RESEARCH ARTICLE

Alternative Competition-Induced Digestive Strategies Yield Equal Growth, But Constrain Compensatory Growth in Red-Eyed Treefrog Larvae

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SARAH S. BOUCHARD[1]*,
CHELSEA J. O'LEARY[1],
LINDSAY J. WARGELIN[1],
WHITNEY B. RODRIGUEZ[1],
KADEEN X. JENNINGS[1],
AND KAREN M. WARKENTIN[2,3]

[1]Department of Biology and Earth Science, Otterbein University, Westerville, Ohio
[2]Department of Biology, Boston University, Boston, Massachusetts
[3]Smithsonian Tropical Research Institute, Balboa, Ancon, Republic of Panama

ABSTRACT

Compensatory growth is well documented across taxa and provides a fitness advantage to animals who would otherwise reach a smaller reproductive size. We investigated the role of competition-induced gut plasticity in facilitating a compensatory response in red-eyed treefrog larvae. We reared larvae at low, medium, and high densities with different per capita resources, environments known to produce individuals with long and short guts. We then transferred larvae to competitively equal environments to determine if longer guts provided an advantage when resources became available. We predicted that larvae from higher densities with longer guts would exhibit hyperphagia and compensatory growth. We measured growth over 1-week, as well as the time to and size at metamorphosis. To assess mechanisms underlying the growth response, we measured diet transit time and intake. Growth, development, and metamorph snout-vent length did not differ between larvae with long and short guts. Instead, different gut lengths were associated with dramatically different feeding strategies. Medium- and high-density larvae fed at rates far below what their guts could accommodate. However, the combination of low intake and longer guts extended diet transit times, presumably increasing digestibility. This unexpected strategy achieved the same results as that of low-density larvae, which ate twice as much food, but passed it more quickly through a shorter gut. The lack of a compensatory response may be attributed to the costs of accelerated growth and weak seasonal time constraints in the tropics. This suggests that although compensatory growth is widespread among animals, expression of the response may vary with environmental context. *J. Exp. Zool. 9999A:XX–XX, 2015.* © 2015 Wiley Periodicals, Inc.

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Growth rate is a critically important life history trait often linked directly to fitness via effects of body size on reproductive output (Blueweiss et al., '78). However, despite selective pressure for large body size, many animals grow submaximally, presumably due to costs associated with rapid growth (Dmitriew, 2011). These costs may be both ecological and physiological, including such factors as increased predation risk, accumulated cellular damage, depressed immune function, decreased resistance to stress, lower reproductive output, and shortened life span (Mangel and Stamps, 2001; Monaghan, 2008; Dmitriew, 2011; Lee et al., 2012; Lee et al., 2013). Although animals typically grow submaximally, growth rate plasticity allows animals to respond to environmental variability, and rapid growth may be advantageous under some circumstances. For example, some species may grow faster during a shortened growing season (Abrams et al., '96; Lindgren and Laurila, 2005) or when predators selectively feed on smaller individuals (Werner and Gilliam, '84).

Animals may also grow rapidly to compensate for a period of growth depression. Such compensatory growth is widespread and has been documented repeatedly across many taxa (Hector and Nakagawa, 2012), including arthropods (De Block and Stoks, 2008; de Almeida Marques and Lombardi, 2011), fish (Ali et al., 2003; Ab Ghani and Merila, 2015), amphibians (Orizaola et al., 2014), reptiles (Radder et al., 2007; Roark et al., 2009), birds (Bize et al., 2006; Criscuolo et al., 2008; Chin et al., 2013), and mammals (Hector and Nakagawa, 2012). The term compensatory growth is often used interchangeably with catch-up growth, but recent papers have been careful to distinguish between the two (Hector and Nakagawa, 2012; Orizaola et al., 2014). Catch-up growth occurs when previously growth-stunted animals obtain the same size as unstunted control animals. It may involve accelerated (compensatory) growth as a mechanism, or it may result from the extension of typical growth rates beyond the normal growth window. Although true compensatory growth is undoubtedly widespread, it may be over-reported because some studies fail to account for non-linear, size-dependent growth in their analyses (Nicieza and Alvarez, 2009).

Compensatory growth allows organisms to change growth trajectories such that they increase body size at sexual maturity. These larger body sizes could provide a significant fitness advantage by allowing initially growth-stunted individuals to meet a minimum size required for reproduction or by better equipping individuals for mate acquisition. However, compensatory growth has also been linked to a wide variety of costs, including those related to predation risk, muscle and skeletal development, starvation resistance, locomotor performance, adult obesity, glucose tolerance, and life span (Metcalfe and Monaghan, 2001; Ali et al., 2003; Yearsley et al., 2004; Mangel and Munch, 2005; Monaghan, 2008; Dmitriew, 2011; Lee et al., 2013). Tradeoffs between these costs and benefits may not be equal across taxa, as mammals and birds are more likely to exhibit compensatory growth than fish and arthropods, presumably because of their determinate growth (Hector and Nakagawa, 2012). Additionally, the costs and benefits can vary within a species depending on specific environmental conditions (Metcalfe et al., 2002; Orizaola et al., 2014; Ab Ghani and Merila, 2015).

Amphibians are a particularly good model for compensatory growth studies because their complex life cycle provides opportunities for compensation both during the larval stage and post-metamorphic juvenile stage. Additionally, size at metamorphosis is an important life history trait that is linked to larval growth and is positively correlated with survival and future reproduction (Smith, '87; Semlitsch et al., '88; Scott, '94; Cabrera-Guzman et al., 2013). Recent studies have demonstrated the capacity of anuran larvae to exhibit compensatory growth after food restriction (Capellan and Nicieza, 2007; Hector et al., 2012), intraspecific competition (Jasienski, 2008), temperature (Orizaola et al., 2014), salinity stress (Squires et al., 2010; Wu et al., 2012), and delayed hatching (Orizaola et al., 2010), but not following the threat of predation (Capellan and Nicieza, 2007). However, few studies have attempted to understand mechanisms underlying these growth patterns. In northern populations of the Common European frog, *Rana temporaria,* accelerated growth following low temperature is achieved via increased feeding rates and higher growth efficiencies (Orizaola et al., 2014). Larvae from northern populations have relatively longer guts than those from southern populations (Lindgren and Laurila, 2005), which do not exhibit compensatory growth (Orizaola et al., 2014). Longer guts provide greater gut capacity and may, therefore, allow larvae to increase food intake without a corresponding decrease in diet retention time which could sacrifice digestibility. Similar connections between gut capacity, hyperphagia, and compensatory growth have been found in Atlantic cod, *Gadus morhua* (Belanger et al., 2002).

The red-eyed treefrog, *Agalychnis callidryas*, provides an interesting opportunity to examine the relationship between gut capacity and compensatory growth. This Neotropical species experiences larval gut length plasticity with higher larval densities inducing longer guts (Bouchard et al., 2015). These differences in gut length carryover post-metamorphosis, and endow small juveniles from high larval densities with relatively

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*Correspondence to: Sarah S. Bouchard, Department of Biology and Earth Science, Otterbein University, 1 S. Grove St., Westerville, OH 43081. E-mail: sbouchard@otterbein.edu

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longer guts than large juveniles from low larval densities (Bouchard et al., 2015). Small juveniles appear hyperphagic relative to large juveniles and grow at a faster rate with higher food conversion efficiencies (Tarvin et al., 2015). Larval gut length plasticity in *A. callidryas* may also play an important role in facilitating growth rates pre-metamorphosis and could enable a compensatory response.

The purpose of this study was to determine if changes in *A. callidryas* larval gut length are associated with differences in diet processing time, and if these differences allow larvae to exhibit compensatory growth. We reared larvae at low, medium, and high densities to induce changes in gut length and then measured the time it took to pass a diet marker. We also switched larvae from high to low density environments (reducing competition) and measured intake, growth, and time to and size at metamorphosis. We predicted that larvae with longer guts would have longer diet transit times and that upon transfer from high to low densities, they would exhibit hyperphagia and grow at an accelerated rate, maximizing size at metamorphosis.

MATERIALS AND METHODS

This study is comprised of three experiments conducted during the 2010, 2011, and 2013 *A. callidryas* breeding seasons at the Smithsonian Tropical Research Institute (STRI) in Gamboa, Panama. The research was conducted under the Boston University IACUC protocol 08-011 and STRI IACUC protocols 100625-1008-15 and 2011-0616-2014-04, with permits from the Autoridad Nacional del Ambiente de Panama (SC/A-16-10, SC/A-13-11, and SC/A-11-13).

For all experiments, we collected eggs from the Experimental Pond at the field station and maintained them in the laboratory until hatching. On the day of hatching, 6 days after oviposition, we transferred hatchlings to 400-L mesocosms filled with a mixture of aged tap water and rainwater, and located at the edge of the rainforest. We used 21, 24, and 18 clutches in 2010, 2011, and 2013, respectively (mean clutch size = 40 eggs). All clutches within a year were laid on the same night and hatched on the same day. For each year, we combined larvae from all clutches and haphazardly selected the number required for each mescososm. In 2010, we reared larvae at 5, 25, and 50 individuals per mesocosm, with each density replicated five times. In 2011 and 2013, we reared them at 5, 25, and 45 individuals per mesocosm; the low density was replicated ten times, and the medium and high densities were replicated five times. Larval densities in Gamboa area ponds range from 2.9 to 90 larva $\cdot$ m^{-3} (Touchon and Vonesh, in press); our low and medium densities fall within this range (12.5 and 62.5 larvae $\cdot$ m^{-3}, respectively), and the high density treatment falls just above it (112.5–125.0 larvae $\cdot$ m^{-3}). Each mesocosm contained approximately 200 g of local leaf litter placed in a mesh bag. We covered mesocosms with a secured screen to prevent colonization by other organisms and predation on larvae. We supplied each mesocosm with a resource supplement of 1.5 g Sera Micron algae every 5 days.

Diet Transit Time (2010 and 2013)

We raised larvae at low, medium and high densities until they reached approximately 40 mm total length. Because larval growth rate is density-dependent, individuals from different treatments reached this size at different ages. Using individuals of similar sizes, rather than equal ages, simplified analyses considerably because differences in response variables did not have to be adjusted for size.

In 2010, we individually photographed 20 larvae from each density in dorsal view to measure head-body and tail lengths, using NIH ImageJ software (Rasband, 2012). We selected 10 of those larvae (2 individuals from each of 5 mesocosms) for transit time measurements. The afternoon before we measured transit times, we transferred larvae into individual 350 ml containers with 75 mg Sera Micron $\cdot$ L^{-1} and allowed them to feed ad libitum overnight. The following morning, we placed larvae in a 0.01% suspension of charcoal powder in aged tap water and allowed them to feed for 1 hr (Warkentin, '92). Each individual was monitored for buccal pumping and the collection of charcoal particles around their mouth to ensure that they consumed the charcoal. After 1 hr, we returned them to their original, individual container where they were maintained in a solution of 75 mg Sera Micron $\cdot$ L^{-1}. We checked feces every half hour for the presence of charcoal. Diet transit time was the time elapsed between ingestion of charcoal and its presence in the feces. Transit times were measured twice for each individual on subsequent days and an average of the two measurements for each individual was taken.

We also measured transit times in 2013 following the same methodology (2–3 larvae from each mesocosm with larvae selected from 5 of the 10 low density mesocosms, Fig. 1). The only change was to add 25 mg Sera Micron after 4 and 8 hr to ensure measurements were not limited by food availability. Because of consistent results in 2010, we measured transit time once per individual. We could only size-match larvae from the medium and high densities because the low density larvae grew more quickly than expected. Comparisons were not made with low-density larvae.

Individual Larval Growth (2011)

We reared larvae at low, medium, and high densities to approximately 45 mm total length and 0.7 g body mass. Using larvae of the same size rather than age, assured that any differences in growth rate could be attributed to difference between treatment rather than size-dependent growth (Nicieza and Alvarez, 2009). Upon reaching the required size, we moved 15 larvae from each density (three from each replicate, using half of the low density mesocosms) to individual housing in 1-L tanks floated within 400-L mesocosms (9–10 1-L tanks per mesocosm). By floating individual tanks in outdoor mesocosms, larvae

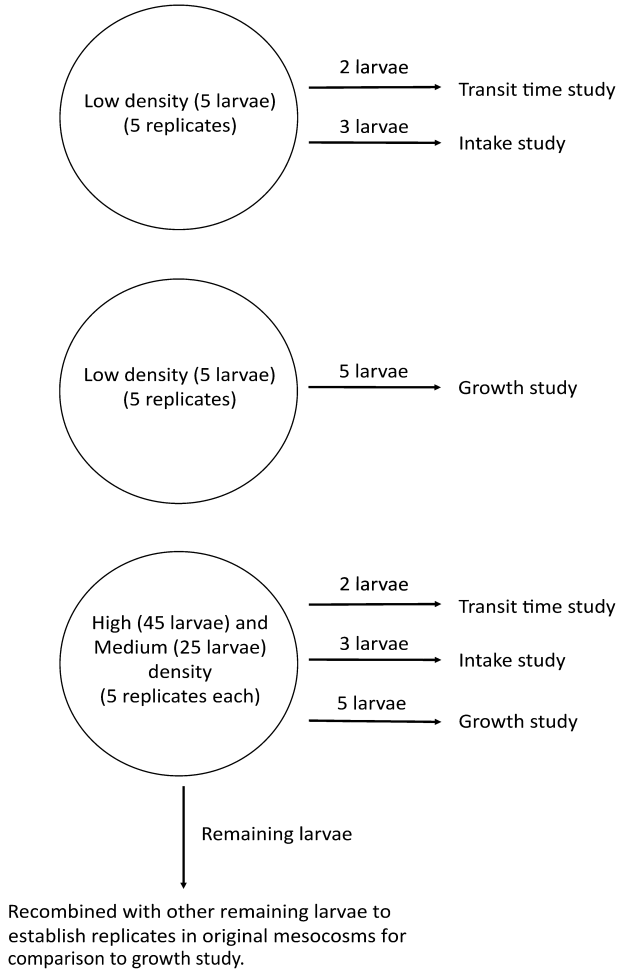

Figure 1. Diagram describing experimental design of 2013 field work. Arrows indicate how larvae were sampled from replicated mesocosms.

experienced more natural lighting and temperature conditions than in the laboratory, and we could monitor individual growth rates. We maintained larvae in these tanks for 1 week and added 150 mg Sera Micron to each tank each day, ensuring ad libitum access to food. We determined growth rate by measuring changes in length and body mass. We also determined developmental stage pre- and post-transfer according to Gosner ('60).

We redistributed the remaining larvae within each treatment to maintain the original densities in the original mesocosms with four replicates. We allowed them to feed and grow for 1 week. We compared the linear growth rates of larvae growing individually and at original densities. For larvae growing individually, we found a mean growth rate for larvae from the same mesocosm. These were compared to the growth rates for the original mesocosms.

Larval Growth, Development, Intake and Gut Size (2013)

We reared larvae at different densities until they reached approximately 40 mm total length and 0.6 g body mass. At this point, we removed five individuals from each mesocosm, using half of the low-density replicates (Fig. 1). Keeping larvae with their tank mates, we transferred larvae to new mesocosms set up exactly as the originals. This transfer moved larvae from competitively variable environments (low, medium, and high density) to competitively equal environments (low density). To determine growth rates, we group photographed larvae from the same mesocosms and individually weighed, and staged (Gosner '60) them on the day of transfer and 1 week later. We maintained them in these mesocosms until they emerged from the water as metamorphs, at which point, we weighed them and measured their snout-vent lengths.

We removed 15 additional larvae from each density (three from each mesocosm, using half of the low density mesocosms) from each of the remaining original mesocosms. Keeping tank mates together in trios, we photographed them and placed them in new mesocosms containing 3 g of algal Sera Micron. The next morning, we moved each trio to a 40 L tank containing a 0.01% suspension of powdered charcoal. Larvae fed on the charcoal suspension for 1 hr, at which point, we returned them to the mesocosms where they spent the previous night. They fed on algae in these tanks for 3 hr when they were euthanized with tricaine methanesulfonate, MS222, and stored in 10% formalin. This process placed a charcoal marker in their gut that was flanked on both sides with algal Sera Micron. Similar to the first growth experiment, the remaining larvae were returned to the original mesocosms. We redistributed individuals to maintain three replicates of the medium density and four of the high density (with 43 individuals each). There were no comparison mesocosms for the low density because of insufficient number of animals.

We weighed and dissected each preserved larva, removing and uncoiling their guts so that we could photograph them from above. Using ImageJ, we determined gut size by analyzing the area of each gut section (manicotto, small intestine, and large intestine). This measure accounts for both differences in gut length and width, but does not refer to the internal surface area of the gut. To determine intake, we measured the area of the charcoal marker within the gut, as well as the area of algae anterior to the marker. We were not able to find charcoal in one low-density, one medium-density, and two high-density guts, so these individuals were omitted from intake analyses. They were included in the analyses of gut size.

Statistical Analyses

All statistical tests were conducted using Statistical Package for the Social Sciences (SPSS) Version 19. Unless otherwise stated, we used mixed effects models in which density was the fixed effect and mesocosm was the random effect. This allowed us to test for treatment effects while controlling for the non-independence of

multiple individuals from a single mesocosm. We report corrected degrees of freedom that account for the random effects. The only exception was when comparisons were made on mesocosm means rather than on individuals within mesocosms (see 2013 growth data below).

We assessed differences in larval body proportions, total body length, body mass, and transit time with an analysis of variance (ANOVA). In 2011, individual growth rates were compared with an ANOVA. We compared differences between growth rates in 1-L tanks and mesocosms with a generalized linear model. In 2013, we could not identify individuals because larvae were maintained in groups of 5. Therefore, growth rates were determined by the difference between mean total length and body mass for each mesocosm. Differences among densities were compared with an ANOVA (a mixed effects model was not used). We used a generalized linear model to compare growth rates between the original and new mesocosms. We also compared time to metamorphosis with a generalized linear model with a Poisson distribution, and size at metamorphosis with an ANOVA (mixed effects model).

Because of differences in body size, we compared total gut area with an ANCOVA using body mass as the covariate. We compared differences in intake among densities with an ANOVA. To determine if intake varied with gut length, we also compared intake using an ANCOVA with total gut area as the covariate. We used least significant difference post-hoc tests with all ANOVAs and ANCOVAs to assess differences among densities.

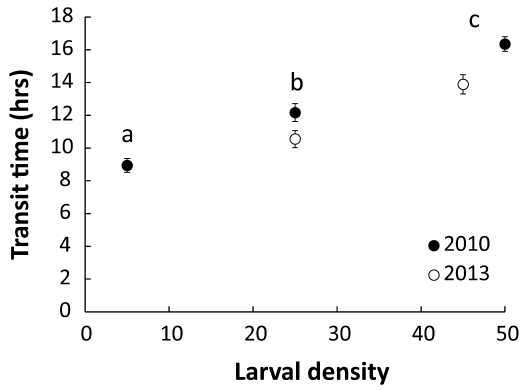

Figure 2. Transit time of a charcoal powder diet marker through the gut of *A. callidryas* larvae reared at low, medium and high densities. Different letters represent significant differences between densities. Differences between the medium and high densities were the same in both 2010 and 2013.

RESULTS

Transit Time

In 2010, the percent of total larval length that was head-body varied significantly with density ($F_{2,112} = 13.12$, $P = 0.001$). Low-density larvae had shorter head-bodies and longer tails ($33.0 \pm 0.3\%$ head-body) than medium and high density larvae which did not differ from each other ($34.9 \pm 0.2\%$ head-body; LSD post-hoc test: $P = 0.697$). Similar differences in body proportions were found for larvae used in 2011 and 2013. Larvae in which diet transit time was measured were size-matched in terms of total length ($F_{2,12} = 1.38$, $P = 0.290$). Diet transit time increased significantly with each larval density, such that high-density larvae took nearly twice as long as low density larvae to pass the marker (16.4 vs. 8.9 h; $F_{2,12} = 44.81$, $P < 0.001$, Fig. 2). Transit time was reassessed in 2013 for medium- and high-density larvae that also did not differ in total length (4.21 ± 0.03 cm, $F_{1,7.0} = 0.70$, $P = 0.430$) or mass (0.64 ± 0.01 g, $F_{1,7.0} = 0.83$, $P = 0.392$). High-density larvae again took significantly longer to pass the marker than medium-density larvae (10.5 vs. 13.9 h, $F_{1,7.0} = 28.42$, $P = 0.001$).

Individual Larval Growth (2011)

Larvae in which individual growth rates were assessed were size-matched for total length ($F_{2,12.4} = 0.24$, $P = 0.794$) and mass ($F_{2,12.3} = 0.21$, $P = 0.816$). They were also matched in developmental stage (Gosner stage: 30.8 ± 0.06, $F_{2,12.6} = 2.36$, $P = 0.134$). Medium-density larvae grew faster than low- and high-density larvae in length ($F_{2,12.3} = 4.71$, $P = 0.030$) and mass ($F_{2,12.5} = 21.52$, $P < 0.001$, Fig. 3). Low- and high-density larvae added the same amount length, but low density larvae gained significantly more mass. High-density larvae were at a slightly lower developmental stage than low- and medium-density larvae (Gosner stage: 34.2 ± 0.25 vs. 34.9 ± 0.17, $F_{2,12.8} = 4.07$, $P = 0.043$). There were also significant differences between larvae that were transferred to the individual growth tanks and those that were maintained at the original densities in the original mesocosms (Fig. 3). The transferred medium-density larvae grew at the same rate as those maintained in mesocosms ($\chi^2 = 0.62$, d.f. = 1, $P = 0.431$). The transferred low-density larvae grew more slowly than those in mesocosms ($\chi^2 = 100.85$, d.f. = 1, $P < 0.001$), whereas transferred high-density larvae grew more quickly than those in mesocosms ($\chi^2 = 9.85$, d.f. = 1, $P = 0.002$).

Larval Growth, Development, Intake and Gut Length (2013)

Larvae initially reared at low, medium, and high densities grew at significantly different rates ($F_{2,12} = 37.92$, $P < 0.001$), and were transferred to competitively equal environments on days 12, 14, and 20, respectively (Fig. 4). Those used in the growth portion of the study did not vary in size at the time of transfer (Length: $F_{2,12} = 0.43$, $P = 0.663$; Mass: $F_{2,12} = 1.05$, $P = 0.381$), but those from the high-density were at a more advanced developmental stage (Gosner stage: 34.2 ± 0.0 vs 33.0 ± 0.2, $F_{2,12} = 24.96$, $P < 0.001$). Once transferred, all larvae grew the same over 1 week (Length: $1.2 \pm 0.04\ \text{cm} \cdot \text{week}^{-1}$; $F_{2,12} = 0.07$, $P = 0.933$; Mass: $0.57 \pm 0.02\ \text{g} \cdot \text{week}^{-1}$, $F_{2,12} = 1.22$, $P = 0.329$), and the

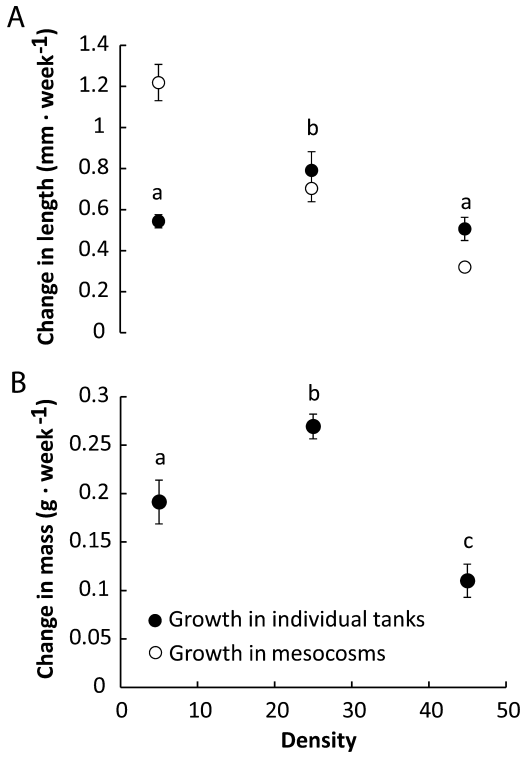

Figure 3. One week growth rates (length and mass) of *A. callidryas* larvae initially reared at low, medium, and high densities and then transferred to individual 1-L tanks (closed circles). Different lower case letters indicate significant differences between densities for transferred larvae. Open circles indicate growth rates of larvae maintained in mesocosms at the original low, medium, and high densities. Growth rates in each of these original densities are significantly different from each other.

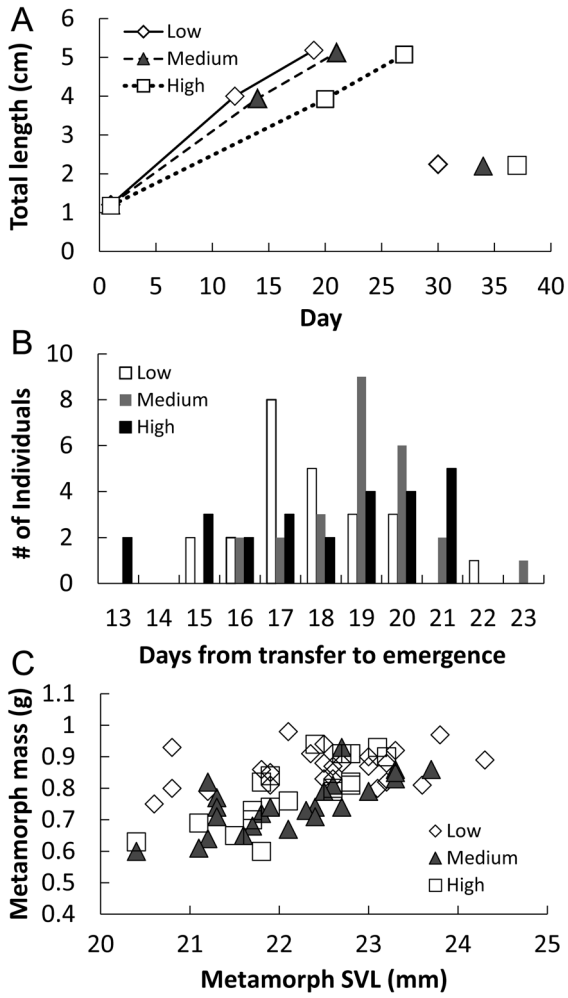

Figure 4. A: Growth rates of *A. callidryas* larvae initially reared at low, medium, and high densities and then transferred to competitively equal, low densities in mesocosms. The second data point in the series represents point of transfer, and the third point is the size 1 week post-transfer. The point disconnected from the line represents metamorph snout vent length. Values are means ± SE, although error bars are too small to see. B: Frequency distribution for the timing of metamorph emergence from the mesocosms. C: Metamorph mass as a function of snout-vent length.

transferred medium- and high- density larvae grew significantly faster than larvae maintained simultaneously in the original mesocosms (Medium: mean growth = 0.41 ± 0.04 cm · week^{-1}, $\chi^2 = 90.19$, d.f. = 1, $P < 0.001$; High: mean growth = 0.29 ± 0.02 cm · week^{-1}, $\chi^2 = 77.28$, d.f. = 1, $P < 0.001$). Transferred larvae were also at different developmental stages after 1 week, with low-density larvae less advanced than medium- and high-density larvae (Gosner stage 37.4 ± 0.2 vs. 39.2 ± 0.2, $F_{2,12.0} = 5.63$, $P = 0.02$). However, metamorphs emerged from the new mesocosms in the same number of days post transfer ($\chi^2 = 1.20$, d.f. = 2, $P = 0.549$; Fig. 4B). Metamorphs did not differ in SVL ($F_{2,12.3} = 1.35$, $P = 0.295$), but those originally reared at medium and high densities were smaller in mass than those originally reared at low density ($F_{2,12.1} = 5.971$, $P = 0.016$; Fig. 4C).

Larvae used to measure intake and gut length were size matched for total length ($F_{2,12} = 2.82$, $P = 0.099$) and head-body length ($F_{2,12} = 0.96$, $P = 0.412$). However, they were not size matched for mass ($F_{2,12} = 10.016$, $P = 0.003$). High-density larvae (0.56 ± 0.02 g) weighed less than medium-density larvae (0.62 ± 0.02 g) which weighed less than low-density larvae (0.67 ± 0.02 g). Although mean differences in mass were significant, there was substantial overlap in mass among densities. Differences became non-significant with removal of the four heaviest low density

individuals and the four lightest high density individuals ($F_{2,34}$= 2.78, P = 0.076). We analyzed our data with and without these eight individuals and found the same results. Here, we present the results of analyses using the complete data set.

Total gut size increased significantly with each increase in density (Density: $F_{2,14.4}$ = 44.97, P < 0.001; Covariate: $F_{1,29}$ = 75.04, P < 0.001; Fig. 5). The differences were due to changes in all parts of the gut (Manicotto: $F_{2,19.4}$ = 14.00, P < 0.001; Small intestine: $F_{2,14.1}$ = 33.30, P < 0.001; Large intestine: $F_{2,17.7}$ = 24.59, P < 0.001; Covariate was significant in each analysis, P < 0.001). Charcoal was found in the guts of 14 larvae from the low and medium densities and 13 larvae from the high density. High-density larvae consumed significantly less of the marker than medium- and low-density larvae ($F_{2,13.1}$ = 25.86, P < 0.001). Algae intake decreased significantly with each density ($F_{2,13.1}$ = 7.170, P = 0.008). Low density larvae ate 47% and 127% more than medium- and high-density larvae, respectively. Medium-density larvae ate 54% more than high-density larvae (Fig. 6).

Larger guts were not associated with higher intake rates. For a given gut area, algal intake decreased significantly with each increase in density (Density: $F_{2,20.7}$ = 13.840, P < 0.001; Covariate: $F_{1,25}$ = 4.47, P = 0.045 Fig. 6). Although guts from high-density larvae were 26% larger than those from low-density larvae, they consumed 72% less algae (adjusted consumption means: Low: 0.83 ± 0.07, Medium: 0.52 ± 0.06, High: 0.23 ± 0.07 cm^2).

DISCUSSION

Gut plasticity is an adaptive response that can help maximize energy and nutrient gains in variable environments. When food resources are low, gut capacity can increase such that mean diet retention time lengthens and digestive efficiency improves. Increases in gut capacity can also accommodate elevated intake rates when nutritional needs are high (Starck, 2003; Naya, 2008; Karasov and Douglas, 2013; McWilliams and Karasov, 2014). We hypothesized that such plastic responses would facilitate compensatory growth in *A. callidryas* larvae when more resources became available after a period of food restriction. However, gut plasticity, intake and growth interacted in unexpected ways that did not support our hypothesis. Rather than exhibit a compensatory response, larvae initially reared at medium and high densities employed an alternative digestive strategy that promoted growth, but reduced foraging costs.

Consistent with a previous study, high and medium larval densities decreased larval growth and developed greater gut capacity (Bouchard et al., 2015). We predicted that the larger guts of medium- and high-density larvae would provide a growth

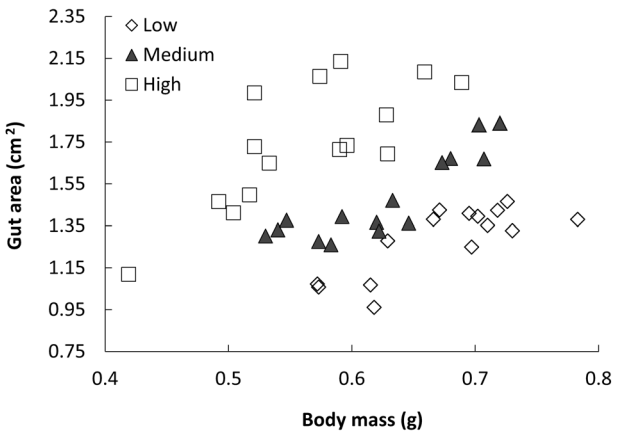

Figure 5. Projected gut area measured from photos as a function of body mass for *A. callidryas* larvae reared at low, medium, and high densities. Larvae were sampled at approximately 40 mm total length.

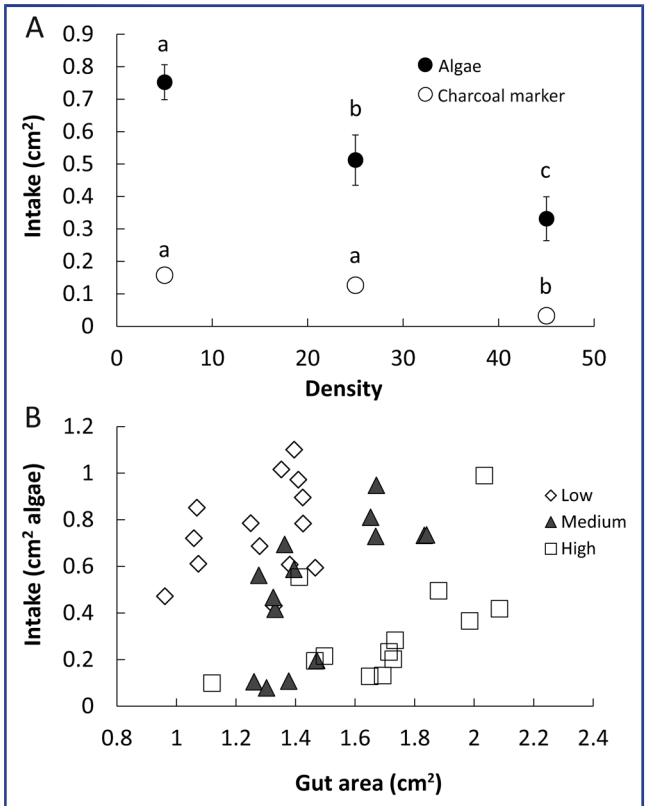

Figure 6. Intake of *A. callidryas* larvae reared at low, medium, and high densities, and then released from competition and fed ad libitum. **A**: Intake of a powdered charcoal marker (open circles) and algal food (closed circles). Values are means ± SE, and different letters represent significant differences in consumption. **B**: Algal intake as a function of gut size. Although gut length varied significantly with density, larval body size was approximately the same.

advantage once larvae were transferred to a high resource environment. However, upon transfer, larvae did not exhibit the hyperphagic response expressed by many animals (Ali et al., 2003). Instead, medium- and high-density larvae continued to feed at low rates that were far below what their enlarged guts could accommodate. These rates were also significantly lower than those of low-density larvae. Because we were only able to measure the two dimensional area of consumed algae, rather than its full volume, it is possible that we underestimated intake differences between treatments. Even with this possibility, the differences are striking and illustrate that high-density larvae fed at a remarkably low level.

The absence of a hyperphagic feeding response could be attributed to a combination of physiological and ecological factors. For example, some animals exhibit metabolic depression as an energy-saving response to food restriction (Brzek and Konarzewski, 2001; Ali et al., 2003; Moe et al., 2004; Burton et al., 2011). *Agalychnis callidryas* larvae from medium and high densities have significantly smaller livers than those from low density (Bouchard et al., 2015). In estivating and hibernating anurans, small livers are indicative of lower metabolic rates (Kayes et al., 2009; Naya et al., 2009). If small *A. callidryas* livers are also associated with lower metabolic rates, medium- and high-density larvae may be physiologically set to consume food at a lower rate. Additionally, the lack of a hyperphagic response could reflect foraging costs. Increased activity required for foraging can significantly increase predation risk (Skelly, '94; Anholt and Werner, '95; Laurila et al., 2008; Touchon et al., 2013a). This may be particularly pronounced in anuran larvae exhibiting gut plasticity because increases in gut size are associated with decreases in tail size (Relyea and Auld, 2004) and a reduction in the ability to evade predators (Van Buskirk et al., '97; Van Buskirk and Relyea, '98).

Despite large differences in feeding rates, all larvae grew at the same rate when transferred to high resource mesocosms (2013 growth study). The combination of low intake and longer guts extended diet transit times in 2010 and 2013 and presumably increased diet digestibility for medium- and high-density larvae. Low-density larvae achieved the same growth rate by eating more food and passing it more quickly through a shorter gut. These different feeding strategies also allowed all larvae to metamorphose in the same number of days post transfer. However, metamorphs initially reared at medium and high larval densities weighed slightly less than those initially reared at low density, despite obtaining the same body length. All larvae were transferred at the same body mass and length, but medium- and high-density larvae had longer guts and smaller fat bodies and livers (Bouchard et al., 2015). Therefore, upon transfer, medium- and high-density larvae had lower lipid reserves than low-density larvae. Because all larvae gained length and mass at the same rate post-transfer, differences in lipid reserves could have been maintained throughout the growth period, and become apparent as a mass difference once metamorphosis was complete.

Although larvae reared at medium- and high-densities did not exhibit compensatory growth, they were able to significantly alter their growth trajectories once more resources became available. Larval density has a strong effect on juvenile body size, and larvae that are continuously reared at high density metamorphose into juveniles that are one third the mass of those reared at low density (Bouchard et al., 2015). High-density larvae in the 2013 growth trial were almost able to eliminate this size difference upon transfer to high resource conditions through a combination of low intake and long guts. The growth period between transfer and metamorphosis was not different among densities; however, comparisons were run asynchronously to eliminate the effects of initial body size on growth (Nicieza and Alvarez, 2009), and the total larval period was longer for those from the higher densities. Although we do not know what would happen if larvae were transferred at the same age rather than size, it seems likely that *A. callidryas* larvae respond to a period of food restriction by extending the larval period (Touchon et al., 2013b). This strategy allows larvae to increase size at metamorphosis without incurring the immediate and long-term costs of compensatory growth (Metcalfe and Monaghan, 2001; Criscuolo et al., 2008; Lee et al., 2012, 2013). In *Rana temporaria*, populations with strong seasonal time constraints on larval growth and development exhibit compensatory growth, whereas less constrained populations do not (Orizaola et al., 2014). As a prolonged breeder in a tropical environment, *A. callidryas* do not experience the same seasonal time constraints as temperate species and may experience less pressure to maximize growth. Tropical species, in general, may be less likely to exhibit compensatory growth than temperate species, unless they grow and develop in ephemeral habitats (Metcalfe et al., 2002).

Interesting insights in larval growth patterns were also revealed in the individual growth rate study (2011) in which larvae were reared at low, medium and high densities and then transferred to individual 1-L tanks. Larvae transferred from the medium density grew faster than those transferred from the low density. However, this was not compensatory growth because transferred medium-density larvae grew at the same rate as larvae simultaneously maintained at medium density in the original mesocosms. Additionally, transferred low-density larvae grew much more slowly than their original mesocosm counterparts. This drop in growth upon transfer for low-density larvae, despite ad libitum food availability, suggests that conditions in the individual 1-L tanks were stressful compared to those in the 400-L mesocosms. Because transferred medium-density larvae still maintained the same level of growth as in the mesocosms, they appeared better equipped to tolerate the poorer conditions than the low-density larvae.

Although individual tanks were a poor environment for low-density larvae, they provided better conditions for high-density

larvae, which were highly food restricted. Transferred high-density larvae grew at a faster rate than those maintained in the original mesocosms. However, they still grew more slowly than those transferred from the medium density. Additionally, despite having the same linear growth as transferred low-density larvae, they did not gain as much mass. This suggests an allocation of resources to structural growth rather than to lipid reserves (Nicieza and Alvarez, 2009). Many animals exhibit the opposite growth pattern after a period of food restriction, favoring the deposition of lipids over structural growth (Jobling and Johansen, '99; Johansen et al., 2003; Nicieza and Alvarez, 2009). However, larvae may metamorphose early if the post-metamorphic environment allows for better growth and survival than the larval environment (Werner, '86). Lipid reserves may not be a critical constraint on the timing of metamorphosis (Beck and Congdon, 2003) therefore, favoring gains in length over mass may be advantageous if it allows larvae to meet the minimum size required for metamorphosis more quickly.

In summary, our data indicate that while compensatory growth may be widespread across animal taxa, there may be significant variation in its expression based on environmental context. Larger guts induced by low resource environments have the potential to facilitate a compensatory response, but may not do so in cases where the life cycle has fewer temporal constraints. Tropical species, in particular, may be less likely to exhibit compensatory growth than temperate species. In *A. callidryas*, gut plasticity better equips larvae to grow and survive under low resource conditions. However, larger guts do not facilitate compensatory growth; instead, larvae with different gut sizes employ dramatically different feeding strategies that produce the same overall growth response. Interestingly, small juvenile *A. callidryas* from high larval densities experience compensatory growth post-metamorphosis (Tarvin et al., 2015). This further suggests that for animals with complex life cycles, the costs and benefits of compensatory growth may vary significantly even with life stage.

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