage embryos will differentiate into the maxillary and mandibular regions of adult skulls and the formation of PA1 represents the earliest stage of jaw morphogenesis (Depew et al., 2005; Minoux & Rijli, 2010; Piotrowski et al., 1996; Schilling et al., 1996; Yelick & Schilling, 2002).

The gene *dlx2a* is expressed by migrated neural crest cells throughout the maxillary and mandibular regions of PA1 in pharyngula-stage fish embryos (Depew et al., 2005; Sperber, Saxena, Hatch, & Ekker, 2008; Talbot, Johnson, & Kimmel, 2010). Labeled *dlx2a* expression was used to visualize PA1 shape in pharyngula-stage embryos from 10 damselfish species (Figure 1a and Figure S2). Whole-mount in situ hybridization (WISH) labeling of gene expression was performed using a standard protocol adapted from Thisse and Thisse (2008). The antisense digoxigenin-labeled riboprobe was synthesized from *dlx2a* complementary DNA (Probe sequence in Supporting Materials).

After WISH labeling embryos were removed from their yolk sacs and photographs of *dlx2a* expression were taken in lateral view using an Olympus DP25 digital camera interfaced with an Olympus SZ61 dissecting microscope (3–5 specimens per species). The computer program tpsDIG2 (http://life.bio.sunysb.edu/morph/; Rohlf, 2006) was used to take the following measurements based on *dlx2a* expression patterns: (a) PA1 height; (b) PA1 length; (c) embryonic head length; (d) 2-dimensional area of PA in lateral view; and (e) 2-dimensional area of the embryonic head in lateral view (Figure 1a). Mean values for these measurements were calculated for each species.

Phylogenetic analysis of variance (pANOVA) analyses was performed using the R package geomorph (Adams & Otárola-Castillo, 2013) to test the first prediction that the shapes of the first jaw precursors to develop are not significantly different in the embryos of damselfishes from different adult trophic guilds. Specifically, pANOVA was used to test for differences in the following values: (a) PA1 length standardized by PA1 height (PA1 length/PA1 height); (b) PA1 length standardized by embryonic head length (PA1 length/head length); and (c) PA1 size (measured as the 2-dimensional area of the first pharyngeal arch in lateral view and standardized by the 2-dimensional area of the embryonic head in lateral view). The phylogenetic relationships reported in Cooper, Smith, and Westneat (2009) were used to correct for differences in relatedness among species. The pANOVA test statistic was calculated from the data and compared with a null distribution generated via 1000 simulations of new dependent variables determined from a single rate matrix on the phylogenetic tree.

Adult head shape is tightly linked to diet in damselfishes (Cooper & Westneat, 2009; Cooper et al., 2017) and

TABLE 1 Diet data, assignments to trophic guilds based on diet or trophic morphology, and the consensus of all dietary and morphological classifications

	Diet data				
Species	% Benthic algae	% Benthic inverts	% Benthic (algae + inverts)*	% Pelagic copepods	References
Abudefduf bengalensis	40–42	13–16	40–47	0	Westneat and Resing (1988)
Abudefduf sexfasciatus	2–64	4–9	2–68	20–98	Frédérich, Fabri, Lepoint, Vandewalle, & Parmentier, (2009); Kuo and Shao (1991); Sano, Shimizu, and Nose (1984)
Amblyglyphidodon curacao	0–48	8–14	8–62	20–65	Kuo and Shao (1991); Sano et al. (1984)
Amphiprion frenatus	35	22	57	19	Sano et al. (1984)
amphiprion percula	33	46	79	1	Sano et al. (1984)—for sister species *A. ocellaris***
Amphiprion perideraion	0	0	0	97	Kuo and Shao (1991)
Dascyllus aruanus	2–53	9–40	9–72	14–56	Frédérich et al. 2009; Limbourn, Jones, Munday, and Srinivasan (2007); Sano et al. (1984)
Dischistodus prosopotaenia	58	1	59	0	Sano et al. (1984)
Pomacentrus amboinensis	70	5	75	7	Sano et al. (1984)
Pomacentrus bankanensis	66–97	1–2	67–99	0–5	Kuo and Shao (1991); Sano et al. (1984)
Pomacentrus moluccensis	5–66	4–12	9–78	11–69	Kuo and Shao (1991); Sano et al. (1984)
Pomacentrus philippinus	5	9	14	61	Kuo and Shao (1991)

Diet Classification 1	Diet Classification 2	Trophic morphology Classification 1	Trophic morphology Classification 2	Consensus
Benthic feeders	**Benthic feeders**	**Benthic feeders**	**Benthic feeders**	**Benthic feeders**
Abudefduf bengalensis	*Abudefduf bengalensis*	*Abudefduf bengalensis*	*Dischistodus prosopotaenia*	*Abudefduf bengalensis*
Amblyglyphidodon curacao	*Amphiprion frenatus*	*Amphiprion frenatus*		*Amphiprion frenatus*
Amphiprion frenatus	*amphiprion percula*	*amphiprion percula*	**Intermediate benthic/pelagic**	*amphiprion percula*
amphiprion percula	*Dischistodus prosopotaenia*	*Dischistodus prosopotaenia*	*Abudefduf bengalensis*	*Dischistodus prosopotaenia*
Dascyllus aruanus	*Pomacentrus amboinensis*	*Pomacentrus amboinensis*	*Amblyglyphidodon curacao*	*Pomacentrus amboinensis*
Dischistodus prosopotaenia	*Pomacentrus bankanensis*	*Pomacentrus bankanensis*	*Amphiprion frenatus*	*Pomacentrus bankanensis*
Pomacentrus amboinensis			*amphiprion percula*	
Pomacentrus bankanensis	**Intermediate benthic/pelagic**	**Intermediate benthic/pelagic**	*Pomacentrus amboinensis*	**Intermediate benthic/pelagic**
Pomacentrus moluccensis	*Abudefduf sexfasciatus*	*Amphiprion perideraion*	*Pomacentrus bankanensis*	*Amblyglyphidodon curacao*
	Amblyglyphidodon curacao	*Pomacentrus philippinus*	*Dascyllus aruanus*	*Dascyllus aruanus*
Pelagic feeders	*Dascyllus aruanus*	*Amblyglyphidodon curacao*	*Pomacentrus moluccensis*	*Pomacentrus moluccensis*
Abudefduf sexfasciatus	*Pomacentrus moluccensis*	*Dascyllus aruanus*		

(Continues)

TABLE 1 (Continued)

Diet Classification 1	Diet Classification 2	Trophic morphology Classification 1	Trophic morphology Classification 2	Consensus
Amphiprion perideraion		*Pomacentrus moluccensis*	**Pelagic feeders**	**Pelagic feeders**
Pomacentrus philippinus	**Pelagic feeders**		*Abudefduf sexfasciatus*	*Abudefduf sexfasciatus*
	Amphiprion perideraion	**Pelagic feeders**	*Amphiprion perideraion*	*Amphiprion perideraion*
	Pomacentrus philippinus	*Abudefduf sexfasciatus*	*Pomacentrus philippinus*	*Pomacentrus philippinus*

Note: Diet Classification 1: maximum % Pelagic copepods >90% or maximum % Benthic (algae + inverts) <15% = Pelagic; all others benthic.

Diet Classification 2: maximum % Pelagic copepods >90% = Pelagic; maximum % Pelagic copepods <20% = Benthic; all others intermediate benthic/pelagic.

Morphological Classification 1: the species with the lowest PC1 score = Pelagic; the six species with the highest PC1 scores = Benthic; all others intermediate benthic/pelagic (see Figure 5).

Morphological Classification 2: the three species with the lowest PC1 scores = Pelagic; the species with the highest PC1 score = Benthic; all others intermediate benthic/pelagic (see Figure 5).

[*] Due to differences between authors, sampling sites, and sampling seasons the data in this column do not necessarily reflect the sum of the two preceding columns.

[**] Head morphology is tightly linked to diet in damselfishes (Cooper & Westneat, 2009; Cooper et al., 2017) and the head shapes of *A. percula* and *A. ocellaris* are nearly identical (Figure 5; Allen, 1991).

both diet composition and trophic morphology were used to classify species to trophic guilds (Table 1). Assigning species to discrete categories (trophic guilds) using continuous data (diet, head shape) is to some degree subjective. To account for this and rigorously test the first prediction multiple pANOVA analyses were performed using five different classification methods (Table 1).

2.3 | Testing for differences in larval head shape and feeding mechanics

Hatchling larvae from 12 species were cleared and stained to visualize skeletal elements using an acid-free protocol (Figure S3; Walker & Kimmel, 2007). These included specimens from the nine species collected at One Tree Island, lab-reared *A. percula*, and the larvae of *A. frenatus* and *A. peridaeraion*. Photographs of cleared and stained larval heads (3–5 per species) were taken in lateral view using an Olympus DP25 digital camera interfaced with an Olympus SZ61 dissecting microscope. A scale bar was included in each photograph.

Larval head shapes were quantified by using the program tpsDIG2 (Rohlf, 2006) to place landmarks (LMs) and semilandmarks (semi-LM) on digital images of larval heads (Figure 1b). Semi-LM were used to capture the shape of curved surfaces between LM. The outline of the dorsoposterior region of the head from the anterior tip of the neurocranium to the ventroposterior juncture of the parachordal and hyosymplectic cartilages (LM 1 and LM 2, Figure 1b), the ventral borders of the hyosymplectic and palatoquadrate cartilages (LM 2 and LM 3, Figure 1b) were used to estimate head shape. The anatomical positions of neither the hyoid arch nor the lower jaw were used to quantify head shape because these highly mobile elements were in a range of positions in different larvae. The programs tpsUtil and tpsrelW (Rohlf, 2007, 2013) were then used to superimpose semi-LM using a chord-distance (Procrustes distance) based "sliders" method and to remove size and orientation differences from LM and semi-LM position data via Procrustes transformations. The program CoordGen8 (Sheets, 2008) was then used to calculate a Procrustes mean shape for each species and to transform the data from tps to IMP format that could be used by the TwoGroup8 application (see below). A phylogenetic principal components analysis (pPCA) of the Procrustes mean head shapes was used to construct a PCA score plot that displayed patterns of head shape variation. The phyl.pca function in phytools (Revell, 2012) was used to perform a pPCA that used the phylogenetic relationships reported in Cooper et al. (2009).

The Procrustes mean head shapes of each species were also used to test the second prediction that larval head shapes are not significantly different in damselfishes from different

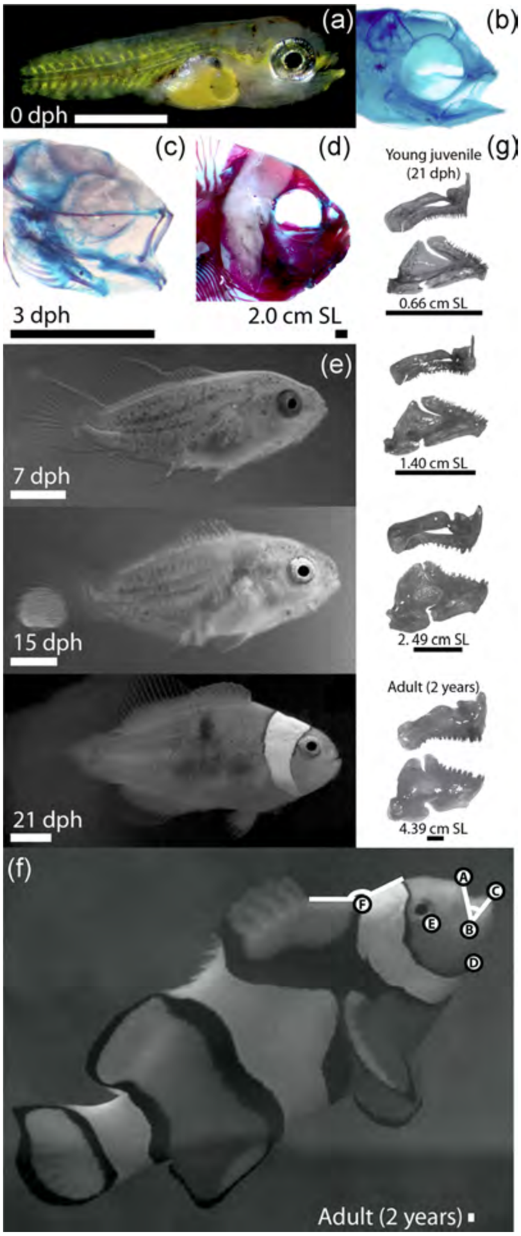

FIGURE 2 Development of *A. percula* from larva to adult and LM used in analyses of feeding strikes. (a) Live larva immediately after hatching (0 dph). (b) Cleared and stained head of a hatchling larva (0 dph). (c) Cleared and stained larval head showing the onset of jaw ossification at 3 dph (bone is stained red; cartilage is stained blue). (d) Cleared and stained juvenile with fully ossified skull. (e) Premetamorphic larva (7 dph), larva undergoing metamorphosis (15 dph), and a recently metamorphosed juvenile (21 dph). (f) Landmarks used in kinematic analyses: A, anterior tip of the upper jaw; B, corner of the mouth; C, anterior tip of the lower jaw; D, anterior tip of the hyoid; E, ventral-most point of the orbit; and F, vertex of the angle used to measure head rotation during cranial elevation. (g) Postmetamorphic upper and lower jaws from specimens that range in age from newly metamorphosed juvenile (21 dph) to adult female (2 years). Younger specimens have more gracile jaws and caniform teeth that are consistent with planktivory (Figure 5; Cooper et al., 2017). Older specimens have more robust jaw bones and blade-shaped teeth that are consistent with feeding on algae and sea anemone tentacles (Cooper et al., 2017; Sano et al., 1984). The SL of each specimen is displayed. Upper jaw above lower jaw in each case. dph, days posthatch; LM, landmarks; SL, standard lengths. All scale bars = 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

adult trophic guilds. The program TwoGroup8 (Sheets, 2006) was used to perform pairwise resampling-based *F* tests (4900 bootstrap iterations) for shape differences between trophic groups (Table 1). Resampling-based *F* tests were also used to test for head shape differences between species from different damselfish lineages. The 12 species examined belong to the monophyletic subfamilies Chrominae, Glyphysodontinae, and Pomacentrinae, which taken together constitute a single branch of the Pomacentridae (Cooper & Santini, 2016).

Measurements of biomechanical relevance to food capture were taken from the mandibles (Meckel's cartilage) of each specimen (Figure 1b). Larval pomacentrids develop a dorsal projection from the posterior region of Meckel's cartilage around which the articular bone will later ossify. This projection is anterior to the palatoquadrate cartilage and can be seen clearly in Figure 1b. The larval adductor mandibulae muscle, which closes the mouth by adducting Meckel's cartilage, attaches to this projection. The simple mechanical advantage (MA) for larval mouth closing (i.e., the degree to which the anatomical configuration of the mandible increases bite force [MA >1] or increases bite speed [MA <1]) can be calculated as the following lever ratio (Westneat, 1994): mouth closing in-lever length (the distance from the lower jaw joint between Meckel's cartilage and the palatoquadrate cartilage to the insertion of the adductor mandibulae; Figure 1b) divided by the mouth closing out-lever length (the distance from the lower jaw joint to the anterior tip of Meckel's cartilage; Figure 1b). The simple MA for larval mouth closing will be referred to as "larval bite MA" hereafter. Mouth closing in-lever and out-lever lengths were measured on each image using tpsDIG2 (Rohlf, 2006) and mean larval bite MA values were calculated for all species. The pANOVA tests were performed as above to test the third prediction that larval bite mechanics are not significantly different in damselfishes from different adult trophic guilds.

2.4 | Kinematic analyses of feeding performance in *A. percula*

High-speed video recordings of *A. percula* feeding strikes were used to measure multiple aspects of biomechanical performance in a wide range of developmental stages: larval (7 and 15 dph), juvenile (21, 40, and 59 dph) and the largest adult specimens available (~2 years of age; female). These data were used to test the fourth prediction that cranial movements during feeding are significantly different in premetamorphic and postmetamorphic *A. percula*. Representative specimens of 7, 15, 21 dph are shown in Figure 2e, an adult female is shown in Figure 2f (juveniles at 40 and 59 dph superficially resemble small adults are not shown). The mean standard lengths (SL) for each age class were as follows (all measurements in mm): 7 dph (4.86 ± 0.23); 15 dph (5.75 ± 0.32); 21 dph (6.56 ± 0.66); 40 dph (14.90 ± 0.68); 59 (19.60 ± 1.43); adult (43.20 ± 4.13). Care was taken to select specimens for each age class that were approximately the same size. All postmetamorphic specimens (21, 40, and 59 dph juveniles and 2-year-old adults) were used for shape analyses after the completion of the video study (see below).

To compare cranial motion between multiple developmental stages it was necessary to use the same food item in all feeding trials. All damselfishes, even those that feed predominantly from the benthos, will consume zooplankton when it is abundant (Westneat & Resing, 1988) and *A. percula* larvae, juveniles, and adults all readily consumed live brine shrimp (*Artemia* sp.). Five specimens from each age class were filmed in lateral view while feeding on brine shrimp and data from three feeding strikes were collected for each specimen. Feeding strikes were recorded at 500 frames/s using an Edgertronic monochrome high-speed video camera (Sanstreak Corp., San Jose, CA). Kinematic analyses of feeding strikes were performed using the ImageJ software program (Schneider et al., 2012).

The maximum values of the following variables were measured for all feeding strikes (see Figure 2f for reference): jaw protrusion (maximum linear distance traveled by LM A over the course of a strike), cranial elevation angle (the angle created by the intersection of a line running along the dorsal edge of the head with a line running along the dorsal edge of the body, with LM F denoting the point of head rotation), hyoid depression (maximum linear distance traveled by LM D over the course of a strike), and gape angle (the angle created between LMs A, B, and C with B as the vertex). The distance from fish to prey at the beginning of a strike, the time elapsed from the beginning of the strike until food capture, the duration of the feeding strike (onset of mouth opening to mouth closure), and the time elapsed from the beginning of the strike until the maximum values for all four variables listed above were reached (jaw protrusion, cranial elevation, hyoid abduction, and gape angle) were also measured.

All measurements were made by the same researcher to minimize the introduction of operator error. Jaw protrusion and hyoid depression were standardized by fish SL. The mean value of every variable in all three feeding strikes was calculated for each specimen. Specimen means were then analyzed using ANOVA to test for differences in the maximum value of each variable among age groups. Tukey's honestly significant difference (HSD) tests were used to determine which age groups were significantly different from each other when ANOVA tests results were significant.

2.5 | Shape analyses—adult damselfishes and juvenile *A. percula*

The heads of adult specimens (2–3 per species) from 56 species (species list in Table S1) were dissected to expose the functional morphology of feeding (Figure 1c; see Cooper et al., 2017 for further details). These specimens included representatives of all damselfish genera and each of the 12 species whose embryos or larvae were examined in this study. The heads of lab-reared *A. percula* from five different size classes (all

TABLE 2 Results of resampling *F* tests for differences in larval head shape

Diet classification 1	Pelagic feeding
Benthic feeding	2.88 0.121

α = 0.05

Diet classification 2	Pelagic feeding	Benthic feeding
Benthic feeding	4.06 0.055	
Intermediate benthic/pelagic	4.88 0.037	2.01 0.190

Bonferroni corrected α = 0.017

Trophic morphology classification 1	Pelagic feeding	Benthic feeding
Benthic feeding	5.23 0.031	
Intermediate benthic/pelagic	2.78 0.115	2.72 0.119

Bonferroni corrected α = 0.017

Trophic morphology classification 2	Pelagic feeding	Benthic feeding
Benthic feeding	5.29 0.030	
Intermediate benthic/pelagic	3.55 0.073	3.33 0.083

Bonferroni corrected α = 0.017

Consensus	Pelagic feeding	Benthic feeding
Benthic feeding	0.98 0.412	
Intermediate benthic/pelagic	2.31 0.155	1.76 0.226

Bonferroni corrected α = 0.017

Lineage comparisons	Pomacentrinae	Chrominae
Chrominae	3.48 0.076	
Glyphysodontinae	2.06 0.183	**8.35** **0.009**

Bonferroni corrected α = 0.017

Note: F-statistics listed above *p* values in all cells. Significant results in bold. See Table 1 for species assignments to trophic guilds (benthic feeding, pelagic feeding, intermediate benthic/pelagic). See Figure 3 for lineage memberships.

postmetamorphic) were also dissected (five specimens per size class). The youngest specimens were collected at 21 dph soon after they had completed metamorphosis. The specimens in each age class were size-matched as closely as possible. Except for the 1-year-old adults (three male specimens size-matched as closely as possible; 34.89 ± 2.73 mm SL), the specimens of *A. percula* that were used for shape analyses were first used in high-speed video studies (see above). The adult cranial morphologies in both males and females of this species were analyzed because they represent sequential developmental stages. Anemonefishes (i.e., clownfishes) are protandrous sequential hermaphrodites that initially mature as males, with larger, dominant individuals subsequently transforming into females (Cooper & Santini, 2016; Fautin & Allen, 1997). The upper and lower jaws from a developmental series of postmetamorphic *A. percula* ranging in age from 21 dph (newly metamorphosed) to 2-year-old adult females were also removed and photographed (Figure 2e).

Photographs of dissected heads and excised jaws were taken in lateral view using an Olympus DP25 digital camera interfaced with an Olympus SZ61 dissecting microscope. A scale bar was included in each photograph. The coordinate locations of 16 anatomical LM of functional importance to feeding (Figure 1c) were obtained from digital images of all dissected heads using tpsDIG2 (Rohlf, 2006).

The positions of LMs on images of all *A. percula* heads were used to test the fifth prediction that the juvenile and adult head shapes of *A. percula* are significantly different. The program CoordGen8 (Sheets, 2008) was used to transform the *A. percula* LM data from tps to Integrated morphometrics package (IMP) format so that they could be analyzed using the TwoGroup8 application (Sheets, 2006). TwoGroup8 was then used to perform pairwise resampling-based *F* tests for differences in head shape (4900 bootstrap iterations).

3 | RESULTS

There was a strong consensus among the different classification methods used to assign species to trophic groups (Table 1). All species were placed in the same trophic group by three of the four classification methods that used either dietary or morphological criteria.

3.1 | Analyses of PA1 shape, larval head shape, and larval bite mechanics

None of the results of the pANOVA tests for differences in PA1 length, PA1 size, or larval bite MA were significant (Table S2). These results support the first prediction that the shapes of the first jaw precursors to develop are not significantly different in the embryos of damselfishes from

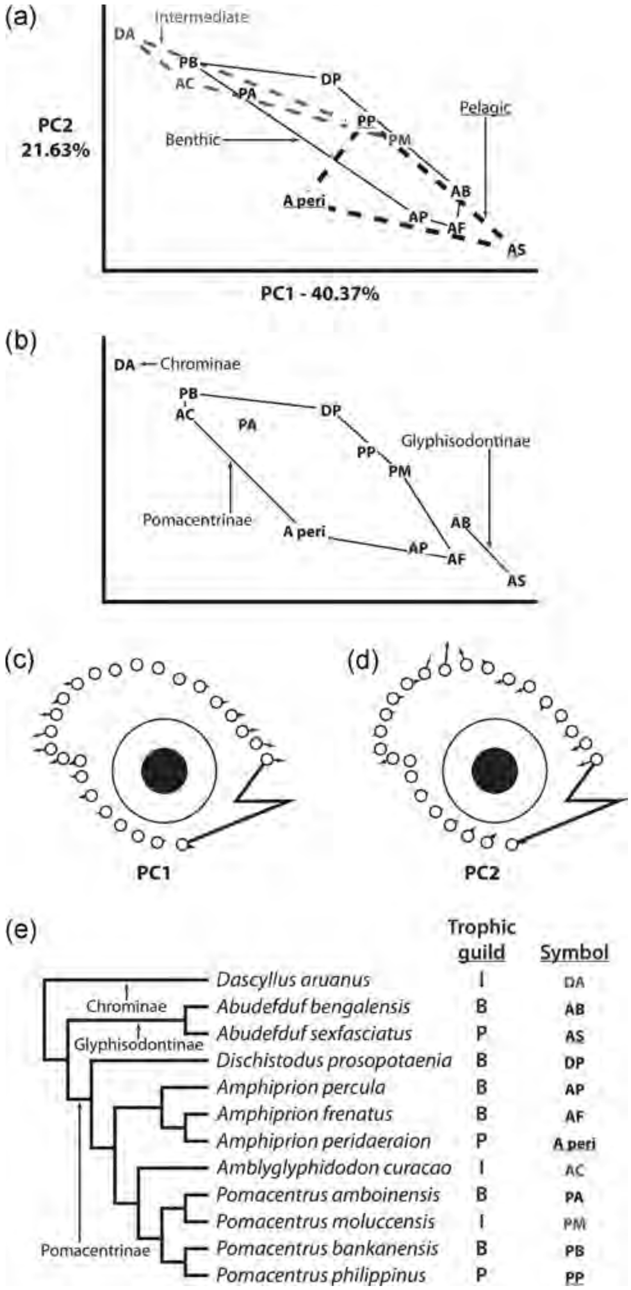

FIGURE 3 Results of pPCA of larval head shape and the phylogenetic relationships of the species examined. (a) PCA score plot depicting adult trophic groups using the consensus trophic guild assignments from Table 1. (b) PCA score plot (the same as in A) with species grouped by lineage (subfamily). (c) Vector diagram depicting the head shape variation associated with PC1 in panels "a" and "b". Higher PC1 scores are associated with greater head elongation. (d) Vector diagram depicting the head shape variation associated with PC2 in panels "a" and "b". Higher PC2 scores are associated with increased dorsal curvature of the head. (e) The phylogenetic relationships and taxonomic classifications of the species whose larvae and embryos were examined, their consensus trophic guild assignments from Table 1 (B = Benthic, I = Intermediate Benthic/Pelagic, P = Pelagic,), and the key to the symbols used in panels "a" and "b". Phylogeny and taxonomy after Cooper et al. (2009) and Cooper and Santini (2016). See Figure S3 for representative images of larval heads from all species examined. pPCA, phylogenetic principal components analysis

different adult trophic guilds. They also support the third prediction that larval bite mechanics are not significantly different in damselfishes from different adult trophic guilds.

There were no significant differences in the head shapes of larvae from different trophic groups (Table 2). These results support the second prediction that larval head shapes are not significantly different in damselfishes from different adult trophic guilds. There was a significant difference between the head shapes of larvae from the Chrominae (*D. aruanus*) and Glyphisodontinae

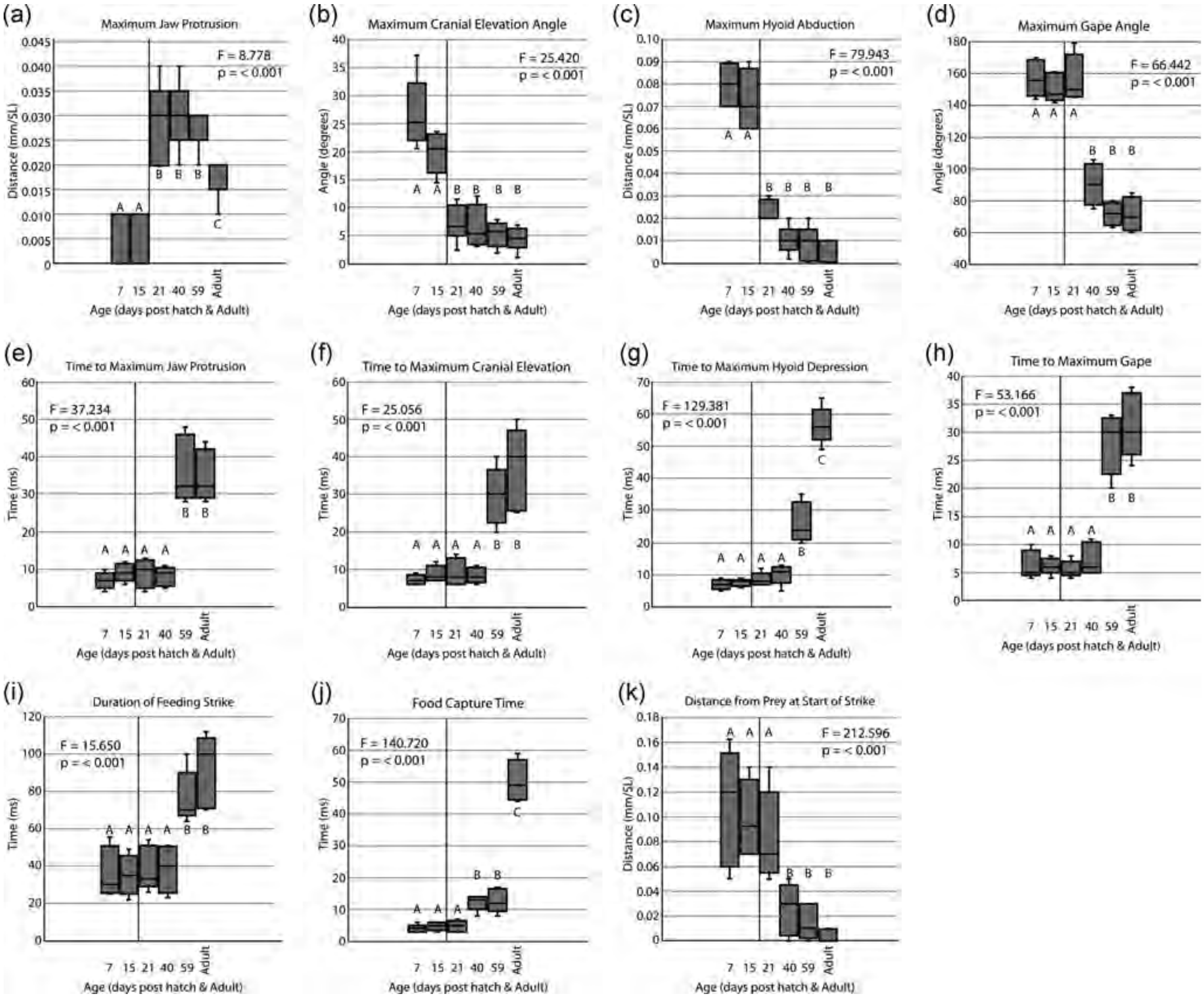

FIGURE 4 The results of ANOVA and Tukey's Honestly Significant Difference (HSD) analyses of kinematic data collected from feeding strikes of *A. percula* at six developmental stages. F-statistics and *p* values for each ANOVA test are displayed in the upper portion of each panel. All ANOVA tests detected significant differences ($\alpha = .05$). The letters A–C within each panel denote developmental stages that fell into significantly different groups based on Tukey's HSD analyses. The vertical lines in the interior of each panel distinguish between larval stages (to the left of the line; 7 and 15 dph) and postmetamorphic stages (to the right of the line; 21, 40, and 59 dph juveniles and adults). (a–d) The maximum extent of each motion examined, measured either by the angle or by distance, was significantly different between larva and all postmetamorphic stages except in the case of gape angle (d), where the youngest juveniles were not significantly different from larvae. (e–j) Adults were always significantly different from larvae and younger juveniles (21 and 40 dph) in regard to the timing of the kinematic events measured. (k) Larvae and the youngest juveniles examined (21 dph) were not significantly different from each other, but were significantly different from older stages, in regard to their distance from prey at the beginning of a feeding strike. The mean SL of the specimens from each age class are listed in the text. ANOVA, analysis of variance; dph, days posthatch; SL, standard lengths

(*A. bengalensis* and *A. sexfasciatus*; Table 2). Score plots generated by pPCA showed a strong degree of overlap in the larval head shapes of species assigned to different trophic groups (Figure 3a). The head shapes of *D. aruanus* (Chrominae) larvae were somewhat distinct from those of larval Pomacentrinae and they were highly distinct from those of larval Glyphisodontinae (Figure 3b).

3.2 | Kinematic analyses of feeding performance in *A. percula*

The results of all ANOVA tests were highly-significant in regard to differences between developmental stages (Figure 4). The results of Tukey's HSD tests showed that the maximum extent of the cranial motions of premetamorphic *A. percula* were significantly different from

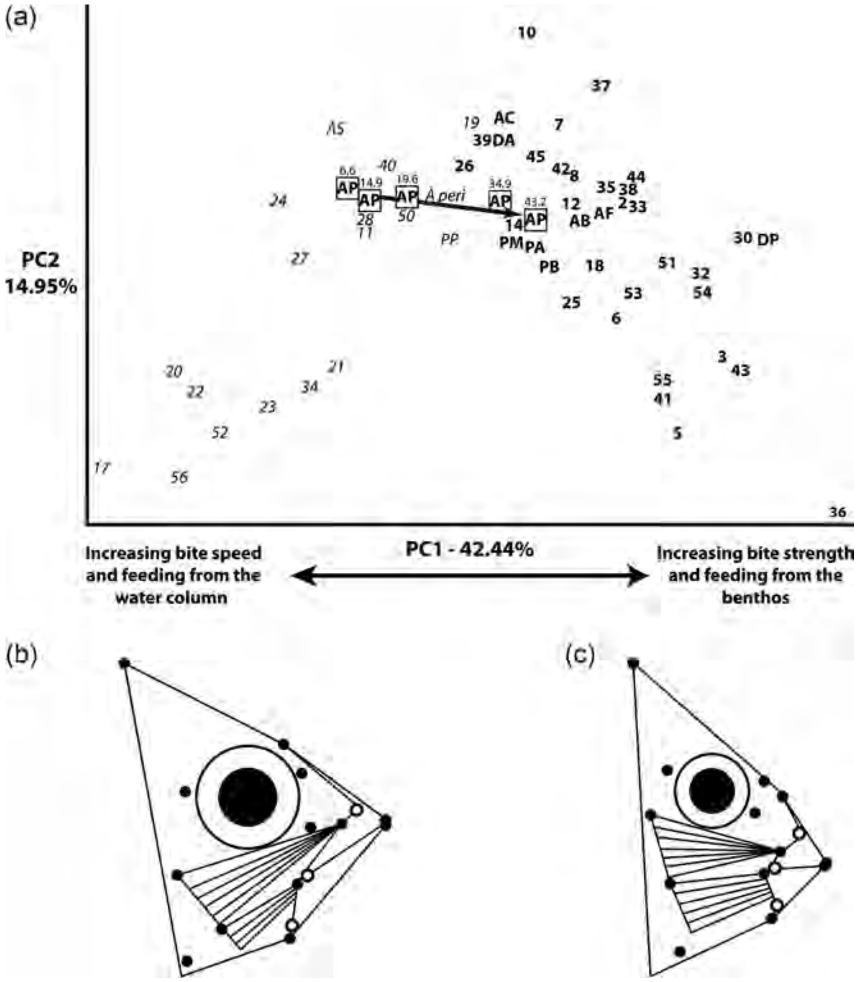

FIGURE 5 Results of a pPCA of adult head shape (56 species) and juvenile head shape in *A. percula*. (a) PCA score plot. PC1 distinguishes pelagic-feeding species that use fast feeding strikes (italics, lower PC1 scores) from benthic-feeding species that use strong bites (bold, higher PC1 scores). The location of the Procrustes mean head shapes of five postmetamorphic stages of *A. percula* are denoted by boxed symbols with the mean SL of each stage above in mm. Arrow denotes the trajectory of postmetamorphic head shape development in *A. percula*. (b) Schematic showing representative LM positions in a damselfish with a low PC1 score. (c) Schematic showing representative LM positions in a damselfish with a high PC1 score. In both "b" and "c" the 16 anatomical landmarks used in the shape analyses are depicted as circles, the upper and lower jaws are outlined with the positions of their rotational joints denoted by open circles, and the *pars malaris* (upper) and *pars rictalis* (lower) divisions of the adductor mandibulae muscle are represented by converging lines. Key to species in Table S1. See Table 1, Cooper and Westneat (2009) and Cooper et al. (2017) for further details of adult damselfish diets. LM, landmarks; pPCA, phylogenetic principal components analysis

those recorded for postmetamorphic specimens in all cases except that of gape angle, where they were not significantly different from those of the smallest postmetamorphic specimens (Figure 3a–d). These results support the fourth prediction that cranial movements during feeding are significantly different in premetamorphic and postmetamorphic *A. percula*.

In regard to developmental changes in both the timing of kinematic events and distance from prey at the start of a feeding strike, the transitions occurred during the juvenile period and not at metamorphosis (Figure 4e–k). The speed of cranial element movement was significantly faster in premetamorphic *A. percula* than it was in both adults and 59 dph juveniles, but larvae were not significantly different from either 21 dph or 40 dph juveniles for any of these measurements (Figure 4e–h). Feeding strikes were of shorter duration in larvae, 21 dph juveniles and 40 dph juveniles in comparison to older specimens (Figure 4i). Food capture was faster in larvae and 21 dph juveniles in comparison to older specimens (Figure 4j). Larvae and 21 dph juveniles were farther away from their prey at the start of a feeding strike in comparison to older specimens (Figure 4k). Adults were always significantly different from larvae and younger juveniles (7–40 dph) in regard to the timing of the kinematic events measured (Figure 4e–j), but in most

TABLE 3 Results of resampling tests for differences between juvenile and adult head shape in *Amphiprion percula*

	Juvenile—21 dph	Juvenile—40 dph	Juvenile—59 dph	Adult male—2 years
Juvenile—40 dph	1.36 0.277			
Juvenile—59 dph	2.98 0.123	0.75 0.412		
Adult male—2 years	**16.94** **0.003**	**15.73** **0.004**	**15.34** **0.004**	
Adult female—2 years	**19.22** **0.002**	**17.98** **0.003**	**17.04** **0.003**	3.57 0.096

Note: F-statistics listed above p values in all cells. Significant results in bold. Bonferroni corrected $\alpha = .005$

cases, they were not significantly different from 59 dph juveniles (Figure 4e,f,h, and I).

3.3 | Head shape analyses and the development of jaw morphology

The damselfish head morphospace defined by pPCA of the combined head shape datasets (adult damselfishes and postmetamorphic *A. percula*) was nearly identical to those reported in Cooper et al. (2017) and Cooper and Westneat (2009), which performed similar analyses using lower numbers of species. As in those studies, the first principal component axis strongly distinguished between pelagic-feeding and benthic-feeding species and there was a close association between head shape and diet (Figure 5). The results of this shape analysis were used to assign species to trophic groups based on morphology (Table 1).

The fifth prediction that the head shapes of juvenile and adult *A. percula* were significantly different was supported (Table 3). The head shapes of juveniles of different ages were not significantly different from one another, nor were male and female head shapes significantly different (Table 3). The three juvenile stages of *A. percula* specimens (6.6–19.6 mm SL) had head shapes that were most similar to the adult head shapes of several planktivorous species (Figure 5): *Chrysiptera tricincta*, 28; *Amblypomacentrus* breviceps; 11; *Neopomacentrus azysron*, 40; *Pomachromis richardsoni*, 50; and *Amphiprion perideraion*; A peri (Allen, 1991; Frederich et al., 2009; Kuo & Shao, 1991; Sano et al., 1984). Adult *A. percula* (34.9–43.2 mm SL) had head shapes that were most similar to the adult head shapes of other benthic-feeding species (Figure 5): *Amphiprion ocellaris*, 14; *Pomacentrus moluccensis*, PM; and *Pomacentrus amboinensis*, PA (Allen, 1991; Galetto & Bellwood, 1994; Kuo & Shao, 1991; Sano et al., 1984; Westneat & Resing, 1988).

The onset of both upper and lower jaw ossification occurred at 3 dph in most specimens of *A. percula* (Figure 2c). The jaws of younger *A. percula* juveniles were more gracile than those from both older juveniles and adult specimens and they also possessed caniform teeth (Figure 2e). These characteristics are consistent with planktivory in adult damselfishes (Cooper et al., 2017). The jaws of older juveniles and adults were more robust and possessed blade-like teeth (Figure 2e), which are consistent with scraping algae from hard surfaces and biting through sea anemone tentacles (Figure 2e; Cooper et al., 2017; Sano et al., 1984).

4 | DISCUSSION

The morphological characters that allow adult damselfishes to specialize in pelagic or benthic food sources appear to be largely a product of postlarval development. Neither embryonic PA1 shapes, larval head shapes, nor larval bite mechanics differed significantly among damselfishes that belong to different adult trophic guilds and the movements of cranial elements during feeding were significantly different in larval and postlarval *A. percula* (i.e., predictions 1–4 were supported; Figures 3 and 4; Table 2 and Table S2). Although damselfish larvae are capable of exogenous feeding, their cranial morphology undergoes considerable remodeling during metamorphosis (McCormick & Makey, 1997; McCormick et al., 2002) and continues to change as juveniles transform into adults (Figures 4 and 5; Table 3; Frederich, Adriaens, & Vandewalle, 2008; Frederich, Parmentier, & Vandewalle, 2006). At least two factors may constrain early cranial development in damselfishes so that important aspects of adult trophic morphology cannot arise before metamorphosis: (a) their larval diet; and (b) the biomechanical requirements for aquatic feeding at small body sizes.

Damselfish larvae begin exogenous feeding soon after hatching and predominantly prey upon evasive copepods (Anto, Majoris, & Turingan, 2009; Buskey, Lenz, & Hartline, 2012; Carassou et al., 2009; Jackson & Lenz, 2016; Sampey et al., 2007). Neither larval nor adult damselfishes use filter-feeding, but instead capture individual zooplankters using rapid feeding strikes (Bradley, Strickler, Buskey, & Lenz, 2013; Cooper et al., 2017; Coughlin & Strickler, 1990; Holzman & Wainwright, 2009; Lenz & Hartline, 1999; Robinson, Strickler, Henderson, Hartline, & Lenz, 2019; Yen, Murphy, Fan, & Webster, 2015). These strikes are significantly faster than those that can be produced by benthic-feeding adult damselfishes (Cooper et al., 2017). Musculoskeletal lever arrangements that increase bite speed do so by sacrificing bite force regardless of body size (Westneat, 2004) and benthic-feeding damselfishes use forceful bites to scrape algae from hard substrates and prey on benthic invertebrates (Cooper & Westneat, 2009; Cooper et al., 2017). Damselfishes that feed heavily from the benthos as adults are, therefore, likely to require different trophic anatomies to feed efficiently as larvae.

A developmental transition from fast, pelagic feeding to benthic feeding using high bite forces has been documented in two damselfish species: *Amphiprion ocellaris* and *A. percula.* Adults of *A. ocellaris* feed heavily from the benthos (Sano et al., 1984) and exhibit slow feeding strikes (Cooper et al., 2017), but their larvae utilize very rapid strikes when capturing copepods (Robinson et al., 2019). Their morphology is nearly identical to that of their sister species, *A. percula* (Figure 5; Allen, 1991), which suggests that their adult diets are highly similar since head shape and diet are closely linked in damselfishes (Cooper & Westneat, 2009; Cooper et al., 2017). The prey capture times and cranial movements of larval *A. percula* are significantly faster than those of adults feeding on the same food item (Figure 4). Evidence from these species and the basic principles of lever mechanics indicate that damselfish larvae will need to remodel their cranial morphology if they are to feed effectively from the benthos as adults.

Damselfishes that feed predominantly on pelagic copepods as adults are also likely to employ different trophic anatomies as larvae. At small body sizes fishes live in a low Reynolds number environment in which viscous forces tend to overcome inertial forces (Hernandez, 1995, 2000). They experience water as a "sticky" fluid that can reduce the effectiveness of many adult feeding strategies (Hernandez, 1995, 2000). Jaw protrusion, for example, facilitates zooplankton capture by pelagic-feeding adult damselfishes (Cooper et al., 2017; Coughlin & Strickler, 1990), but jaw protrusion would likely interfere with zooplanktivory in a viscous fluid by pushing prey away from a larval fish (Galindo et al., 2019). Zebrafish (*Danio rerio*) use protrusile jaws to capture zooplankton as adults, but employ little or no jaw protrusion when feeding on zooplankton as larvae (Galindo et al., 2019; McMenamin, Carter, & Cooper, 2017). The extent and speed of other cranial motions are also significantly different in larval and adult zebrafish during zooplankton capture (Galindo et al., 2019; McMenamin et al., 2017). Because the biomechanical requirements for zooplanktivory are different for adult and larval fishes, pelagic-feeding damselfishes are also likely to require postlarval cranial remodeling.

4.1 | Postlarval skull remodeling and the evo-devo of fish feeding

It is likely that evolutionary divergence in the trophic morphology of adult damselfishes has required changes to postlarval development. Like most marine fishes, pomacentrids have dispersive planktonic larvae and their larva-to-juvenile metamorphosis is frequently accompanied by simultaneous and rapid changes in habitat, morphology, behavior, coloration, and diet (Benoit, Pepin, & Brown, 2000; Chambers & Leggett, 1987; Juanes, 2007; McMenamin & Parichy, 2013). The subsequent development of juvenile fishes into adults also typically involves important changes in diet that are accompanied by a remodeling of skull shape (Daly, Brodeur, & Weitkamp, 2009; de la Moriniere, Pollux, Nagelkerken, & van der Velde, 2003; de la Moriniere et al., 2003; Frederich et al., 2008; Frederich et al., 2006; Graham, Grubbs, Holland, & Popp, 2007) and significant postmetamorphic shifts in the functional morphology of feeding were seen in *A. percula* (predictions four and five were supported; Figures 2, 4, and 5; Table 3). Although these aspects of development are not universal among ray-finned fishes (Actinopterygii), they are common to many thousands of species (Bernal, Olivar, Maynou, & de Puelles, 2015; de la Moriniere et al., 2003; Leis, 2010; McMenamin & Parichy, 2013; Nunn, Tewson, & Cowx, 2012). These factors indicate that a greater understanding of the developmental changes that have shaped the evolution of fish feeding can be gained by further investigation of two transitions: (a) the transformation of larvae into juveniles; and (b) the remodeling of fish skulls that occurs over the course of juvenile development.

Although the larvae-to-juvenile transition in fishes frequently involves metamorphosis (i.e., rapid anatomical reorganization), in many species this transformation is more protracted, but thyroid hormone (TH) regulates both metamorphosis and metamorphosis-like processes in large numbers of vertebrates (Das et al., 2006; Hu et al., 2019; Laudet, 2011; McMenamin & Parichy, 2013; Paris et al., 2010; Wojcicka, Bassett, & Williams, 2013) and sharp increases in TH production are known to accompany the larvae-to-juvenile transition in multiple fish species (Campinho, 2019; Chang et al., 2012; McMenamin & Parichy, 2013). TH is also known to play an important role in skull morphogenesis throughout development and to have large effects on the growth and remodeling of both endochondral and intramembranous bone (Bassett & Williams, 2003; Hanken & Hall, 1988; Harvey et al., 2002; Hirano, Akita, & Fujii, 1995; Waung, Bassett, & Williams, 2012; Wojcicka et al., 2013). Experimental work with genetically modified zebrafish (*D. rerio*; Cyprinidae) has shown that individuals which cannot manufacture TH will retain many aspects of larval feeding mechanics into adulthood (Galindo et al., 2019; McMenamin et al., 2017). There is also evidence that changes in TH signaling have played an important role in the evolution of skull mechanics within the cypriniform lineage to which the zebrafish belongs (Galindo et al., 2019; McMenamin et al., 2017; Shkil & Smirnov, 2015; Shkil et al., 2015; Shkil, Kapitanova, Borisov, Abdissa, & Smirnov, 2012). Further investigation into the mechanisms through which TH affects larval skull reorganization is likely to inform our understanding of trophic evolution in fishes.

The cranial bones of many fish species undergo substantial remodeling throughout juvenile development (Figures 2 and 5; Table 3; Cooper, Wirgau, Sweet, & Albertson, 2013; Fischer-Rousseau, Cloutier, & Zelditch, 2009; Frederich & Vandewalle, 2011; Frederich et al., 2008). Multiple signaling pathways have been identified as regulators of bone remodeling in fish skulls (bone morphogenic protein, calcium/calmodulin, fibroblast growth factor, Hedgehog, TH) and the potential for these pathways to influence adaptive changes in trophic morphology has been recognized in each case (Cooper et al., 2013; Galindo et al., 2019; Hu & Albertson, 2017; Okada, Tanaka, & Tagawa, 2003; Parsons et al., 2016; Parsons, Taylor, Powder, & Albertson, 2014). Manipulation of Hedgehog signaling in African rift-lake cichlids and elimination of TH signaling in zebrafish has been shown to alter fish skull remodeling so as to create functional convergence with closely related species that have different feeding mechanics. (Galindo et al., 2019; Hu & Albertson, 2014; Hu & Albertson, 2017; McMenamin et al., 2017). These findings suggest that changes in skull remodeling may have played a role in the trophic diversification of other fish species.

5 | CONCLUSIONS

Postlarval development is an important period of skull morphogenesis for damselfishes. Changes in skull shape during metamorphosis and juvenile development have significant consequences for the development of feeding mechanics in pomacentrids and many other fish species. Damselfish diversification has repeatedly involved shifts between pelagic feeding using fast bites and benthic feeding using high bite forces. The mechanisms that direct skull metamorphosis and cranial bone remodeling have likely played a large role in shaping this evolutionary pattern. Recent work has shown that changes in TH signaling, which is a major regulator of skull development, bone remodeling and metamorphosis in many vertebrate species, can have large effects on the postlarval development of fish feeding mechanics (Galindo et al., 2019; McMenamin et al., 2017). Additional research has identified several more signaling pathways that regulate the remodeling of fish cranial bones (Albertson & Yelick, 2004; Cooper & Albertson, 2008; Cooper et al., 2013; Hu & Albertson, 2014; Hu & Albertson, 2017; McMenamin & Parichy, 2013; Parsons, Andreeva, Cooper, Yelick, & Albertson, 2010).

The evolution of fish feeding is one of the most thoroughly studied subjects in the field of functional morphology and patterns of trophic evolution have been described in detail for a large number of fish lineages. This work can serve as a useful guide for investigations targeted at determining how changes in skull development have shaped these patterns. There is an increasing recognition that the controls of cranial morphogenesis during postlarval development have played an important role in the diversification of fish skulls (Albertson & Yelick, 2004; Cooper & Albertson, 2008; Cooper et al., 2013; McMenamin & Parichy, 2013; Parsons et al., 2010). The findings presented here support this perspective. Additional exploration of the connections between skull organization during late development and transitions in trophic ecology are likely to make useful contributions to the field of evolutionary developmental biology.

CONFLICT OF INTERESTS

The authors have no conflicts of interest in regard to the manufacturer or distribution of this manuscript, neither do they have any corporate funding or affiliations.

AUTHOR CONTRIBUTIONS

R. V. H., E. S., and H. M. performed gene expression studies. W. J. C., R. V. H, and H. M. collected shape data from embryos and larvae. W. J. C., R. V. H., and H. M. performed shape analyses. W. J. C., H. M., Z. D., A. D., D. M., and O. L. performed fish care and reared captive-bred *A. percula*. Z. D., A. D., and D. M. collected video data. W. J. C. and O. L. analysed video data. W. J. C. and E. S. oversaw graduate and undergraduate student training in the techniques used during this research. R. V. H. and W. J. C. wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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