The effects of preformed vitamin A and provitamin A carotenoid supplementation on tadpoles of the poison frog *Phyllobates vittatus*

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Data Availability

The data and code used for analyses are available on a Figshare repository accessible at https://doi.org/10.6084/m9.figshare.22149266.v1.

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Conflict of Interest

The Authors declare no conflict of interest.

Ethics Approval

All animal care and use procedures were approved by the University of Michigan's Institutional Animal Care and Use Committee (protocol # PRO00010325).

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Abstract

Understanding the nutritional requirements of captive animals is necessary for proper animal husbandry, however, the specific dietary requirements for many amphibian species commonly kept in captivity are unknown. Like most vertebrates, frogs cannot synthesize carotenoids and must therefore obtain these essential nutrients through diet. It is unclear if amphibians can cleave provitamin A carotenoids to form vitamin A metabolically within the body, so common practice is to supplement their captive diets with both preformed vitamin A and provitamin A carotenoids. We carried out a feeding experiment in tadpoles of *Phyllobates vittatus*, a commonly kept poison frog species, to test the effects of supplementing a fish flake diet with a provitamin A carotenoid (2.5mg/g β-carotene) and vitamin A (0.033-0.066µg/ml retinyl acetate), both individually and in combination. Contrary to our expectations, supplementation had either no effect or adverse effects on tadpole growth and survivorship. Tadpoles reared under supplemented diets with vitamin A showed higher mortality rates, coupled with symptoms of hypervitaminosis A. Survivors had a smaller body size and mass at metamorphosis. β-carotene supplementation alone had no detectable effect. The vitamin A and β-carotene levels in our supplemented diet have been shown to be harmless or benefit tadpoles of other species, yet our results indicate that adding these amounts to what is found in a generalist fish flake mix can have detrimental effects on *P. vittatus* tadpoles. More broadly, this study highlights the importance of creating husbandry guidelines based on the specific physiological needs of the species (or species groups) being kept in captivity, rather than general ones for all amphibians, as is often done.

Introduction

As wild populations of a considerable number of amphibian species decline (Campbell et al., 2020), captive populations of some of them are increasing. This is due to both their increasing popularity in the pet trade and captive breeding programs (Edmonds, 2021; Tapley et al., 2015). Proper husbandry is required for all captive animals to ensure that their physiological needs are met. An important aspect of proper husbandry is animal nutrition. However, the nutritional requirements of most amphibians are unknown (Tapley et al., 2015). Breeders of captive frogs often work under the assumption that a diet based on one or a few species of feeder insects, such as Drosophila or crickets, does not contain all of the nutrients that amphibians require and, thus, enhance diets with commercially available supplements. Despite the wide variety of products available for amphibian diet supplementation, there is very little research to inform their use in particular species.

Carotenoids can be powerful antioxidants that quench and stabilize reactive oxygen species produced during periods of growth and development (Goodwin, 1984; Keogh et al., 2018; McInerney et al. 2019). This limits the damage that reactive oxygen species cause to cells, which is believed to result in increased cell division and differentiation (Keogh et al., 2018; McInerney et al. 2019). In amphibians, antioxidants are especially important during the larval stage and metamorphic climax because this is when the production of reactive oxygen species is greatest (Keogh et al., 2018; McInerney et al. 2019). Furthermore, carotenoids and their metabolism are involved in coloration in a variety of species across the tree of life (Goodwin, 1984; Toews et al. 2017). Since frogs, like most animals, are unable to produce carotenoids, they must be obtained exclusively through their diet (Densmore and Green 2007; Keogh et al., 2018; McInerney et al. 2019). It is therefore common practice amongst frog breeders to add carotenoid supplements to the captive diets of most species. However, breeders must be careful not to over supplement with carotenoids as they can have negative effects when given in excess (Keogh et al., 2018; Palozza et al. 2003). For instance, one of the most commonly used carotenoids, β-carotene, can be toxic at high concentrations and has been found to have a pro-oxidant effect that can cause a reduced growth rate (Keogh et al., 2018; Palozza et al. 2003).

For many animals, carotenoids also act as vitamin A precursors (Brenes-Soto and Dierenfeld, 2014; Clugston and Blaner, 2014). This means that provitamin A carotenoids can be cleaved to form vitamin A metabolically within the body. Whether this is the case across amphibians remains unclear. The few studies that have addressed the problem *in vivo* have not found evidence for conversion of specific carotenoids (i.e. β-carotene) to vitamin A (McComb 2010; Collins et al., 1953; Wright, 2006), although there is evidence that at least some species may be able to convert β-carotene and some xanthophylls (e.g. lutein) to retinoids (Baruah and Goswami 2012, Brenes−Soto and Dierenfeld 2014). Vitamin A plays important roles in gene expression, vision, limb regeneration and embryonic development (McInerney et al. 2019; Clugston and Blaner 2014), and in anurans its deficiency has been linked to squamous metaplasia of the mouth, which is commonly referred to as “short tongue syndrome” (Clugston and Blaner 2014). Frogs affected by short tongue syndrome have difficulty catching prey, resulting in lethargy, weight loss, and eventually death if untreated (Clugston and Blaner 2014). To prevent hypovitaminosis A, a common practice amongst breeders today is to co-supplement with carotenoids and preformed vitamin A.

Nevertheless, since vitamin A is fat-soluble, excessive intake poses a risk for toxicity (Densmore and Green 2007). Hypervitaminosis A has been recorded in African clawed frogs (*Xenopus laevis*) at different stages of their life cycle (Clugston and Blaner 2014). This condition causes weight loss and negatively affects the skin and liver of adult frogs (Pessier 2013), and has been reported to cause abnormal development in tadpoles, with notable observations of hyperpigmented, hemorrhagic skin, and chronic diarrhea (Weissmann 1961; Weissman et al. 1963). In tadpoles of Duttaphrynus melanostictus defective mouth parts and edema were also

observed (Jangir et al. 1994). Proper dosages of preformed vitamin A have not been determined for most species, even if under or over-supplementation can lead to hypovitaminosis A or hypervitaminosis A, both of which can be life threatening if untreated (Clugston and Blaner 2014).

With the goal of improving husbandry practices of captive amphibians, this study investigated the effects of supplementing feed with β-carotene (a commonly used provitamin A carotenoid), vitamin A, and their combination on tadpoles of the poison-dart frog *Phyllobates vittatus*, a species commonly kept in captivity. Through observation and measurement of tadpoles during development, we evaluated the effect of the above supplementation regimes on tadpole survivorship, growth rates, final size at metamorphosis, and time to metamorphosis. Our results provide somewhat unexpected initial insights into the dietary carotenoid requirements of *Phyllobates* poison-dart frog tadpoles, and highlight the need for further species-specific research on the diets of captive amphibians.

Methods

Study Species

P. vittatus is a diurnal, terrestrial species that is endemic to the Golfo Dulce Region of Costa Rica, where it can be found in lowland moist and wet forests. Adults range in size from 22.5mm to 31mm (snout-to-vent length), with females being on average larger than the males. *P. vittatus* is black with a broad gold, red-orange, or orange dorsolateral stripe that runs continuously from the dorsal base of each thigh to the tip of the snout, and blue-green speckling on its limbs. Their diet is made up of a variety of small arthropods. Females will lay clutches of 7-21 eggs every two to three weeks. The male cares for the eggs by providing them with moisture, and once the eggs hatch, males will transport the tadpoles to a pool of water (Silverstone 1976), where they likely maintain an omnivorous diet (Grant et al. 2006). Although this has not been studied in *P. vittatus*, predatory behaviors, including tadpole cannibalism, have been observed in multiple closely related species (Caldwell and de Araujo 1998, Márquez 2023). Tadpoles are uniformly dark brown on the dorsal side, and are lighter brown ventrally, and gradually develop orange dorsolateral stripes, starting around Gosner stage 37 (Gosner 1960). Metamorphosis occurs approximately ten weeks after hatching (Savage 2002; Paitz and Dugas 2021; R. Márquez pers. obs.; R. Arkin pers. obs.).

Experimental Animals

153 A total of 35 *P. vittatus* eggs, from 6 different clutches, were collected from a single
154 captive colony of 7 adults (4M, 3F) maintained at the University of Michigan. Adults are fed
155 *Drosophila hydei* and *D. melanogaster* fruit flies three times a week, supplemented with
156 Repashy Calcium Plus, Rep-Cal Herptivite multivitamin, and Rep-Cal Calcium with Vitamin D
157 (one supplement per feeding). All eggs were laid between 1/20/22 and 2/11/22. They were
158 removed from the terrarium and reared in petri dishes until hatching. Thirteen eggs from 2
159 clutches had been de-jellied shortly after laying. We found no differences between these and
160 other eggs in any of the measured parameters (Table S1). All eggs hatched within the time frame
161 typical of our lab colony (mean 16.4 days, s.d. 2.65, range 14-23 days). One tadpole died within
162 2 days of hatching and before ever being fed, so it was removed from the study.

163 *Experimental diets*

164 All four diets were composed of of 99.7% ground fish flakes (75:25 mixture of
165 SeraFlora/SeraSans; SERA, Germany), which was previously analyzed for nutrient content
166 (Byrne and Silla, 2017), and contributes 0.01454 mg g^{-1} total carotenoids and 0.00188 mg g^{-1}
167 preformed vitamin A. The remaining 0.03% varied between four treatments as follows: Diet 1
168 (control) was unsupplemented. Diet 2 was supplemented with 2.5mg g^{-1} β-carotene (Sigma
169 Aldrich, Cat. No. C9750), Diet 3 was supplemented with 0.5 mg g^{-1} vitamin A acetate (i.e. retinyl
170 acetate; Sigma-Aldrich Cat. No. R4632), and Diet 4 was supplemented with 2.5mg g^{-1} β-carotene
171 and 0.5 mg g^{-1} vitamin A. Cellulose powder (Sigma Aldrich Cat. No. 435236), which is odorless
172 and has been found to have no impact on the growth, survivorship, or development of tadpoles
173 (Keogh et al., 2018; Dias et al., 1998; Deng et al., 2006), was added to treatments 1, 2, and 3 to
174 ensure that all experimental diets contained the same amount of feed. Table 1 shows the total
175 amounts of carotenoids and vitamin A in each diet treatment, and Table S2 summarizes the
176 recipes used in each diet. All diet mixes were suspended to a 1:10 ratio in deionized H_2O,
177 wrapped in aluminum foil and mixed on a rocker for 48 hr at room temperature. Once mixed,
178 diets were loaded onto 3ml syringes, wrapped in foil, and stored at 0 to -4°C until used.

179 β-carotene was selected as the provitamin A carotenoid due to its commercial availability
180 and presence in popular supplements. Retinyl acetate was chosen because it is the form of
181 vitamin A commonly found in commercial vitamin A supplements for both animals and humans
182 (e.g. Repashy vitamin A plus). Dosages of β-carotene and vitamin A were chosen based on
183 previous studies on anurans. Multiple studies have reported benefits or no effects from feeding
184 diets containing 0.1-20mg g-1 of β-carotene or other carotenoids (Byrne and Silla, 2017; Keogh),
185 which fall in the range of carotenoid contents found in freshwater microalgae (Soares et al.,
186 2019; Wang et al., 2022). Considering two studies found that doses of 10mg g-1 were excessive

in some species (Cothran et al., 2015; Keogh et al., 2018; McInerney et al., 2019; Ogilvy and Preziosi, 2011; Ogilvy et al., 2012; Umbers et al., 2016), we picked a dosage of 2.51mg g-1, on the lower end of this range. Vitamin A has mainly been investigated with regards to hypervitaminosis in anuran tadpoles. Therefore, studies use dosages higher than the optimal for normal development. Classic studies on Xenopus tadpoles found that dosages of 1.5-2.5µg per milliliter of water in the enclosure added twice per week produced symptoms of hypervitaminosis (Weissmann, 1961; Weissmann et al., 1963). Other studies using similar or higher dosages found negative effects on traits such as limb regeneration ability and mouthpart development in Xenopus and other amphibians (Scadding and Maden, 1986a; 1986b; Jangir et al. 1994). With this in mind, we selected a dose of 0.033-0.066µg ml-1, nearly two orders of magnitude lower. To achieve this dosage we added 500µg g-1 to our base diet, resulting in 501.88µg g-1 total Vitamin A. This was diluted 1:10 in water, of which 0.1-0.2ml were provided to each tadpole (see details below), for a total of 5.0188-10.0376µg per feeding. Tadpoles were housed in 150ml of water, resulting in 0.033-0.066µg ml-1, equivalent to 0.1IU ml-1 vitamin A.

Feeding experiment

After hatching, individuals were randomly assigned to one of four treatments, and housed in individual 230 ml plastic cups filled with approximately 150ml of reverse-osmosis (RO) water. They were fed triweekly: Monday, Wednesday, and Friday. Before each feeding, syringes were thawed and the feed was homogenized using a blunt tip needle. Although in some cases individual syringes were used for more than one feeding and refrozen in between feedings, we do not believe freeze-thaw cycles had a major effect on nutrient concentrations, since a syringe was used at most during two feeding sessions. Tadpoles received 2 drops of food (~0.1 ml) for the first month. After that time, individuals received 4 drops of feed (~0.2 ml). Tadpoles were photographed using a Canon EOS R6 camera with a Canon RF24-105mm F4 L IS USM lens after each feeding. The body (from tip of snout to base of tail) and tail length of each individual were measured using image analysis software (ImageJ, version 1.53; Schneider et al. 2012). Feeding and photographing stopped once tadpoles began the metamorphic climax at Gosner's stage 42, as evidenced by the emergence of forelimbs (Gosner, 1960). Once both arms emerged, tadpoles were moved to a tilted 1L cup with ~75ml of new RO water to facilitate leaving the water. Once out of the water, froglets were moved to plastic containers (10 cm x 20 cm x 10 cm) lined with sphagnum moss and dried magnolia leaves. Froglets were monitored every two days until they reached Gosner's stage 46, marked by the full absorption of their tail (Gosner, 1960). At that time, individuals were photographed (NIKON D5100 camera with an AF Micro-Nikkor 60mm f/2.8D lens) and their body length and weight were recorded. Two tadpoles had not yet developed arms after 81 days, so they were put into new 230 ml plastic cups and a partial water

change was performed. This was done to clear the algae buildup that made it difficult to observe and photograph the tadpole. We did not observe evidence that these tadpoles had consumed the algae (e.g. scrape marks), and although they were among the last to undergo metamorphosis, we do not believe the algae affected their growth trajectory in a significant way, since enclosures were replaced as soon as accumulation was visible. Our statistical analyses produced qualitatively identical results with or without these individuals (Table S3), so they were retained for the analyses presented in the main text.

Statistical analyses

To compare mortality over time between treatments, we computed survival curves using the Kaplan-Meier method (Kaplan & Meier 1958) and tested for differences between survival curves using the chi-squared approach described by Harrington & Fleming (1982). Survival curves were fitted using the survfit() function and comparisons were made using the survdiff() function, both implemented in the R package survival (Therneau 2022).

We then evaluated the effect of diet treatment on tadpole growth as a function of age by fitting logistic curves to each tadpole's growth trajectory and comparing the best-fitting growth rate and maximum size parameters between treatments. The logistic equation was used in the following form:

$$S_t = \frac{M}{1 + \left(\frac{M - m}{m}\right) e^{-rt}}$$

where S_t is the size at a given age, M is the maximum size asymptote, m is the minimum size asymptote, r is the growth rate, and t is the age. Growth curves were fit to each individual using non-linear least squares as implemented in the function nlsLM() of the minpack.lm R package (Elzhov et al. 2022). We tested for differences in maximum size and growth parameter estimates between treatments using bi-variate anovas with treatment and whether tadpoles survived to metamorphosis as fixed factors. To further explore differences between treatments and survivorship categories, we conducted post-hoc Tukey-Kramer tests. Finally, we tested for between-treatment differences in our empirical measurements of size, weight and age at metamorphosis also using anovas and Tukey-Kramer tests. The data and code used for all analyses and visualizations are available on the figshare repository associated with this paper.

Results

Effects of diet on survivorship

Overall, 82.35% (28/34) of tadpoles survived to complete metamorphosis. Tadpoles that were fed diets supplemented with vitamin A (i.e. diets 3 and 4) had higher mortality rates, especially the treatment group that was supplemented with both vitamin A and β-carotene (i.e. Diet 4), which showed a clear excess of observed deaths relative to the expectation under the Kaplan-Meier model ($\chi^2 = 9.67$, df = 3, p = 0.02; Fig 1A-B). Remarkably, tadpoles that died in the treatment groups supplemented with vitamin A (1 in treatment 3, 4 in treatment 4), were observed to have skin and gastrointestinal symptoms (persistent diarrhea and edema of the body), that reflect what was described in *X. laevis* and *Duttaphrynus melanostictus* tadpoles with hypervitaminosis A (Fig 1C; Jangir et al. 1994; Weissmann 1961; Weissman et al. 1963).

Effects of diet on growth

All of the tested tadpoles displayed growth trajectories characterized by initial rapid growth that gradually slows down towards metamorphosis (Fig. 2A, S1). We found significant effects of treatment, survivorship, and their interaction on both maximum body size (Fig. 2B; treatment: $F_{3,27} = 32.5$, p = 4.2e-9; survivorship: $F_{1,27} = 171.3$, p = 3.3e-13; treatment × survivorship: $F_{2,27} = 16.9$, p = 1.7e-5) and growth rate (Fig. 2C; treatment: $F_{3,27} = 15.9$, p = 3.7e-6; survivorship: $F_{1,27} = 67.4$, p = 8.2e-9; treatment × survivorship: $F_{2,27} = 12.4$, p = 1.5e-4). However, most of the signal in these tests is due to the tadpoles that did not survive to metamorphosis, most of which displayed faster initial growth spurts but stopped growing soon after, at considerably smaller sizes, where they remained for several days before dying (Fig. 2A-C; Fig. S1). The only significant post-hoc comparisons were either between non-surviving tadpoles of different diet treatments, or between surviving and non-surviving tadpoles, both between and within treatments (Table 2).

Among the tadpoles that survived to metamorphosis, diet treatment had moderate effects on body length ($F_{3,24}$= 3.41, p = 0.034) and body mass ($F_{3,24}$ = 2.68, p= 0.069) at metamorphosis, but not on age at metamorphosis ($F_{3,24}$ = 1.06, p = 0.38; Fig 2D-F). Individuals fed the control diet (Diet 1) were on average the largest and heaviest at metamorphosis, while those supplemented with just vitamin A (Diet 3) were smaller and weighed less than those in other treatments. Individuals supplemented with β-carotene (Diet 2) and β-carotene combined with vitamin A (Diet 4) were found to have intermediate sizes and weights (Fig 2D-E). However, the only statistically significant pairwise comparisons in both metamorphic size and weight were between the vitamin A and control treatments (Diet 3 vs. Diet 1; Table 3), possibly due to our moderate sample sizes.

Discussion

Our goal in this study was to examine the effects of supplementing the diets of *P. vittatus* tadpoles with additional carotenoids (β-carotene) and preformed vitamin A. This practice is commonly carried out by captive amphibian owners and caretakers, but the safety, benefits, and appropriate dosages of these supplements have only been studied in a limited number of species. The results of our feeding experiment suggest that, at least in the case of *P. vittatus*, supplementing with additional β-carotene and/or vitamin A than what is found in our base fish flake feed (5.466 μg/g β-carotene and 0.00188 mg/g preformed vitamin A, respectively) does not provide any benefits, and can have detrimental effects in terms of tadpole mortality, and probably also on pre-metamorphic growth.

The most severe negative effects were observed in the tadpoles fed Diet 4, which contained both supplemental β-carotene and vitamin A. This treatment resulted in a significantly lower survival rate than the other tested diets, and the affected individuals displayed clearly different growth trajectories, as well as symptoms reminiscent of descriptions of hypervitaminosis A in tadpoles of other frog species (Fig 1C; Jangir et al. 1994; Weissmann 1961; Weissman et al. 1963), suggesting that these tadpoles died due to a diet-associated disease, most likely hypervitaminosis A. The same was true for the single tadpole that died under Diet 3 (only vitamin A supplement). The fact that tadpoles supplemented with only vitamin A were much less likely to die or show symptoms than those supplemented with both β-carotene and vitamin A could be due to some of the β-carotene being converted endogenously to vitamin A. While it is commonly assumed that amphibians are not capable of cleaving provitamin A carotenoids to form vitamin A, there is evidence that suggests that at least some species can do so (Baruah and Goswami 2012; Brenes-Soto and Dierenfeld 2014). For instance, false tomato frogs (*Dyscophus guineti) fed a diet devoid of vitamin A but* supplemented with a mixture of carotenoids showed increased vitamin A plasma levels, indicating they are likely able to convert carotenoids to vitamin A (Brenes-Soto and Dierenfeld 2014). In vitro experiments on the β-carotene metabolism of Asian common toad (*Duttaphrynus melanostictus*) and Indian bullfrog (*Haplobatrachus tigerinus*) tadpoles also suggest that they are able to convert β-carotene to vitamin A (Baruah and Goswami 2012). However, it is unknown if this is the case for *P. vittatus* tadpoles, so further research is necessary to isolate the physiological causes of this result. In the end, if a species is able to convert carotenoids into vitamin A, then proper carotenoid supplementation could eliminate the need for additional preformed vitamin A supplements, highlighting the importance of species-specific research for husbandry practices.

We based the dosing of supplemented diets on previous work with other (distantly related) frog species in an attempt to avoid over-supplementation (see Methods section for citations and details). Yet, it seems clear that tadpoles in this study did experience adverse effects associated with over-supplementation. This highlights the importance of knowledge on the biology of focal species for adequate captive breeding. The lack of research on the nutritional requirements of most amphibian species kept in captivity (or their close relatives) is concerning, considering the increasing number of species kept in the pet trade, in zoos, and in *ex-situ* conservation efforts. Understanding the nutritional requirements of captive amphibians is especially important for endangered species since adequate husbandry is the keystone of captive breeding programs. Implementing the proper diet with proper levels of supplementation leads to larger, healthier, more genetically diverse populations for release, which is essential for reintroduction success (McInerney et al. 2019).

More generally, our findings highlight that the dietary requirements of frogs vary between species. This is not surprising considering the clade made up by anurans shared a common ancestor approximately 221.8 million years ago (Kumar et al. 2022). Despite their degree of evolutionary divergence, it is common for all frog species to be grouped as a single category in husbandry contexts, which leads to and this belief is reflected by assumed similarities in husbandry practices, which may be unwarranted. This frame of thinking is not usually applied to more "mainstream" species kept in captivity. For instance cats *(Felis catus*) and dogs (*Canis lupus familiaris*), for which clearly distinct husbandry practices are in place, diverged approximately 55 million years ago (Kumar et al. 2022). In contrast, dendrobatids and mantellids, two families of frogs commonly kept in captivity, diverged approximately 150 million years ago (Kumar et al. 2022), but are assumed to have very similar dietary and other husbandry needs. Considering the phylogenetic diversity and wide breadth of trophic ecologies of amphibians, and especially of tadpoles, there is a clear need for further research to establish adequate husbandry and veterinary guidelines and practices that reflect the specific needs of each species (or at least taxonomic group). This information can improve the success of ex-situ conservation efforts, as well as the health of amphibians kept as pets, in zoos, and in laboratories. Our study is a step in this direction for *Phyllobates* poison-dart frogs, and possibly other dendrobatid frogs kept in captivity.

Author Contributions

RA and RM conceived and designed the study. RA obtained the data. RA and RM analyzed the data. RA wrote the manuscript with contributions from RM. RM obtained funding. Both authors read and approved the manuscript.

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Tables

Table 1. Total carotenoid, β-carotene, and Vitamin A contents of the four tested diets. Baseline contents were obtained from Byrne and Silla's (2017) liquid chromatography analyses of the same commercially-available feeds used for the control diet (i.e. Diet 1).

	Total Carotenoids	**β-carotene**	**Vitamin A**
Treatment 1	0.01454 mg/g	0.005466 mg/g	0.00188 mg/g
Treatment 2	2.51454 mg/g	2.505466 mg/g	0.00188 mg/g
Treatment 3	0.01454 mg/g	0.005466 mg/g	0.50188 mg/g
Treatment 4	2.51454 mg/g	2.505466 mg/g	0.50188 mg/g

Table 2. Pairwise comparisons between diet treatments and survivorship categories in growth curve parameters obtained through a Tukey-Kramer post-hoc test. Maximum size and growth rate comparisons correspond to panels B and C of Figure 2, respectively.

Comparison	**Maximum size (mm)**				**Growth Rate**			
	Difference	**Lower 2.5%**	**Upper 97.5%**	**p-value**	**Difference**	**Lower 2.5%**	**Upper 97.5%**	**p-value**
D2 Surv. – D1 Surv	-0.03	-0.68	0.62	1.00	0.01	-0.04	0.06	1.00
D3 Surv. – D1 Surv	-0.37	-1.03	0.28	0.58	0.01	-0.04	0.06	1.00
D4 Surv. – D1 Surv	-0.12	-0.86	0.62	1.00	0.01	-0.05	0.06	1.00
D3 Surv. – D2 Surv	-0.34	-0.98	0.29	0.64	0.00	-0.05	0.04	1.00
D4 Surv. – D2 Surv	-0.09	-0.81	0.63	1.00	0.00	-0.06	0.05	1.00
D4 Surv. – D3 Surv	0.26	-0.46	0.98	0.93	0.00	-0.05	0.05	1.00
D3 Died – D1 Died	-1.84	-3.63	-0.05	**0.04**	0.05	-0.08	0.18	0.86
D4 Died – D1 Died	-2.89	-4.31	-1.48	**8.34e-6**	0.17	0.06	0.27	**3.20e-4**
D4 Died – D3 Died	-1.05	-2.47	0.36	0.26	0.11	0.01	0.21	**0.026**
D1 Surv. – D1 Died	0.65	-0.70	2.00	0.76	-0.02	-0.11	0.08	1.00
D2 Surv. – D1 Died	0.62	-0.72	1.96	0.79	-0.01	-0.10	0.09	1.00
D3 Surv. – D1 Died	0.28	-1.06	1.62	1.00	-0.01	-0.10	0.09	1.00
D4 Surv. – D1 Died	0.54	-0.85	1.92	0.90	-0.01	-0.11	0.09	1.00
D1 Surv. – D3 Died	2.49	1.14	3.85	**4.56e-5**	-0.07	-0.17	0.03	0.31
D2 Surv. – D3 Died	2.46	1.12	3.81	**4.87e-5**	-0.06	-0.16	0.04	0.49
D3 Surv. – D3 Died	2.12	0.78	3.46	**4.36e-4**	-0.06	-0.16	0.04	0.45
D4 Surv. – D3 Died	2.38	0.99	3.76	**1.37e-4**	-0.06	-0.17	0.04	0.44
D1 Surv. – D4 Died	3.55	2.75	4.34	**5.89e-13**	-0.18	-0.24	-0.12	**1.89e-9**
D2 Surv. – D4 Died	3.52	2.74	4.29	**4.12e-13**	-0.17	-0.23	-0.12	**3.80e-9**
D3 Surv. – D4 Died	3.17	2.40	3.95	**4.86e-12**	-0.17	-0.23	-0.12	**2.99e-9**
D4 Surv. – D4 Died	3.43	2.58	4.28	**6.64e-12**	-0.18	-0.24	-0.11	**1.52e-8**

Table 3. Pairwise comparisons between diet treatments in size and weight of surviving tadpoles at metamorphosis. Weight and length comparisons correspond to panels E and F of Figure 2, respectively.

	Weight at Metamorphosis (g)				**Body Length at Metamorphosis (mm)**			
	Difference	**Lower**	**Upper**	**p-value**	**Difference**	**Lower**	**Upper**	**p-value**

		2.5%	97.5%			2.5%	97.5%	
D2 – D1	0.00	-0.02	0.01	0.82	-0.10	-0.53	0.33	0.93
D3 – D1	-0.02	-0.03	0.0001	**0.052**	-0.45	-0.88	-0.02	**0.038**
D4 – D1	-0.01	-0.03	0.01	0.55	-0.32	-0.80	0.17	0.31
D3 – D2	-0.01	-0.03	0.005	0.24	-0.35	-0.77	0.06	0.11
D4 – D2	-0.004	-0.02	0.01	0.94	-0.22	-0.69	0.26	0.59
D4 – D3	0.01	-0.01	0.02	0.68	0.14	-0.34	0.61	0.86

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Figures

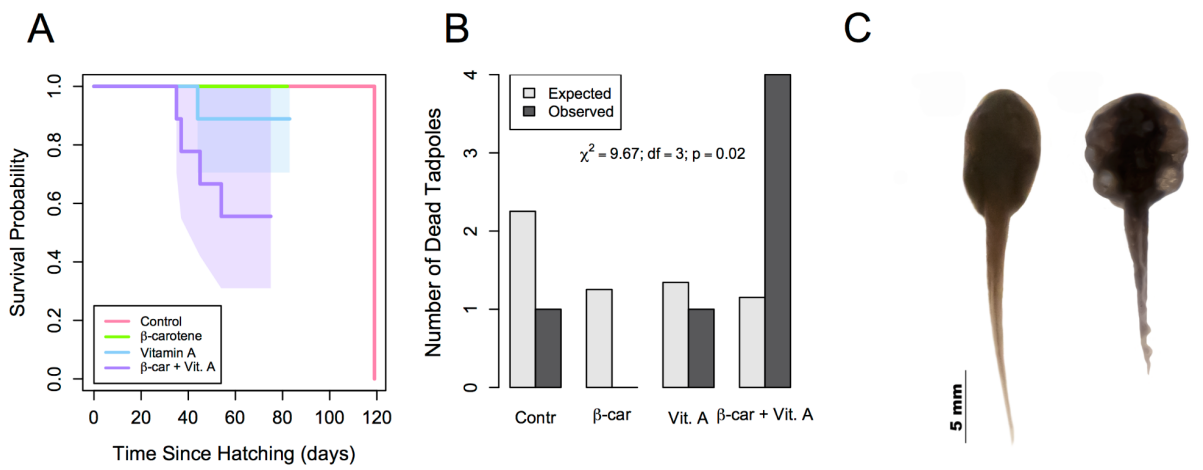
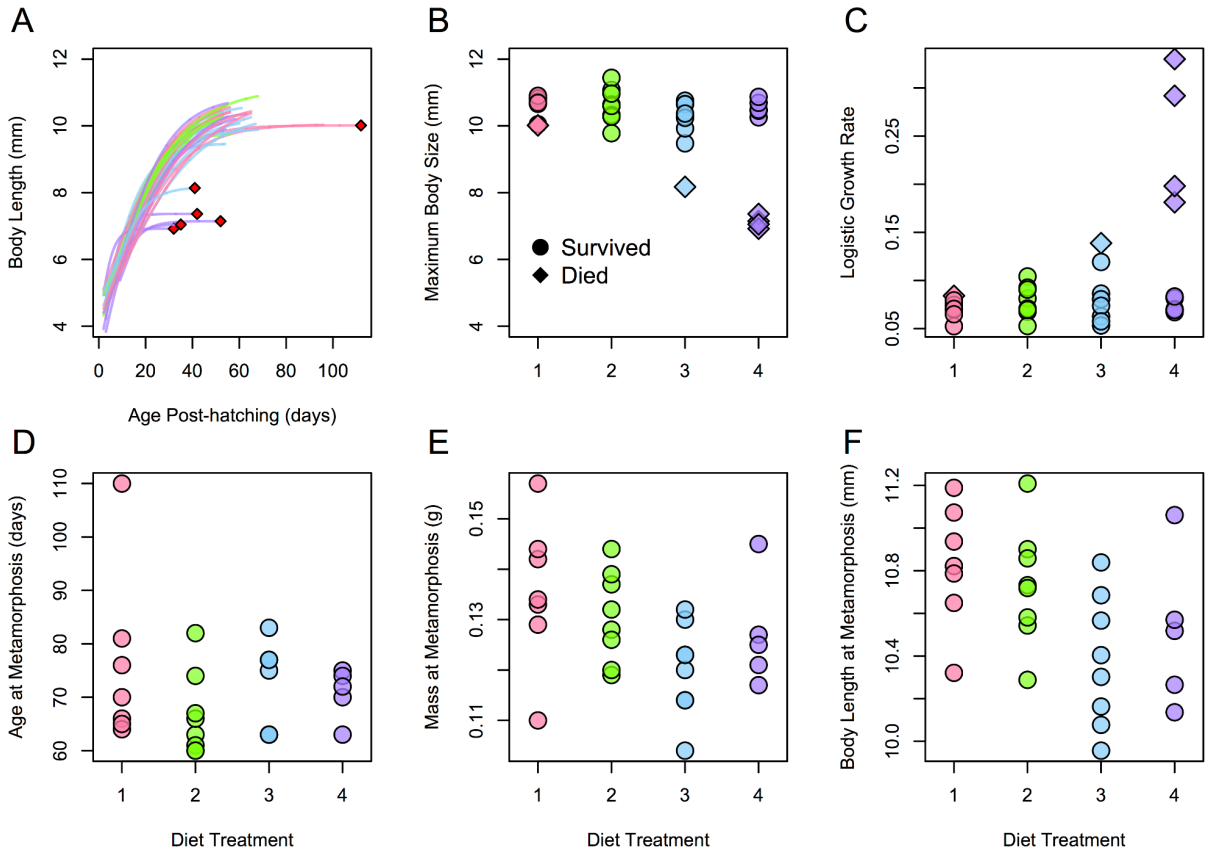


Figure 1. The effect of dietary supplementation on tadpole survivorship. A) Kaplan-Meier (KM) survivorship curves for each of the four treatments. Translucent polygons represent 95% confidence intervals. B) Observed and expected mortality by treatment under a KM model with equal survivorship by across treatments. C) Examples of a healthy tadpole (left), and one exhibiting the symptoms associated with A hypervitaminosis (right). Photos were taken around Gosner stage 30, 24 days after eclosion.



379

380 **Figure 2.** The effect of diet supplementation on tadpole growth. A) Best-fitting logistic growth curves for the 34
381 tadpoles used in the experiment. Tadpoles that did not survive to metamorphosis are marked with a red diamond. B-
382 C) Best-fitting maximum size and growth rate parameters for the curves in panel A grouped by treatment and
383 survivorship status. D-F) Metamorphic age, mass, and body length of individuals that successfully survived past
384 metamorphosis, grouped by treatments. Colors in all figures match those in Figure 1.

385

Supplementary Materials

Supplementary tables

Table S1. Survivorship and growth parameters compared between tadpoles born from jellied and de-jellied eggs. Mortality was compared using Fisher's exact test, and all other parameters were compared using one-way anovas. OR = Odds Ratio.

Parameter	Test Statistic	df	p-value
Mortality	OR = 0.565	-	0.642
Maximum Size	F = 0.512	1, 32	0.479
Growth Rate	F = 1.699	1, 32	0.202
Time to Metamorphosis	F = 0.095	1, 26	0.760
Mass at Metamorphosis	F = 0.714	1, 26	0.406
Body Length at Metamorphosis	F = 0.664	1, 26	0.422

Table S2. Recipes for the four tested diets. The flake mix was a 75:25 by weight mixture of Sera Flora and Sera Sans fish flakes.

Prepared Diet					
	Flake Mix	**β-carotene**	**Vitamin A**	**Cellulose**	**Total**
Control (Diet 1)	3.988 g	0 mg	0 mg	12.0 mg	4 g
β-carotene (Diet 2)	3.988 g	10.0 mg	0 mg	2.0 mg	4 g
Vitamin A (Diet 3)	3.988 g	0 mg	2.0 mg	10 mg	4 g
β-carotene and Vitamin A (Diet 4)	3.988 g	10.0 mg	2.0 mg	0 mg	4 g

Table S3. Results from statistical analyses including and excluding the two individuals whose enclosure briefly had algae.

Statistical test	**Results Including**	**Results Excluding**
Kaplan-Meier chi-squared	$\chi^2 = 9.67$, df = 3, p = 0.02	$\chi^2 = 8.80$, df = 3, p = 0.03
Max Size ~ Diet +	$F_{3,27} = 32.5$, p = 4.2e-9	$F_{3,26} = 37.2$, p = 1.48e-9
Survivorship +	$F_{1,27} = 171.3$, p = 3.3e-13	$F_{1,26} = 211.75$, p = 5.23e-14
Diet × Survivorship	$F_{1,27} = 16.9$, p = 1.7e-5	$F_{1,26} = 7.93$, p = 0.009
Growth Rate ~ Diet +	$F_{3,27} = 15.9$, p = 3.7e-6	$F_{3,26} = 15.0$, p = 7.29e-6
Survivorship +	$F_{1,27} = 67.4$, p = 8.2e-9	$F_{1,26} = 78.4$, p = 2.51e-9
Diet × Survivorship	$F_{1,27} = 12.4$, p = 1.5e-4	$F_{1,26} = 10.1$, p = 0.004
Age at Meta. ~ Diet	$F_{3,24} = 1.1$, p = 0.38	$F_{3,23} = 0.86$, p = 0.47
Weight at Meta. ~ Diet	$F_{3,24} = 2.7$, p = 0.069	$F_{3,23} = 5.0$, p = 0.008
Length at Meta. ~ Diet	$F_{3,24} = 3.4$, p = 0.034	$F_{3,23} = 3.5$, p = 0.033

Supplementary figure

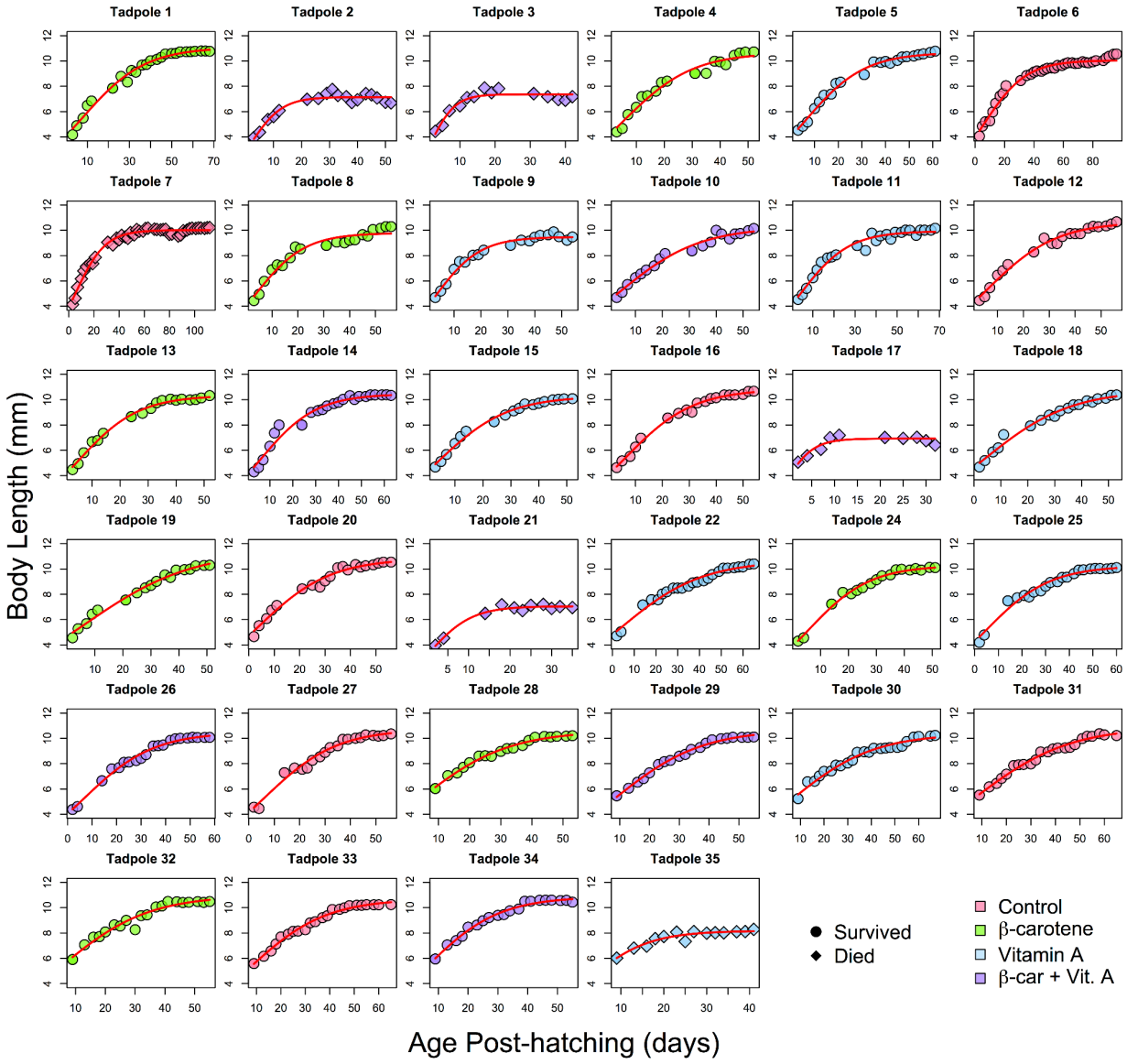


Figure S1. Individual growth trajectories for all tadpoles used in the study. Points represent raw measurements, and red lines are the best-fitting logistic growth curves for each individual.

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