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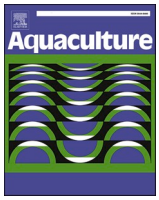

Larviculture, allometric growth patterns, and gape morphology of the Florida blenny, *Chasmodes saburrae*

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A B S T R A C T

Blennies are cryptobenthic reef fishes (CRFs) that occupy a critical functional group in the trophodynamics of their respective ecological systems and for many of these species there has been renewed interest in their diversity and evolution. Here we provide a robust larviculture methodology for combtooth blennies (f. Blenniidae), that may have applications for both ornamental aquaculture and scientific research. Larval ontogeny, pigmentation, and allometric growth patterns, including gape morphology, of the Florida blenny, *Chasmodes saburrae,* from the northern Gulf of Mexico (GoM) are described from a complete larval series from hatch to settlement (notochord length/standard length, NL/SL = 3.37–14.49 mm; 1–21 days post hatch, dph). Larvae/settlers were assigned intervals of development using a suite of morphological characters. Allometric growth rates and inflection points of rate change were computed with piecewise linear regressions. Pigmentation and general patterns of development followed that of other blenniid species in the GoM. Growth of all but one morphometric character measured followed a positive allometric growth rate relative to size (NL/SL) after hatching and followed a mostly biphasic growth pattern with inflection points corresponding to phenotypic changes during notochord flexion or prior to settlement and the completion of metamorphosis. A feeding gape size of 26.6% that of maximum gape size was calculated using size at first feeding of prey items and used to estimate an optimized feeding protocol. Based on this gape informed diet, larger and more nutrient rich prey items may be implemented in the feeding protocol between four and eight days earlier than previous study recommends. Captive breeding techniques used during this study show the practical advantages of culturing blenniid larvae with life histories similar to *C. saburrae* compared with that of most marine fishes currently being cultured and suggests that blenniid larvae may serve as sensible candidates as a model study group for marine teleosts.

1. Introduction

As one of the ten most traded marine ornamental families, combtooth blennies (f. Blenniidae) are popular in the marine aquarium trade throughout the world and continue to command a sizeable portion of this market (Von Linden et al., 2020). Additionally, many species are currently cultured and additional research is underway to bring new species to the market (Rhyne et al., 2012). Despite this popularity, few studies have been published regarding the breeding and rearing of combtooth blennies, and information on protocol and feeding strategies is scarce and almost nonexistent for all blenny families. The few publications available detailing blenniid larviculture pertain to the culture of genera popular in the aquarium trade such as *Meiacanthus, Ecsenius*, and *Salarias* (Olivotto et al., 2010; Moorhead and Zeng, 2017). Outside of interest as ornamental fishes, there has been recent interest in studying the evolutionary and demographic changes, habitat invasions, and trophodynamics of blennies (Eytan and Hellberg, 2010; Hundt et al., 2014; Brandl et al., 2018). For these researchers, the option of purchasing wild-caught individuals for study can carry a high price tag. An alternative approach is captive production. For researchers exploring the potential of a species as a "model species", culturing methods that make the subsequent study of evolution and development possible must be refined (Lencer and McCune, 2018). Once a tested and reliable protocol is established to produce a model study species, the culture thereof can lead to investigation of early life history aspects of development and improvement of existing culture methodologies.

Several characteristics make combtooth blennies an appropriate choice for aquaculture and application as a model study group of marine teleosts. Their relative abundance and high site fidelity in shallow, easily accessible habitats make capturing them easier than that of many other marine fishes (Ditty, 2002). Combtooth blennies are equipped to handle changes in water chemistry and water flow as many species reside in intertidal zones that experience frequent disturbance (Tellock and Alig, 1998). The majority of aquacultured ornamental marine species are demersal spawners, making the ease of egg handling and sequestering of larvae to the larval growout tank much easier than pelagic spawners (Von Linden et al., 2020). The larval period is marked by the immediate onset of exogenous feeding and is generally short between 17 and 35 days post hatch (dph) (Von Linden et al., 2020). Once larval settlement is reached, newly settled individuals can be transferred to a suitable

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growout environment in relatively high densities. Lastly, some blennies may reach sexual maturity shortly after the juvenile stage is reached at around 20 mm SL or after the initial bifurcation of the primary caudal elements (Ditty et al., 2005). These aspects of blenniid reproductive biology make them particularly equipped for aquaculture-based research activities and present the researcher with a potential model study organism for marine teleosts.

Establishing a captive-breeding protocol first relies on the application of prior research and demonstrated culture success coupled with an understanding of early larval development in the species of interest. Successful culture can provide a complete growth series for detailed morphological study and may complement existing knowledge of larval development. Studies involving wild-caught larvae may encounter gaps in a larval series or see a deficit in counts of larvae during various stages of development (Ditty et al., 2005). Captive-breeding and/or captive-rearing can supplement existing knowledge and reduce the need to conduct collections of wild-caught larvae and juveniles (Stevens and Moser, 1982) by providing complete growth series and filling gaps in development stages missing from wild-collected sampling (Ditty et al., 2003; Osse and van den Boogaart, 2004; Rowlands et al., 2006; Solomon et al., 2017). Successful, repeated larviculture of a species can provide information on the ontogenetic development, timing of settlement, and allometric changes to traits such as mouth gape and structures associated with swimming ability in larval fish that may not be obtainable from wild-caught specimens.

Understanding the development of gape morphology during early development is key to establishing culture protocols and dietary needs of a cultured species, as gape size is the major limiting factor for size of prey consumed at the onset of exogenous feeding (Shirota, 1970). Studies have shown that fish larvae prefer prey smaller than what is predicted by gape size-prey size relationships (Krebs and Turingan, 2003). There is general consensus that fish larvae feed on prey items 25–50% that of the maximum gape size as was first reported by Shirota (1970) and supported by a number of studies (Yúfera and Darias, 2007; Krebs and Turingan, 2003). Furthermore, as larval development proceeds through ontogenetic growth changes, gape morphology in particular can exhibit allometric growth changes to accommodate larger prey sizes and adverse effects of competition for survival (Harding, 1999; Osse and Van den Boogaart, 1995; van Snik et al., 1997; Fuiman and Higgs, 1997). In general, as gape size increases the range of ingestible prey increases linearly in a progression towards larger prey capacity (Schael et al., 1991). The relationship between gape size and body size (SL) varies between taxa and measurements are absent for a majority of culture ornamental species, making it difficult to determine potential larval diets aimed at maximizing growth and survival (Burgess and Callan, 2018). A large body of research has been conducted on measurements of maximum gape size (S_G) and gape width (GW) the estimation acceptable prey sizes during early development (Shirota, 1970; Guma'a, 1978; Rowlands et al., 2006).

Here we present a larviculture protocol for the Florida blenny, *Chasmodes saburrae*. Through the successful implementation of this protocol we are able to characterize larval ontogeny, pigmentation, and allometric growth patterns including gape morphology from a complete larval series. We then implement the methodology developed by Ditty et al. (2003) for small scaleless fishes (like blenniids) to assign individuals into development intervals. The objective of this research was to examine allometric growth changes during larval development and use standardized scaling methods and age of larvae to estimate an optimal feeding protocol based on gape size and observations during culture.

2. Methods

All methods of collection and handling of live fish for this study were done so in accordance with Texas A&M University at Galveston IACUC protocol under permit 2018–0365.

2.1. Larval rearing and data collection

2.1.1. Broodstock care

Broodstock pairs of *C. saburrae* (55–70 mm SL) were collected from Pensacola Bay, Florida in summer of 2016–2017 and transported to the Texas A&M University at Galveston Sea Life Facility. After drip acclimatization, fish were added to three of eight 75-l (20-gal) aquaria on a shared recirculating seawater system with established life support and biological filtration. Broodstock tanks were painted black on all but one side for observation. A single open-ended white PVC pipe of 100-mm length and 25-mm diameter with removable clear transparency film placed inside the tube as a spawning surface was provided to each pair. A photoperiod of 14 L:10D was maintained with ambient overhead compact fluorescent lights. Broodstock, larval growout, and juvenile growout tanks were kept on a shared recirculating system to minimize handling, temperature, and salinity stress when transferring eggs or fish between tanks. 10% water changes were performed every week in addition to water replacement after siphoning. System water quality parameters were maintained at salinity of 28–30 ppt, temperature of 26–28 °C, pH of 8.0–8.2, NH_3 levels of 0–0.25 ppm, and undetectable levels of NO_2 and NO_3. Three broodstock pairs were fed a varied diet of krill, clam, mysid shrimp, squid or omnivore gel-based diet 3 times a day (0800 h, 1100 h, and 1500 h). Broodstock were fed to satiation and after fifteen minutes excess food was siphoned from tanks. Males began to occupy the tube and inspect spawning surface almost immediately and a new egg clutch would be laid 2–3 days after a new tube was introduced. Under these conditions, spawning was continuous with new eggs being laid down daily until the tube was removed for hatching. Tubes were checked for eggs each morning and observations of courtship behavior were made which was usually followed by spawning activity between morning and noon similar to that described by Tavolga (1958) and Peters (1981).

2.1.2. Live food culture

C. saburrae were cultured using small rotifers (*B. rotundiformis, S*-Type, Reed Mariculture, USA) and three size classes of *Artemia salina* nauplii (INVE technologies, Thailand LTD; GSL) (Peters, 1981; Sorgeloos et al., 2001). Rotifers were continuously cultured in 20-l Compact Culture Systems (Reed Mariculture, USA) at 24–28 °C to allow for easy cleaning and harvesting. Three species of live phytoplankton (*Nannochloropsis oculata*, T-*Isochrysis galbana,* and *Rhodomonas salina*) were cultured to feed rotifer cultures twice daily between 0800 and 1000 h and between 1600 and 1800 h at a concentration of 50,000 cells ml^{-1} (salinity 30–35 ppt, pH 7.8–8.2, NO_2 and $NO_3 < 0.03$ ppm). A 5-l (25%) volume was harvested daily from each culture bucket to maintain density and production. *Artemia* nauplii were decapsulated and hatched for 15 h followed by harvesting into one container left at room temperature (25 °C) for further growth and another container refrigerated below 10 °C to slow nauplii metabolism and retain hatching size (Léger et al., 1983). This was repeated for nauplii at 24 h and 48 h metanauplii stages for feedings of larger larval sizes after a daily 30-min phytoplankton enrichment period with a 1:1:1 mix of species above. This method provided ever increasing size classes of nauplii with sustained nutritional value of newly hatched yolk-bound and later metanauplii size enriched with phytoplankton and Vitachem (Boyd Enterprises). A sample of 50 individuals was collected from each live food culture for size measurements.

2.1.3. Larval rearing

Larval hatching and growout were carried out in 68-l round, black plastic pond planter containers with drain screens and submersible heaters with a total water volume of 54.5 l. Once observed, eggs remained in the broodstock tank under continued male care for 144 h (6 days), after which the spawning tube was carefully transferred to larval growout tanks. During this time, spawning continued. Tubes were secured to the standpipe drain screen with rubber bands and positioned

vertically over the perforated bubble ring tubing to allow constant water flow over the eggs. Gentle laminar aeration with fine bubbles through the tube mimicked fanning by the male and creates water flow away from the standpipe screen in a vertical circular motion throughout the tank. A 4.0 cm strip of transparency film was secured to the top of drain screen to cover the water line and prevent suction of larvae to the screen after hatching. Once transferred, a portion of the larvae began hatching immediately after introduction and hatching continued through the following 24 h after which time the tube was removed. The amount of larvae to hatch out during this 24 h period was variable, and exact estimates of larval density were not known. Water flow was set to 2 drip/s during the first 10 days for around a 16% daily water exchange rate and increased to a slow constant flow for the remainder of the rearing period. Harvested rotifers and nauplii were transferred to 4-l polycarbonate cambro containers with concentrations of 15 ind. ml $^{-1}$ and 5 ind. ml $^{-1}$, respectively. This concentration dilutes to a final concentration of 3 ind. ml $^{-1}$ and 1 ind. ml $^{-1}$ in the larval tank. *Nannochloropsis oculata* and *I. galbana,* were used in a 1:1 ratio for the preparation of green water at a volume of 2-l and added to the feed bucket. The feed buckets containing rotifers, nauplii and green water were topped off with seawater to a final volume of 3 l, they were then connected to the larval growout containers using airline tubing and volume control valves. Larval feeds were performed twice daily between 0900 and 1000 h and between 1600 and 1800 h at a drip rate of 2–3 drips per second. Prior to feeding, larval tanks were flushed of remaining food by lowering the water level with the Hartford loop line and switching to a larger-size drain screen (Fig. 1). Larvae were fed algae-enriched *S*-type rotifers (*B. rotundiformis*) (10 ind. ml^{-1}) from day 1 at hatch to 11 days post hatch (dph), newly hatched

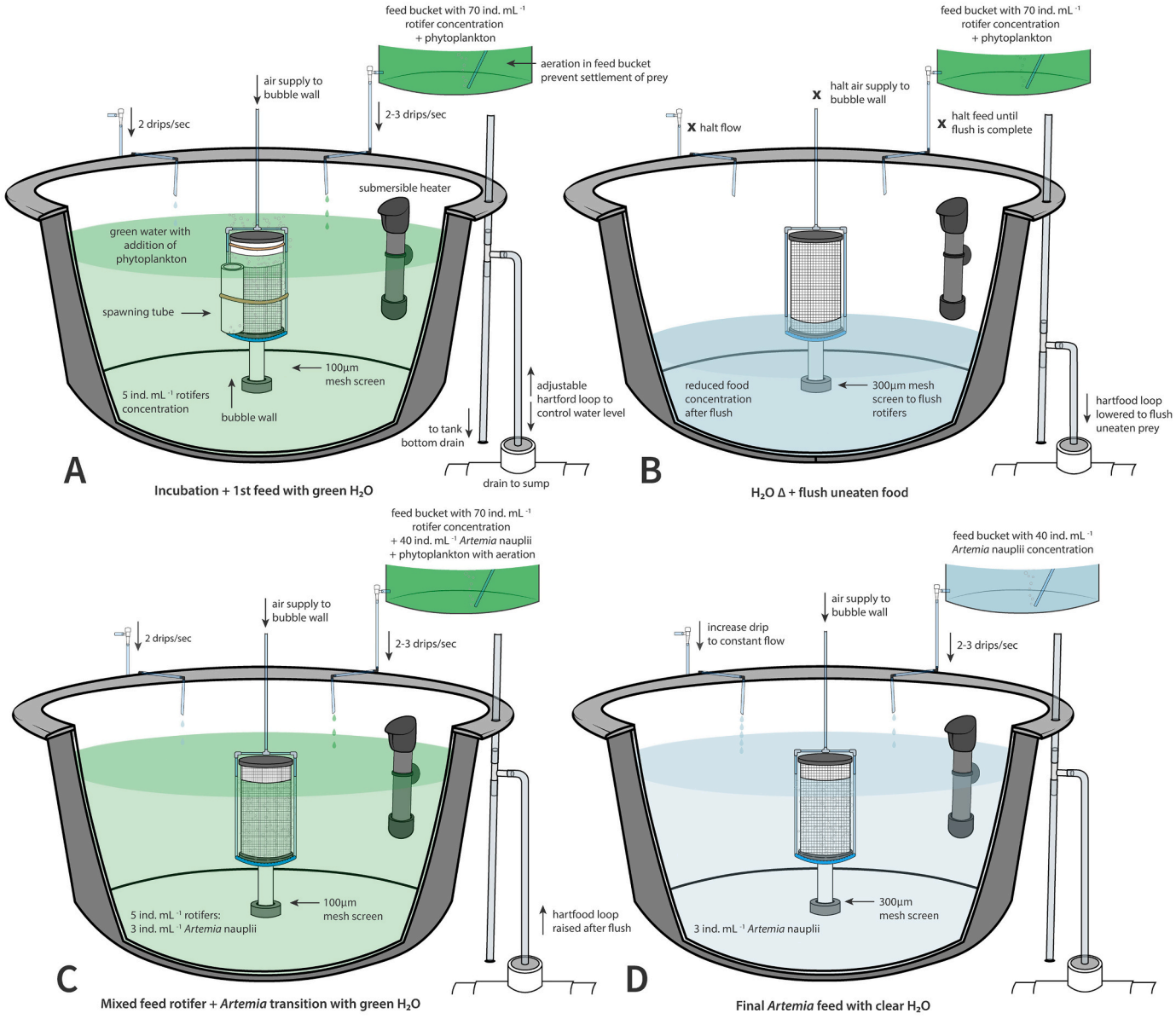

Fig. 1. Diagram of larval culture tank construction and culture protocol used during this study. A) Initial egg incubation and hatching step with spawning tube secured vertically to 100 µm drain screen. First feed of S-type *B. rotundiformis* rotifers at a concentration of 5 ind. ml^{-1}. Addition of phytoplankton during feed as designated by the green-water technique of culture. B) Water volume exchange and flush of uneaten live foods by lowering hartford loop and changing drain screen to a 300 µm mesh size to clear tank of food items too large for consumption and maintain appropriate food densities. Water and air flow are turned off during this phase to allow adequate suction of live foods through drain screen. C) Mixed live food transition period and introduction of first *A. salina* nauplii live food at concentration of 3 ind. ml^{-1} with continued use of phytoplankton in green-water technique. D) 48-h *A. salina* only feeds with increased water flow and discontinued used of green-water technique. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A. salina nauplii (3 ind. ml^{-1}) from 6 to 11 dph during the transition from rotifers to nauplii and (6 ind. ml^{-1}) from 12 to 14 dph, algae-enriched 24 h *A. salina* nauplii (3 ind. ml^{-1}) from 15 to 21 dph, and algae-enriched 48 h *A. salina* nauplii (3 ind. ml^{-1}) starting at 20 dph until settlers were weaned onto an artificial pellet diet (*Otohime Marine Larval and Weaning Feed, Marubeni Nisshin Feed Co., Ltd*). Two groups of larvae (Group A and Group B) were cultured during this study.

2.1.4. Sampling and specimen preservation

During the course of development (1 – 21dph), larvae were sampled daily from group A and B to facilitate a complete larvae growth series for morphological analyses ($n = 5$ per day). Ten pre-juvenile settlers were also collected after the 21-day period to extend the size range of the growth series beyond settlement. Larvae were anesthetized with MS-222 and placed into individual tubes and fixed in 4% buffered formalin (sodium acetate trihydrate) solution to reduce larval shrinkage (Hay, 1981; Rowlands et al., 2006). After 24 h larvae were transferred to a final storage solution of 70% ethanol for long-term preservation (Ditty et al., 2005).

2.2. Analyses

2.2.1. Morphometric characters

Photographs of developing eggs and larval specimens were taken using a *Leica* S6D Stereo Microscope with a *Moticam* 10.0+ MP camera (MOTIC). To provide the best view of all morphometric characters used in the analyses, images of three perspectives were taken for each specimen at either 1×, 2×, or 4× magnification (lateral head view, ventral head view, and lateral body view). 10 morphometric characters associated with feeding and locomotion were measured (±0.00 mm) for each the 115 specimens included in this study. In addition to notochord length (NL) and standard length (SL), these measurements include head length (HL), eye diameter (ED), preanal length (AL), pectoral length (PL), snout length (SNL), depth at pelvic (DAP), depth at anus (DAN), upper jaw length (UJL), lower jaw length (LJL), and gape width (GW). Pectoral length was not measured for a portion of specimens due to damage or position of the fin when photographed. Measurements were taken using *TPSDig* software and each image was scaled with a 1.0 mm scale bar applied to each image when captured (*TPSDig*) (Fig. 2).

2.2.2. Gape size calculation

Maximum gape size (S_G) was calculated using the traditional gape size Eq. (A) given by Shirota (1970) and the later modified Eq. (B) by Guma'a (1978):

$S_{GS} = \sqrt{2}(L_{UJ})$ (A)

$S_{GG} = \sqrt{(L_{UJ}{}^2 + L_{LJ}{}^2)}$ (B)

Larvae were held under the microscope with forceps, while inserting a dissection pin into the mouth until a jaw angle of ~90° was achieved and an image was captured at the appropriate magnification with a set scalebar of 1.0 mm in the imaging software. A mouth angle of 90° was selected as this is assumed to be the maximum gape for larval fishes (Shirota, 1970). To validate gape size calculated using the Guma'a equation, landmarks were placed on the 1) anterior tip of the premaxilla, 2) point of jaw articulation, and 3) anterior tip of the dentary in *TPSDig* (Guma'a, 1978; Wittenrich and Turingan, 2011; Burgess and Callan, 2018). Landmark placement was adjusted to a common angle of 90° by rotating the landmark at the tip of the dentary about the point of jaw articulation and the distance calculated between landmarks at the tip of upper and lower jaws (Fig. 3).

2.2.3. Ontogenetic index and intervals of development

Assigning multiple discrete states to individual ontogenetic events can help define natural intervals of development (Ditty et al., 2003). Multivariate analyses of these character state variables reveal early development information useful to identifying the position along the progression of ontogeny, characterize timing and rate of ontogeny for a given species, and allow direct comparisons between species for defining interspecies characteristics. Each specimen ($n = 115$) was scored for a suite of twelve characters following the methodology developed by Ditty et al. (2003) with each score representing a discrete ontogenetic event or character state. This procedure can be used to determine natural intervals of development and characterize the state of ontogeny in small scaleless combtooth blennies. 'State' designates an instantaneous position within an ontogenetic sequence and 'stage' represents an interval of development traditionally defined in early life history. To improve contrast of anatomical structures such as preopercular spines, cephalic

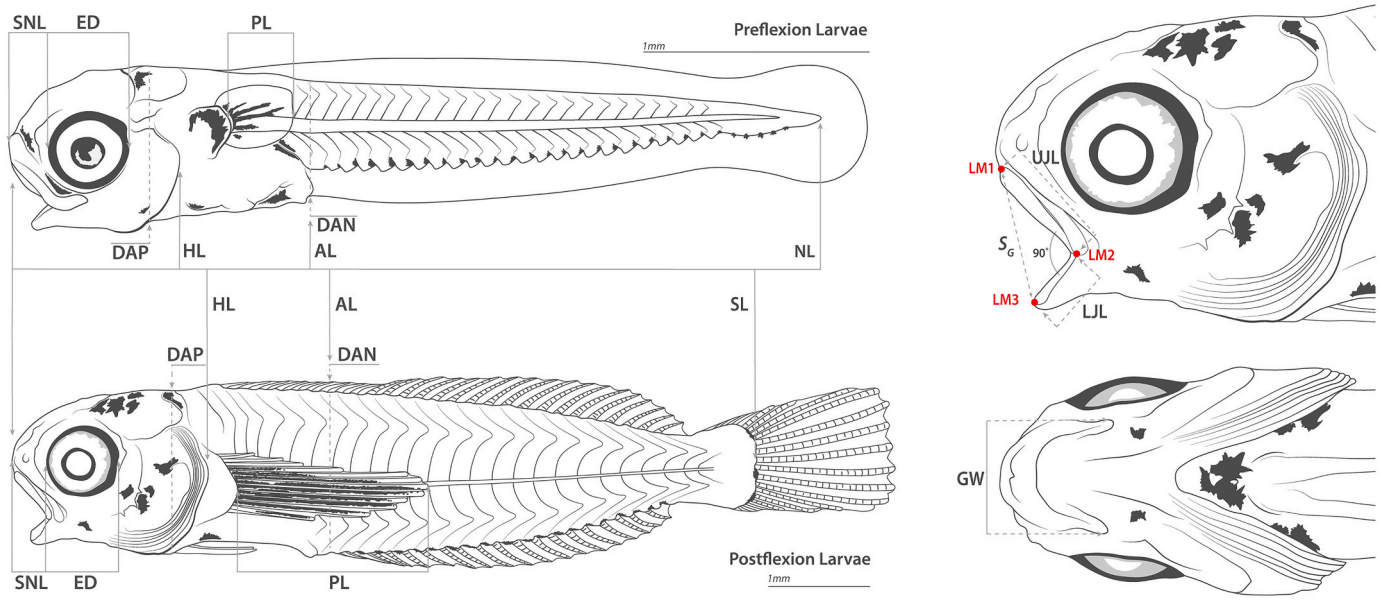

Fig. 2. Morphometric characters measured in Florida blenny *Chasmodes saburrae* larvae. Standard length (SL) and notochord length (NL) measured for body size; snout length (SNL), eye diameter (ED), pectoral length (PL) depth at pelvic (DAP), head length (HL), anal length (AL), depth at anus (DAN) measurement for single character assessment; upper jaw length (UJL), lower jaw length (LJL), and gape width (GW) used for gape morphology character assessment and calculation of maximum gape size (S_G); landmarks (LM1, LM2, and LM3) used as secondary measure of gape size.

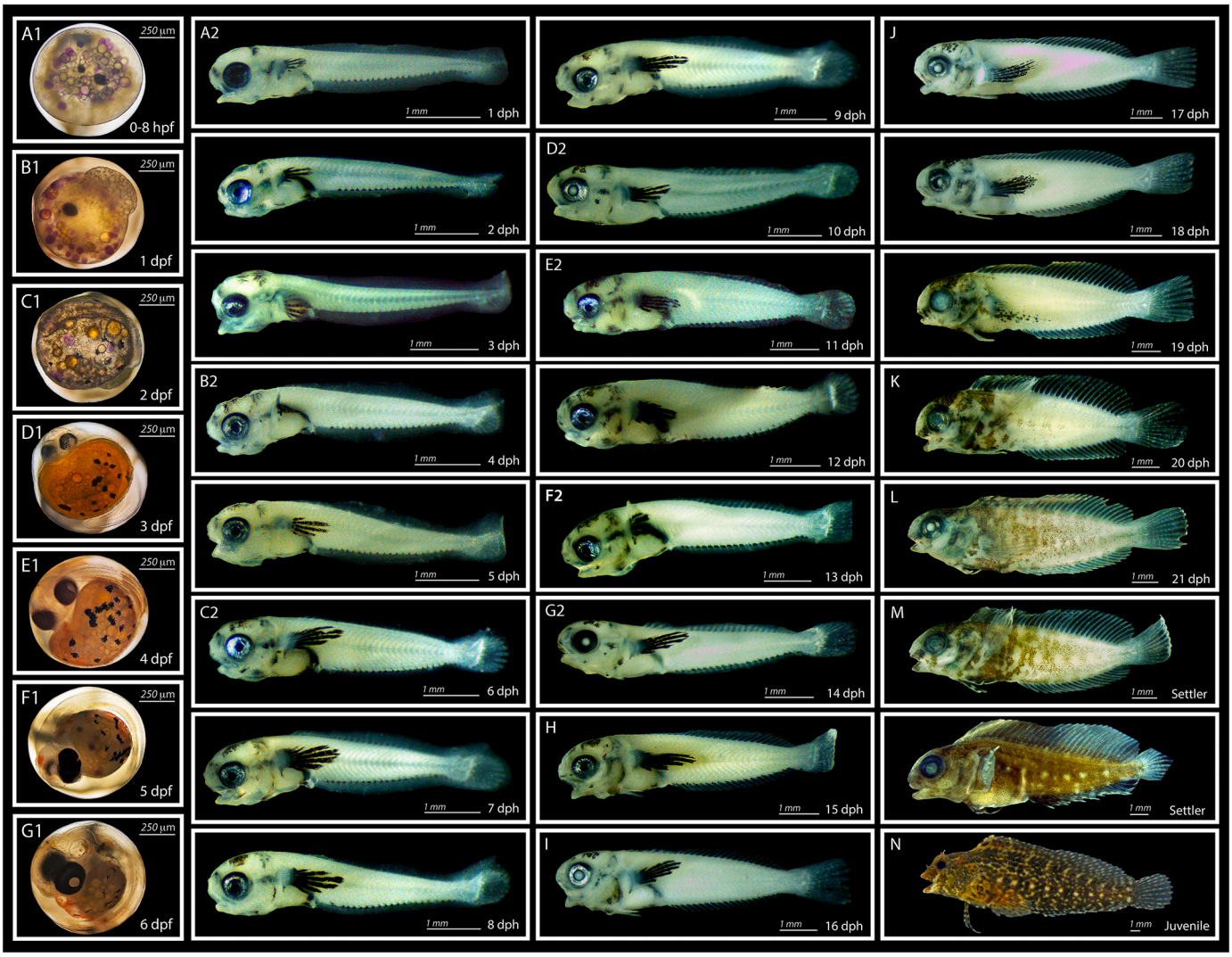

Fig. 3. Embryonic of eggs and larval development series of Florida blenny Florida blenny *Chasmodes saburrae*. Shown are multiple individuals sampled from two larval batches, Group A and B. Scale bars = 1.0 mm. (A1 – G1) embryonic incubation period from 8 hours post fertilization (hpf) to 6 days post fertilization (dpf) prior to hatching. (A2): 1 day post hatch (dph) preflexion larva with functional mouth, absent yolk reserve, medium fin fold, and ventral stellate melanophore pigmentation. Pigmentation present on pectoral fin bud (B2): 4-dph flexion larva with initial upward flexion of notochord with initial formation of caudal fin ray elements. Stellate melanophores visible atop cranium (C2): 6-dph flexion larva with anteroposterior elongation of the head and melanophore present at jaw hinge intersection of maxillary and dentary bones (D2): 10-dph flexion larva with complete resorption of caudal fin fold and median fin folds along the trunk. Dorsal ray formation appears to proceed those of anal fin. (E2): 11-dph postflexion larva with hyplural plate and caudal fin rays. Increased cephalic pigmentation. (F2): 13-dph postflexion larva with emergence of pelvic fin bud. (G2-I): 14–16-dph postflexion larvae with continued formation of dorsal, anal, pelvic, and caudal fin elements. Larvae begin to stay close to vertical structure of culture tank wall indicating soon to occur bottom settlement and metamorphosis (J): 17-dph postflexion larva with all dorsal and anal spines and rays visible with dorsal indentation. Settlement begins to occur with fish settling on bottom of larval culture tank. (K): 20-dph settler with metamorphosis complete, increased cephalic pigmentation and emergence of pelvic soft rays. Snout and maxillary elongation resulting in slight downward turn of mouth. (L): 21-dph settler with complete formation of all fin elements, deepening of body depth, beginnings of juvenile/adult coloration with gradient of cryptic pigmentation fading towards the posterior. (M): 21+ dph settler with complete coverage of body coloration and loss/absence of pectoral fin pigmentation. Continue elongation of snout and maxillary bones. (N): juvenile fully pigmented with complete juvenile coloration.

cirri, and fin rays, specimens were dipped in Acid Blue 113 stain (Cyanine Blue 5R stain) (Sigma-Aldrich Corp) prior to character state assignment (Saruwatari, 1997).

Agglomerative hierarchical cluster analysis was performed on character scores using complete linkage and Manhattan distance rules to organize and map 'interval' structure using the *pvclust* R-package (Maechler, 2019; Kassambara and Kassambara, 2020). The *pvclust* package also allows the user to test cluster validation and stability using a bootstrap resampling method (Suzuki and Shimodaira, 2006). A bootstrap resampling of 5000 iterations was used to determine the probability distribution and obtain nonparametric estimates of standard error (Pillar, 1999). A stability threshold with a confidence level of $\alpha =$ 0.10 was used to reject the null hypothesis of stable group structure for the proposed number of clusters ($n = 3$) (Pillar, 1999). Resulting clusters were assigned descriptive labels (larval, metamorph, and settler) according to Ditty et al. (2003). After assigning individuals to an interval of development, a discriminant function analysis (DFA) was performed on character scores to summarize group differences and intraspecific criteria that discriminated intervals using the lda() function in *MASS* R-package and the *candisc* R-package (Ripley et al., 2013; Friendly et al., 2021). Multivariate normality and outliers were assessed using a chi-squared quantile-quantile plot with Mahalanobis squared distance (D^2) values. The larvae interval was further divided into traditionally defined stages of 'preflexion', 'flexion', and 'post-flexion' based on direct

observation of specimens assigned to the interval.

2.2.4. Allometric growth analyses

Patterns of growth for measured morphometric characters were modeled by a power function of NL or SL obtained from $\log_{10}$ transformation of each character. Patterns in allometry were described using the growth coefficient (i.e. power function exponent) with the eq. $Y = aX^b$, where y is the dependent variable (measured character), and x, the independent variable (NL or SL), a is the intercept and b, the growth coefficient (Fuiman, 1983). The intercept and power exponent were obtained from linear or piecewise linear regressions on the log-transformed data (Nowosad et al., 2021). A growth coefficient of $b = 1$ indicates isometric growth while a growth coefficient of $b > 1$ or $b < 1$ indicates positive or negative allometric growth, respectively. Null hypothesis of isometric growth (H_0: $b = 1$) was tested for each linear model using the t-test with $\alpha = 0.05$. Assessment of regression model performance and output residuals plots for each character indicated that some data followed a nonlinear relationship, thus piecewise linear regressions were performed to determine inflection points. Piecewise regression models were performed using the *segmented* R-package (Muggeo, 2008) which uses an iterative procedure to estimate the breakpoint and summarize generalized linear models with segmented relationships. This package allows the user to estimate the initial guess for number and position of the breakpoint(s) in order to quickly assess possible differences with the computational efficiency of the algorithm (Muggeo, 2008). Slope comparison t-tests ($\alpha = 0.05$, $n - 4$ degrees of freedom) were performed to check if the slopes (i.e. growth coefficients) of the two linear segments (3 in the case of SNL) were significantly different from each other using the following equations:

$$t = \frac{b1 - b2}{S_{b1-b2}}$$

$$S_{b1-b2} = \sqrt{S_{b1}^2 + S_{b2}^{2.}}$$

b1 and b2 are the slopes of the line segments, and S_{b1-b2} is the standard error of the difference between the two slopes. $S_1 + S_2$ is the standard error of the segment slopes. To compare model performance AIC (*Akaike's Information Criterion*) scores were compared with single regression model scores and F-tests were performed ($n - 4$ degrees of freedom) to determine whether piecewise linear functions were a better fit than a single linear regression. To avoid bias during back-transformation of data, once a regression model was chosen, back-transformed predictor values were multiplied by a correction factor computed by the *logbtcf*() function in R (Sprugel, 1983).

2.2.5. Estimating feeding protocol with gape size

Calculated gape size (S_G) was used to estimate the timing at which to introduce a new food item during the progression of development. A single linear regression analysis was performed between gape size and dph for both larval groups and a t-test was performed to compare the slopes of each model. Food size was recorded by measuring the width of each prey item size class from samples of each culture ($n = 50$) and calculating mean size and standard deviation. Width of each prey item was the smallest dimensional measurement and assumed to be the limiting size for consumption (Krebs and Turingan, 2003). Percent of maximum gape size during feeding was estimated using the mean gape size for 5-dph larvae as a baseline for the transition from rotifers to *Artemia* nauplii as was observed during both group cultures. Based on this information, a more optimal feeding protocol was estimated which can then be tested with feeding trials.

3. Results with discussion

3.1. Morphological development

Pigmentation patterns mostly followed that described by Peters (1981) and Ditty et al. (2003). Laterosensory pores were first present behind the head with bony ossicles development in tandem but did not appear until early settlement, much later than those reported for the five species studied by Ditty et al. (2003) (Fig. 3).

Caudal fin ray bifurcation used for the approximation of juvenile stage (Randall, 1966) was not observed in the growth series of this study. Instead, wild-caught individuals from the same regional locality as the broodstock were examined. Specimens between 16.0 and 24.0 mm SL were examined, and a SL of 20.0 mm was determined to be the size at which caudal element bifurcation begins. Mean size and I_O values for observed settlement of *C. saburrae* was 8.93 mm SL ($I_O = 73.1$) and smallest size for individuals assigned to the settler interval was 9.27 mm SL ($I_O = 74.3$). Timing of settling to the bottom of the larval culture tank and assignment to a settler interval indicates that *C. saburrae* settle sooner than that of other GoM species as reported in Ditty et al. (2003). This is likely due to the estuarine habitat occupied by *C. saburrae* relative to the coastal fully marine habitats occupied by other reported species. Species adapted and reproducing in estuarine habitats tend to reach settlement more quickly in age and size than those occupying fully marine coastal habitats.

3.2. Intervals of development

Cluster analysis of characters scores was carried out on 11 of the 12 characters examined, excluding nasal cirrus which did not appear in samples for this study. Three clusters (I – III) were identified and tested for stability with multiscale bootstrap resampling (Fig. 4). After 5000 replicate samples, the three clusters were partitioned with high approximately unbiased (AU) p-values (>90%) indicating stability in these clusters. AU p-values ≥90% were considered strong evidence for a cluster, thus the null hypothesis of stable group structure is consistent with the suggested confidence level of $\alpha = 0.10$. Resulting clusters were assigned the same descriptive labels for interval of development (larvae, metamorph, and settler) following Ditty et al. (2003). Cluster I, termed larvae, contained individuals with total character state scores ≤ 9 and O_L values less than 62. Cluster II, termed metamorphs, contained specimens with total character state scores between 13 and 26 and O_L values >61.3. Cluster III, termed settlers, included individuals with total character state scores >28 and O_L values >72.1 (Table 1). The period of development characterized by both 'larvae' and 'metamorph' intervals are commonly defined as the larval period but require these artificial labels to more clearly illustrate changes in ontogeny along scaling metrics of time (dph), SL, and ontogenetic index (Table 2). The applied methodology developed by Ditty et al. (2003) designed for assigning small scaleless fishes like blennies to a developmental interval based on individual characters states provided a robust framework under which to characterize the state of ontogeny in *C. saburrae*. Using this method permitted direct comparison to other GoM blenniid species included in Ditty et al. (2003) and added to this body of work on early blenny development in the northern GoM. This study demonstrates the utility of this methodology to characterize development of blenniid larvae.

To distinguish which characters contributed most to the grouping of individuals to an interval, a discriminant function analysis (DFA) was performed on the 11 characters used in the cluster analysis. Chi-squared qq-plot of the character score data confirmed the assumption of multivariate normality with only three outliers with high D^2-values. MANOVA results (wilks' $\lambda = 0.00665$; F-statistic = 116.02; $p < 0.0001$) indicated a large F-statistic and correspondingly small p-value providing support to reject the null hypothesis of equality of interval group means. Two canonical roots extracted all within-group variability. Canonical root 1 discriminated settlers from metamorphs and larvae (71.2%

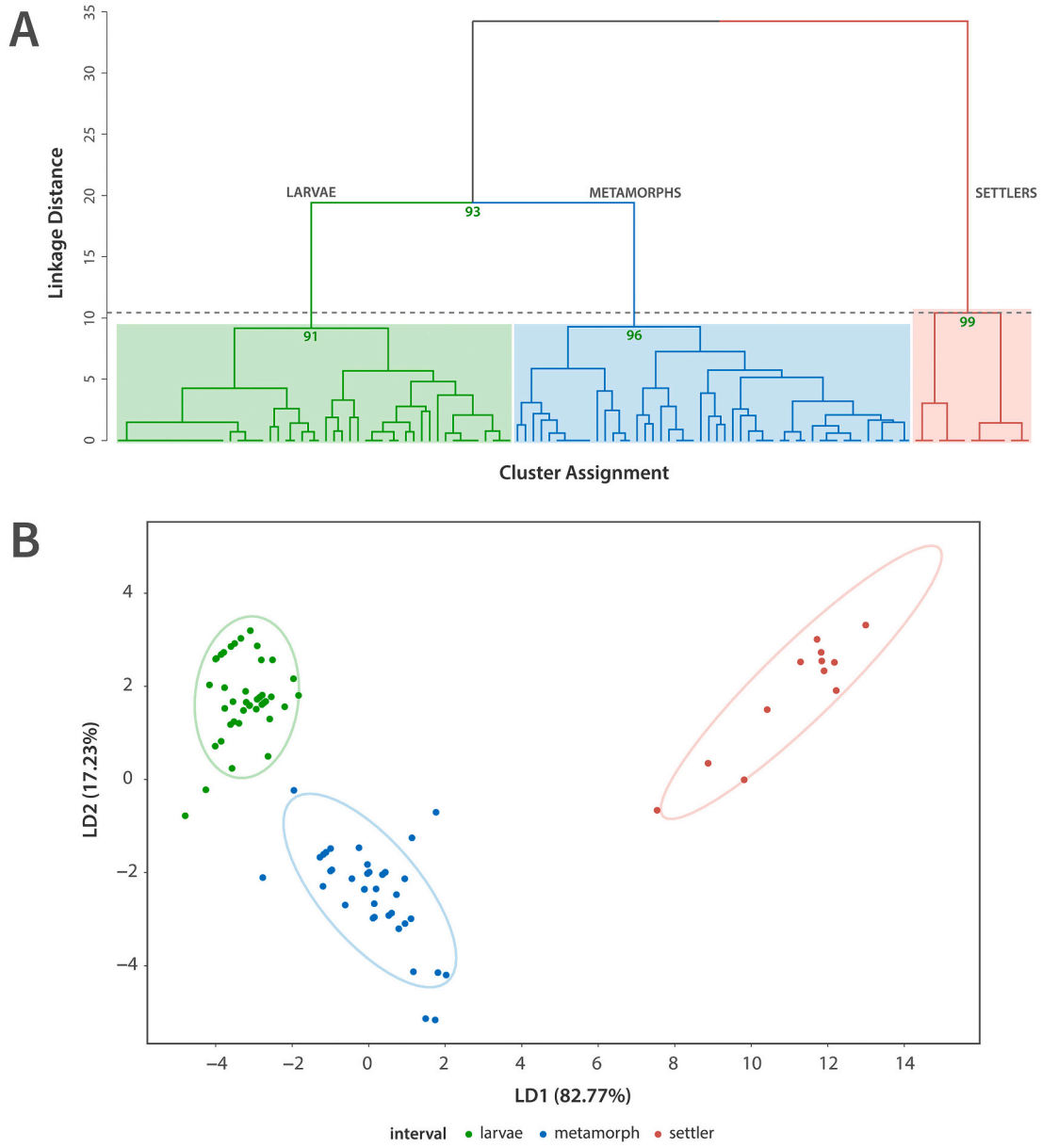

Fig. 4. A) Dendrogram from hierarchical cluster analysis of character state scores from Florida blenny *Chasmodes saburrae* larval development. Approximately unbiased (AU) *p*-values indicate support of cluster stability ($\alpha = 0.90$); results from cluster analysis indicate three well-supported stable clusters designated as intervals of development (larvae, metamorph, and settler). B) Plot of canonical variates (canonical roots 1 and 2) computed from discriminant function analysis and their relative contribution (% variability) to discriminating intervals of development assigned to *C. saburrae* specimens following cluster analysis.

explained variability), and canonical root 2 separated between metamorphs and the other two intervals to a lesser extent (28.8% explained variability) (Fig. 4).

Characters with the most contribution to discriminating ability of root 1 were orbital cirrus, number of teeth, and body pigmentation as explained by the initiation of lateral body pigment, orbital cirri size and pigmentation, number of teeth in settlers. Discriminating ability of root 2 was largely driven by the state of the preopercular spine growth or resorption, caudal fin skeletal development and pigmentation, body pigmentation, and number of dorsal spine elements formed. Metamorphs of *C. saburrae* are defined by a higher tooth count, proliferation of pigment on the caudal fin and pectoral fin, initiation of dorsal spine formation, and resorption of preopercular spines. Settlers are defined by proliferation of trunk, orbital cirrus, and pelvic fin pigmentation, reduction in pectoral fin pigmentation, and complete incisiform dentition.

Following assignment of development intervals, the larvae interval was further divided into three periods of preflexion, flexion, and postflexion based on direct observation. Preflexion stage began at hatch (0–3 dph) between (3.37–4.32 mm SL) and completed with the onset of flexion of the notochord. Flexion was observed in larvae at 4 dph (4–10 dph) at 5.05 mm SL (4.12–5.75 mm SL). Prior to the metamorph interval, larvae transitioning after flexion to metamorph but still assigned to the larvae interval featured proliferation of pigmentation on the head and pectoral fin, initiation of pigment along the dorsal rays, extension of the preopercular spines, and initial segmentation of primary and formation of secondary caudal elements. The period of postflexion occurred at 5.68 mm SL (5.17–6.40 mm SL) occurring simultaneously with many individuals still undergoing flexion. The metamorph interval began on 11 dph (11–20 dph) at 7.63 mm SL (6.28–9.27 mm SL) with a high degree of transformation taking place to prepare for settlement and taking on juvenile characteristics. Individuals were observed settling to

Table 1
Summary information for ontogenetic index values and character state scores for Florida blenny *Chasmodes saburrae*. Intervals (Larvae, Metamorphs, Settlers) were determined by cluster analysis of total character scores for 10 discrete morphological characters. Total character score is the sum of scores assigned each of the 10 characters listed in Appendix Table 1. All sizes are mm SL and statistics provided are mean and (range). Size at juvenile (L_{juv}) used to designate the start of the juvenile period was determined using wild-caught specimens of *C. saburrae* collected from the same region as that of broodstock used in this study.

Category	*C. saburrae*
Overall summary	
Sample size	115
Size range	3.37–14.49
Range of ontogenetic index	41–89
Range of total character score	0–35[1]
All Larvae	
Cluster Interval	Larvae
Sample size	52
Size	4.87 (3.37–6.40)
Ontogenetic index	52.3 (40.6–62.0)
Total character score	4.6 (0−11)
Preflexion Larvae	
Cluster Interval	Larvae
Sample size	16
Size	3.81 (3.37–4.32)
Ontogenetic index	44.5 (40.6–48.8)
Total character score	0.4 (0.0–2.0)
Flexion Larvae	
Cluster Interval	Larvae
Sample size	19
Size	5.05 (4.12–5.75)
Ontogenetic index	53.9 (47.3–58.4)
Total character score	4.9 (1.0–8.0)
Postflextion Larvae	
Cluster Interval	Larvae
Sample size	17
Size	5.68 (5.17–6.40)
Ontogenetic index	57.9 (54.8–62.0)
Total character score	8.2 (6.0–11.0)
Metamorphs	
Cluster Interval	Metamorph
Sample size	48
Size range	7.63 (6.28–9.27)
Ontogenetic index	67.7 (61.3–74.3)
Total character score	17.4 (13.0–26.0)
Settlers	
Cluster Interval	Settler
Sample size	15
Size range	11.24 (8.67–14.49)
Ontogenetic index	80.3 (72.1–89.2)
Total character score	32.1 (28.0–35.0)
Size at juvenile (L_{juv})	20.0

the bottom of the culture tank and remaining there during feeding at 17 dph (8.5 mm SL). This was preceded by metamorphs often swimming or resting in a vertical orientation along the sides of the tank indicating the impending settlement. Physical settlement was completed by all remaining individuals by 20 dph (9.00 mm SL). Individuals assigned to the settler interval that featured ontogenetic character states defining this interval acquired these traits after physical settlement to the tank bottom. The settler interval began on 21 dph at 11.24 mm SL (8.67–14.49 mm SL) with all remaining individuals completing metamorphosis. Note, ten individuals with ages beyond the 21-day culture period were included to extend the dataset and included description settler characteristics prior to entering the juvenile stage.

3.3. Allometric growth patterns

Ten of 11 morphometric traits measured followed a positive allometric increase after hatching and featured at least one inflection point of change in allometric growth. Eye diameter maintained isometric growth throughout development ($b = 1.0633$). Gape size based on the equation given by Shirota (1970) followed the same allometric growth pattern as UJL as this was the measurement used to calculate the gape size. The Shirota equation did not represent the true growth pattern of gape size without the inclusion of the LJL as is the case with the Guma'a (1978) equation. Thus, gape size calculated based on UJL alone was not considered further. Snout length (SNL) was the only character with two inflection point changes in allometric growth rate with the third segment of growth experiencing negative allometry ($b = 0.537$). Residuals plots were assessed for nonlinear relationships and heterogeneity of variance and in all cases necessitated application of a nonlinear piecewise model. All *t*.test slope comparisons between piecewise segments were significantly different ($\alpha < 0.05$) and all piecewise regressions featured a high relative *F*-statistic and lower AIC score than that of single regressions, thus piecewise regression models were considered to be superior to a single linear regression for the 10 characters (Table 3).

Inflection points of allometry occurred at two points when scaled with standard length during progression of development. One growth condition featured a rapid positive allometric growth during the larvae interval during preflexion, flexion, and postflexion before slowing at the transition to the metamorph interval with either a lower positive rate, isometric, or negative rate. The second condition occurred at the transition between the metamorph to settler interval prior to settlement beginning with a lower positive allometric growth rate and shifting to a higher positive rate through the settler interval indicating preparation for the energetically expensive flexion period. SNL featured both of these conditions but with a negative allometric growth rate once becoming settlers. Size at inflection for HL (6.09 mm SL), PL (5.81 mm SL), DAN (6.01 mm SL), DAP (5.81 mm SL), UJL (5.86 mm SL), and S_{GG} (4.94 mm SL) all followed condition one with rapid growth until the start of the metamorph interval. Condition two characterized GW (8.33 mm SL), AL (8.57 mm SL), and LJL (8.28 mm SL) with higher positive allometric growth just prior to the settler interval and coinciding the metamorphosis (Fig. 5). Changes in allometric growth of gape size was characterized by the shortest rapid period of positive growth ($b = 1.515$) before transitioning to isometric growth ($b = 1.043$) for much of the development period. This is likely due to the dichotomy between allometric growth patterns of the upper and lower jaws as these were used in the calculation of gape size and feature opposing conditions of growth change. This dichotomy appears to stabilize the gape size throughout early development likely allowing *C. saburrae* to consume new prey sizes at a relatively unchanging rate to the juvenile stage.

Allometric growth has been observed during larval development in many fish species, including triplefin blennies (Solomon et al., 2017). Morphological variation expressed by larvae due to allometric growth and quantification of shape change can help delimit intervals of development (Strauss and Fuiman, 1985). These changes in shape often occur at discontinuities in the course of development, such as postflexion or metamorphosis. Here we report allometric growth patterns for the development of *C. saburrae* larvae corresponding to thresholds between the three intervals (larvae, metamorph, settler). All but one of the morphometric characters examined followed changes in allometric rates ($b \neq 1$) corresponding with either the threshold between larval and metamorph intervals or prior to the transition to settlement and metamorphosis, thus supporting the hypothesis of ontogenetic priorities and reflections of successive functional priorities during development (Osse and Van den Boogaart, 1995).

Together, ontogenetic changes as characterized by morphological

Table 2
Threshold estimates for timing and variation of settlement as indicated by three scaling metrics applied to Florida blenny, *Chasmodes saburrae,* in this study and five blenniid species from the northern Gulf of Mexico included in Ditty et al. (2005). Thresholds were calculated from means and (coefficients of variation) of the three largest metamorphs and three smallest settlers based on standard length (SL).

Species	Habitat	Development threshold	SL (mm) mean (CV)	Total character state score mean (CV)	Ontogenetic index mean (CV)	Author
C. saburrae	Estuarine	Larvae Metamorph	6.32 (1.3%)	11.3 (3.3%)	61.5 (0.7%)	this study
C. saburrae	Estuarine	Metamorph - Settler	9.2 (2.3%)	23.7 (24.9%)	74.2 (1.0%)	this study
Hypsoblennius invemar	Coastal	Metamorph - Settler	12.5 (6.0%)	25.3 (17.2%)	87.4 (2.4%)	Ditty, 2002
Hypsoblennius ionthas	Coastal/ Estuarine	Metamorph - Settler	11.7 (5.1%)	25.0 (32.0%)	86.8 (2.0%)	Ditty, 2002
Hypleurochilus multifilis	Coastal	Metamorph - Settler	12.5 (4.6%)	30.2 (16.6%)	86.6 (1.8%)	Ditty, 2002
Scartella cristata	Coastal	Metamorph - Settler	11.0 (6.2%)	25.7 (14.3%)	82.8 (2.6%)	Ditty, 2002
Parablennius marmoreus	Coastal	Metamorph - Settler	20.1 (7.1%)	29.4 (18.6%)	86.8 (0.5%)	Ditty, 2002

Table 3
Regression analysis of measure morphometric characters from Florida blenny *Chasmodes saburrae*. Power functions computed from linear and nonlinear regression analysis on log10 transformed data, coefficient of determination (R^2), associated ANOVA with F statistic, AIC scores and back-transformed inflection point in mm SL are provided. All significances were at the $p < 0.01$ level.

Character	Regression model	R^2	AIC	Segment	n	b	SL: point of inflection (mm)	Regression equation
Head length (HL)	Single	0.977	−466.6	–	115	1.39	–	$y = 0.125x^{1.39}$
	Segmented	0.981	−484.5	segment 1	49	1.58	<6.09 mm SL	$y = 0.094x^{1.58}$
		–	–	segment 2	66	1.26	>6.09 mm SL	$y = 0.167x^{1.26}$
Pectoral length (PL)	Single	0.943	−283.7	–	100[1]	1.59	–	$y = 0.068x^{1.59}$
	Segmented	0.959	−317.8	segment 1	40	2.14	<5.81 mm SL	$y = 0.029x^{2.14}$
		–	–	segment 2	60	1.32	>5.81 mm SL	$y = 0.123x^{1.32}$
Depth at anus (DAN)	Single	0.964	−400.1	–	115	1.48	–	$y = 0.088x^{1.48}$
	Segmented	0.975	−440.7	segment 1	49	1.82	<6.01 mm SL	$y = 0.051x^{1.82}$
		–	–	segment 2	66	1.24	>6.01 mm SL	$y = 0.145xs^{1.24}$
Depth at pelvic (DAP)	Single	0.983	−514.3	–	115	1.32	–	$y = 0.129x^{1.32}$
	Segmented	0.984	−523.2	segment 1	46	1.45	<5.81 mm SL	$y = 01.06x^{1.45}$
		–	–	segment 2	69	1.24	>5.81 mm SL	$y = 0.152x^{1.24}$
Anal length (AL)	Single	0.985	−543.9	–	115	1.23	–	$y = 0.272x^{1.23}$
	Segmented	0.988	−572.4	segment 1	93	1.17	<8.57 mm SL	$y = 0.302x^{1.17}$
		–	–	segment 2	22	1.48	>8.57 mm SL	$y = 0.155x^{1.48}$
Gape width (GW)	Single	0.961	−393.8	–	115	1.45	–	$y = 0.041x^{1.45}$
	Segmented	0.964	−401.5	segment 1	89	1.37	<8.33 mm SL	$y = 0.047x^{1.37}$
		–	–	segment 2	26	1.73	>8.33 mm SL	$y = 0.022x^{1.73}$
Snout length (SNL)	Single	0.902	−283.5	–	115	1.44	–	$y = 0.024x^{1.44}$
	Segmented	0.954	−364.8	segment 1	46	2.13	<5.81 mm SL	$y = 0.008x^{2.13}$
		–		segment 2	58	1.18	5.81 mm SL < X < 9.55 mm SL	$y = 0.044x^{1.18}$
		–		segment 3	11	0.54	>9.55 mm SL	$y = 0.187x^{0.54}$
Eye diameter (ED)	Single	0.966	−482.7	–	115	1.06	–	$y = 0.093x^{1.06}$
Upper jaw length (UJL)	Single	0.940	−418.0	–	115	1.05	–	$y = 0.090x^{1.05}$
	Segmented	0.968	−487.7	segment 1	47	1.46	<5.86 mm SL	$y = 0.048x^{1.46}$
		–	–	segment 2	68	0.80	>5.86 mm SL	$y = 0.153x^{0.80}$
Lower jaw length (LJL)	Single	0.923	−337.2	–	115	1.31	–	$y = 0.034x^{1.31}$
	Segmented	0.929	−343.3	segment 1	88	1.21	<8.28 mm SL	$y = 0.043x^{1.21}$
		–	–	segment 2	27	1.63	>8.28 mm SL	$y = 0.018x^{1.63}$
Gape size Guma'a (G_{SG})	Single	0.940	−437.3	–	115	1.15	–	$y = 22.33x^{1.15}$
	Segmented	0.963	−453.6	segment 1	25	1.52	<4.94 mm SL	$y = 52.772x^{1.52}$
		–	–	segment 2	90	1.04	>4.94 mm SL	$y = 112.253x^{1.04}$

[1] Linear and segmented regressions were performed on a portion ($n = 100$) of larval specimens based on measurability of pectoral length from captured images.

character state changes coupled with allometric growth changes of morphometric characters indicate the time at notochord flexion and the time proceeding settlement and completion of metamorphosis are critical periods of development for *C. saburrae* larvae. Similar patterns of development can be seen in larval fish of other species. A biphasic pattern of growth inflection exhibited in *C. saburrae* was seen in all but two morphometric traits examined with snout length following a triphasic growth pattern and eye diameter following isometric growth throughout development. Growth coefficients for all biphasic or triphasic characters followed pre-inflection positive allometry, indicating a rapid growth of those elements prior to transition to the metamorph interval or before settling. Positive allometric growth was followed by continued but reduced positive allometry in all but two biphasic characters (upper jaw and lower jaw lengths) during post-inflection development. Upper jaw length experienced a negative post-inflection allometric growth while lower jaw length showed an increase in post-inflection growth rate. The only character with two inflection points (snout length) experienced a negative post-inflection growth rate during the third segment of development after settlement occurred.

3.4. Gape-informed feeding protocol

Gape size at feeding was estimated from the maximum gape size calculation (S_G), mean width measurements of live food items, and the observation of age at first feeding of 0 h *Artemia* nauplii. Slope comparison *t*-tests indicated a significant difference in slopes between the

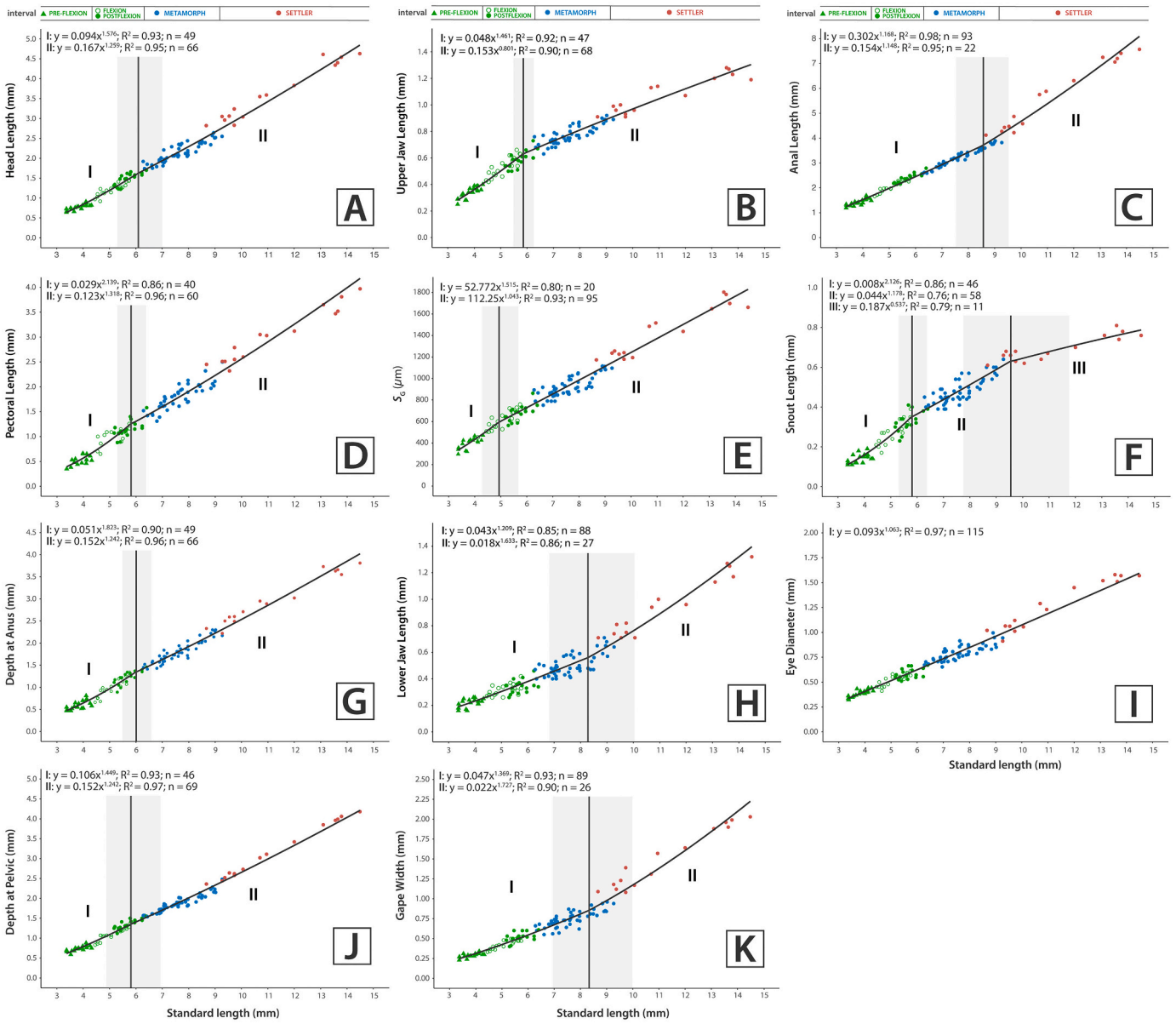

Fig. 5. Allometric growth equations for morphometric characters and relationship to standard length scaling metric of Florida blenny *Chasmodes saburrae* larvae during early stages of development. Each point represents measurements from a single specimen. Interval colors indicate interval of development assigned to each specimen. Larvae interval further divided into preflexion, flexion, and postflexion stages of development based on direction observation. Power functions feature growth coefficients for allometric growth rates and vertical lines indicate the inflection point of change in growth rate between segments. (A) head length, (B) gape width, (C) pectoral length, (D) anal length, (E) depth at anus, (F) snout length, (G) depth at pelvic, and (H) eye diameter.

two larval groups and difference in intercept indicated that group B hatched at a smaller size that group A. Accordingly, group A was used to estimate an optimal feeding protocol as this group included samples from 1-dph, the age capable of transition from rotifers to *Artemia* nauplii (5–6 dph) and the end of the larval series at 21 dph. A mean size of 183 μm (±17.33) was calculated for 0 h *Artemia* nauplii indicating a feeding gape size at 26.6% that of the maximum gape size. The transition from rotifers to 0 h *Artemia* nauplii occurs at approximately the same point as the inflection point for allometric to isomeric growth in gape size. Thus, a consistent rate of growth was assumed with the introduction of larger sized prey items in a linear fashion with isometrically increasing gape size. Adjusted gape size plotted on scales of SL and dph allowed for an estimation of timing for introduction of progressively larger food through the course of development (Fig. 6). At a feeding gape angle of 26.6% that of max gape size, newly hatched larvae are capable of feeding on *S*-type rotifers of mean size 113 μm (±13.29) immediately after hatching as confirmed by observations during culture and a mean max gape size of 0.383 (±14.56) mm or 383 μm (±47.77) within the range needed to consume *S*-type rotifers. Diet transition to enriched 24 h Artemia nauplii is possible prior to the metamorph interval at 9 dph and to enriched 48 h nauplii soon after becoming a metamorph at 12 dph. A weaning diet of 300 μm artificial pellets can be introduced as early as 15 dph; however, the pellets used in this study immediately sink to the bottom and so should not be introduced until metamorphs begin to settle permanently to the bottom at 17 dph. Original feeding protocol was based on previous study of blenniid larviculture (Peters, 1985; Olivotto et al., 2010; Moorhead and Zeng, 2017). This included *S*-type rotifers from 0 to 11 dph, newly hatched 0 h *Artemia* nauplii for 6–14 dph, enriched 24 h *Artemia* nauplii for 13–21 dph, and enriched 48 h *Artemia* nauplii for 21 dph onward with introduction of artificial pellet diet later after settlement. According to gape size a more optimal diet is proposed here: *S*-type rotifers from 0 to 8 dph, newly hatched 0 h *Artemia* nauplii

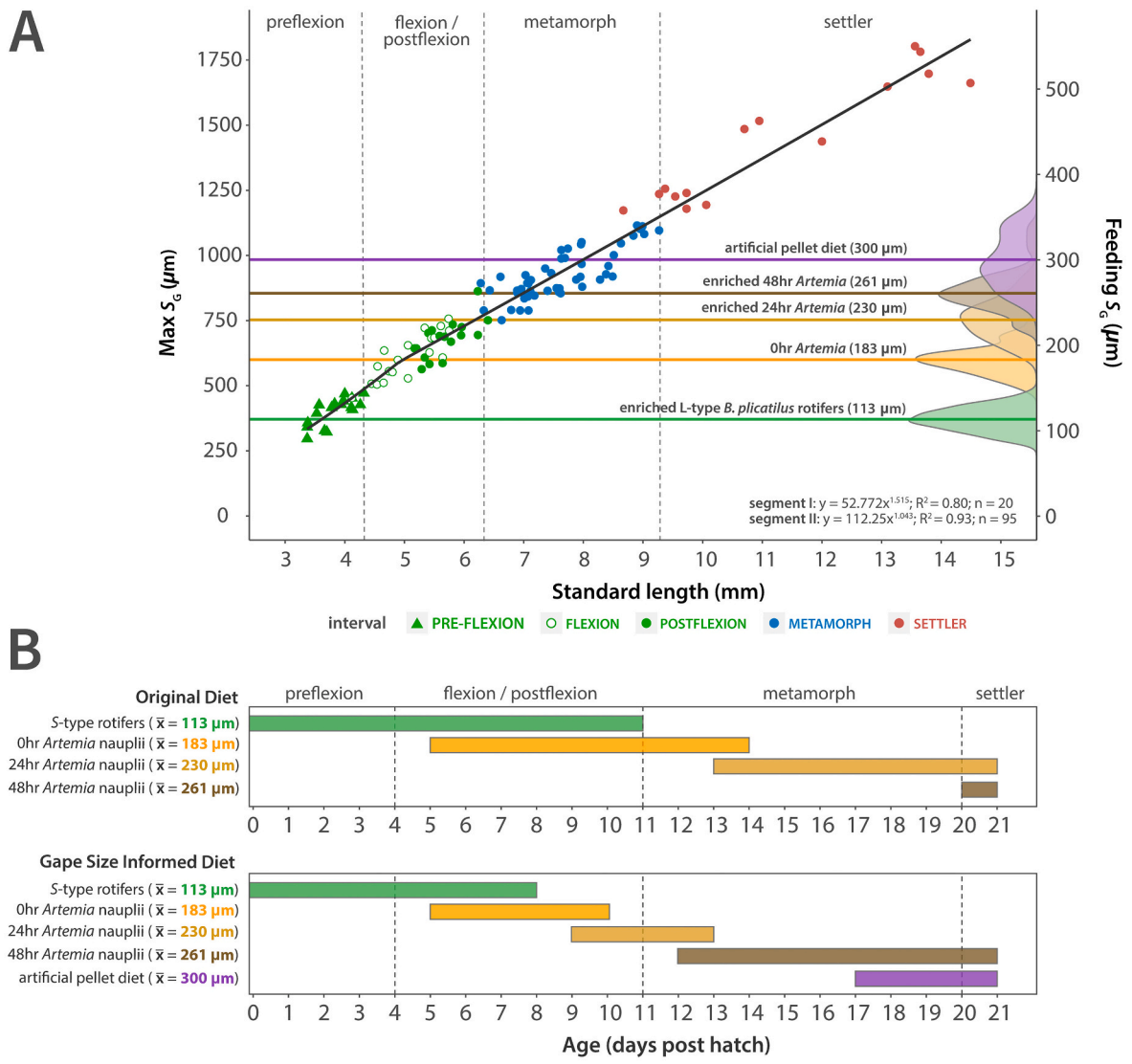

Fig. 6. A) Plot of maximum gape size (max-S_G) and estimated feeding gape size (feeding-S_G) and relationship to standard length (SL) scaling metric for all specimens. Inflection point indicates change in allometric growth rate; dashed lines demarcate intervals of development. Mean width of live food items fed to Florida blenny *Chasmodes saburrae* larvae during culture are applied to the feeding gape size (feeding-S_G) scale on the secondary y-axis. B) Mean width of live food items fed to Florida blenny *C. saburrae* during larviculture are applied to the feeding gape size (feeding-S_G) scale on the secondary y-axis. Original feeding protocol applied during larviculture of Florida blenny *C. saburrae* during this study and estimated optimal feeding protocol based on live food item size and feeding gape size (feeding-S_G).

for 5–10 dph, enriched 24 h *Artemia* nauplii for 9–13 dph, enriched 48 h *Artemia* nauplii for 12–21 dph and a weaning diet of 300 µm artificial pellet at 17+ dph. This proposed diet includes a 3-day transitional overlap period with both rotifers and 0 h nauplii during which variable rates of flexion is occurring followed by 1-day transition period between subsequent nauplii size and period of overlap between enriched 48 h nauplii and artificial pellet until all settlers are weaned from live food (Fig. 6). Observation and protocol along with changes in morphology and allometric growth patterns are summarized in Fig. 7.

Maximum gape size (S_G) is an important variable to measure for studies of feeding ecology and can be a useful metric to estimate timing to introduce successive food sizes in larviculture. Study of S_G and upper and lower jaw lengths used in the calculation thereof for *C. saburrae*, further reveals allometric growth patterns of growth corresponding to the onset of flexion, in agreement with other body proportions with a similar timing of rate change. This study compared both gape size equations as presented by Shirota (1970) and Guma'a (1978) and found that Shirota's use of only the upper jaw length results in an overestimation of actual maximum gape (as measured directly) and that using the Guma'a equation. Thus, the Guma'a equation was used for this study based on a consistently shorter lower jaw length relative to upper jaw length which matched that of direct measurement. Therefore, lengths of both upper and lower jaws were included in the calculation of gape size. Allometric change in S_G of *C. saburrae* exhibited a rapid positive allometry prior to flexion followed by a near isometric growth post flexion. An explanation for this could be that newly hatched larvae require rapid growth in order to reach a greater size spectrum of prey rapidly to avoid competition from more recently hatched larvae and avoid predation. This agrees with strategies of most fish larvae studied and may be particularly important for estuarine-dependent species, like *C. saburrae*, encountering a greater prey variety and elevated competition from other estuarine-dependent teleosts occupying estuaries during the early part of life (López-Herrera et al., 2021). Post-inflection isometric growth of S_G began at the point of transition from initial *S*-type rotifer feed to 0 h nauplii indicating a linear model of development proportional to increasing fish size (SL) for this trait. Thus, larger food items can be introduced according to a constant rate of growth until metamorphosis. Using this data, we estimated an optimized diet

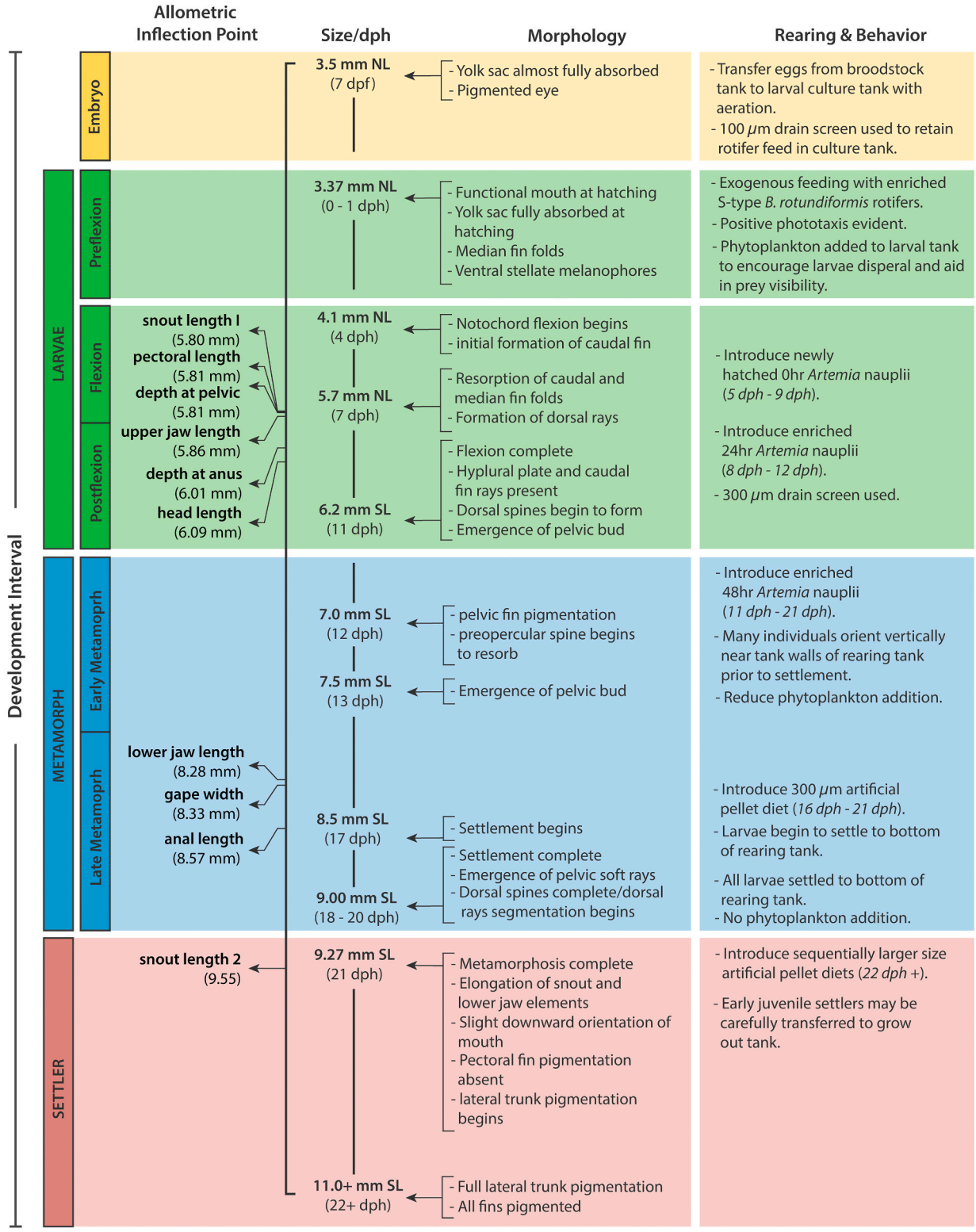

Fig. 7. Chart of morphological change and allometric growth patterns summarizing general ontogeny of Florida blenny *Chasmodes saburrae*.

consisting of S-type rotifers fed from 0 to 8 dph, 0 h Artemia nauplii from 5 to 10 dph, enriched 24 h nauplii from 9 to 13 dph, enriched 48-h nauplii from 12 to 21 dph followed by introduction of weaning artificial pellet diet (300 μm) at 20 dph. This "gape-informed diet" may reduced mortality at crucial stages of growth and may also support faster growth in these larvae, however more research is required to understand how this diet would affect these metrics.

4. Conclusions

Above we have presented a larviculture protocol for rearing blenniids which may have scalable applications in both research and commercial ornamental aquaculture. Through a series of morphometric measurements, we have also proposed potential changes to this protocol based upon the larval gape size. Using our larviculture techniques we are

also able to report a complete larval growth series for *C. saburrae* and assign individuals to development stage intervals based on the protocol established by Ditty et al. (2003). These findings are intended to complement research on wild-caught individuals and serve as a foundation for future studies of combtooth blenny larval biology. Based on the demonstrated larviculture, traits such as short larval duration times and less demanding dietary requirements compared to those of many other marine teleosts, combtooth blennies may serve as sensible model organisms for use in future studies of evolution, ecology, and physiology of marine fishes.

CRediT authorship contribution statement

Joshua E. Carter: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Megan A. Sporre:** Investigation, Formal analysis, Writing – review & editing, Visualization. **Ron I. Eytan:** Supervision, Funding acquisition, Writing – review & editing, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738153.

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