



## LJMU Research Online

Slight, DJ, Nichols, HJ and Arbuckle, K

**Are mixed diets beneficial for the welfare of captive axolotls (*Ambystoma mexicanum*)? Effects of feeding regimes on growth and behavior**

<http://researchonline.ljmu.ac.uk/id/eprint/1347/>

### Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Slight, DJ, Nichols, HJ and Arbuckle, K (2015) Are mixed diets beneficial for the welfare of captive axolotls (*Ambystoma mexicanum*)? Effects of feeding regimes on growth and behavior. *Journal of Veterinary Behavior: Clinical Applications and Research*. 10 (2). pp. 185-190. ISSN 1558-7878**

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact [researchonline@ljmu.ac.uk](mailto:researchonline@ljmu.ac.uk)

<http://researchonline.ljmu.ac.uk/>

# Accepted Manuscript

Are mixed diets beneficial for the welfare of captive axolotls (*Ambystoma mexicanum*)? Effects of feeding regimes on growth and behavior

Dean J. Slight, Hazel J. Nichols, Kevin Arbuckle

PII: S1558-7878(14)00130-0

DOI: [10.1016/j.jveb.2014.09.004](https://doi.org/10.1016/j.jveb.2014.09.004)

Reference: JVEB 834

To appear in: *Journal of Veterinary Behavior*

Received Date: 8 May 2014

Revised Date: 15 September 2014

Accepted Date: 19 September 2014

Please cite this article as: Slight, D.J., Nichols, H.J., Arbuckle, K., Are mixed diets beneficial for the welfare of captive axolotls (*Ambystoma mexicanum*)? Effects of feeding regimes on growth and behavior, *Journal of Veterinary Behavior* (2014), doi: 10.1016/j.jveb.2014.09.004.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



1        **Are mixed diets beneficial for the welfare of captive**  
2        **axolotls (*Ambystoma mexicanum*)? Effects of feeding**  
3        **regimes on growth and behavior**

4  
5  
6        Dean J. Slight<sup>a,b</sup>, Hazel J. Nichols<sup>a,c</sup> and Kevin Arbuckle<sup>d,e</sup>

7  
8  
9  
10     <sup>a</sup> School of Natural Sciences and Psychology, Liverpool John Moores University, Byrom  
11     Street, Liverpool, U.K., L3 3AF

12     <sup>b</sup> deanslight@gmail.com

13     <sup>c</sup> h.j.nichols@ljmu.ac.uk

14     <sup>d</sup> Institute of Integrative Biology, Biosciences Building, Crown Street, University of Liverpool,  
15     Liverpool, U.K., L69 7ZB

16     <sup>e</sup> Corresponding author, k.arbuckle@liverpool.ac.uk, Tel: +44 151 795 4532

17

18 **Abstract**

19 Good nutritional husbandry is crucial to maintain high welfare standards in captive animals.  
20 Both direct effects of diet on growth, development, and maintenance, and indirect effects of  
21 feeding regimes on behavior may be important. Despite this, many questions remain as to  
22 how we should best feed many of the species that are commonly kept in captivity. There is a  
23 great deal of speculation amongst animal keepers as to issues such as whether a mixed diet  
24 is better than an invariant one, but little research is available to inform this question. In this  
25 study, we investigate the impact of mixed versus invariant diets on growth and behavior in  
26 the axolotl (*Ambystoma mexicanum*), an aquatic amphibian of severe conservation concern  
27 that is frequently maintained in captive collections. We then use our results to provide advice  
28 on feeding management in the context of improved welfare. We maintained juvenile axolotls  
29 under one of three 'diets' (feeding regimes): bloodworm (invariant), *Daphnia* (invariant), and  
30 alternating these two prey items between feeds (mixed). Morphological and behavioral data  
31 were collected over a period of 15 weeks and analyzed using generalized linear mixed  
32 models to determine whether our feeding treatments influenced growth and behavior. We  
33 find that axolotls grew fastest on our bloodworm diet and slowest on our *Daphnia* diet, with a  
34 mixed feeding regime leading to intermediate growth rates. Diet treatment did not  
35 significantly influence our measured behaviors, but feeding and locomotion events were  
36 more frequent (and resting less frequent) on feeding days than non-feeding days. These  
37 data suggest that providing a mixed diet is not necessarily beneficial to either growth or  
38 welfare of captive animals. In the case of axolotls, an invariant diet of bloodworm should  
39 increase growth rates but the diet (mixed versus invariant) does not influence behavior.  
40 Overall, our results suggest that mixed diets in themselves may not be beneficial to the  
41 growth or welfare of axolotls as compared to a high-quality invariant diet.

42

43 **Keywords:** Development; Nutrition; Folklore husbandry; Aquatic amphibian; Environmental  
44 enrichment; Activity

45 **Introduction**

46 Studies of diets and feeding regimes are important to promote good nutrition in captive  
47 animals by allowing an evidence-based husbandry approach. Adequate nutrition is  
48 necessary for optimal growth, maintenance, health and reproduction (Ofstedal and Allen,  
49 1996); therefore failure to provide suitable diets can negatively impact captive breeding  
50 programs and animal welfare. For instance, many common veterinary conditions including  
51 metabolic bone diseases, obesity, anorexia, nutrient deficiencies and toxicities, and some  
52 infectious diseases are a direct result of poor dietary management (Donoghue, 2006;  
53 Rosenthal and Mader, 2006). Furthermore, indirect benefits of good nutritional resources are  
54 also evident. For instance, Venesky et al. (2012) found that leopard frog tadpoles (*Lithobates*  
55 *sphenocephalus*) fed a high-protein diet had greater immune function and resistance to the  
56 cosmopolitan epizootic chytrid fungus (*Batrachochytrium dendrobatidis*) when compared to  
57 tadpoles fed a low-protein diet. Therefore, nutrition is a vital consideration for animal  
58 husbandry if we are to maintain high welfare conditions (Hadfield et. al., 2006).

59 Evidence-based husbandry is an important goal, but there remains limited research  
60 available upon which such approaches can be built. While this applies to captive animals in  
61 general, ecological and husbandry-related research suffers from a taxonomic bias towards  
62 mammals (Bonnet et al., 2002; Anderson et al., 2008; Arbuckle, 2009; Hosey et al., 2009),  
63 and amphibians are particularly poorly represented in nutritional studies (Arbuckle, 2009). As  
64 such, if we are to implement evidence-based husbandry regimes to improve welfare of  
65 captive amphibians (and other animals) we must first generate a good research platform  
66 from which to start. Indeed, many non-evidence-based (or 'folklore') husbandry practices  
67 and claims concerning exotic animals have been found to be poorly justified upon academic  
68 scrutiny (e.g. Arbuckle, 2010).

69 The animal care literature is replete with claims that mixed diets are better than  
70 invariant, single prey-species, diets for carnivorous species (e.g. Greene et al., 1997; Preece,  
71 1998; Barrie, 1999; Calvert, 2004; Barten, 2006; Diaz-Figueroa, 2008). However, few studies  
72 have investigated whether mixed diets provide advantages for the growth, development or

73 behavior of captive animals, and so assertions of increased welfare are generally examples  
74 of folklore husbandry (Arbuckle, 2013). Mehrparvar et al. (2013) investigated whether single  
75 or multiple aphid species fed to insect predators improved the development or survival of the  
76 predators, and in fact found that mixed diets were inferior to a good single prey species.  
77 Borg and Toft (2000) used a gradient of mixed diets (aphids and grasshoppers) from 0% to  
78 45% aphids plus a 'free choice' condition to feed grey partridge chicks. Their study was  
79 designed to test optimal foraging predictions with regard to diet choice, but the data  
80 suggested that a small amount of aphids in the diet was much better than a high proportion  
81 of aphids and slightly better than no aphids (an invariant diet of grasshoppers) in terms of  
82 growth. This suggests that there may be a slight benefit to mixed diets for some species,  
83 although Borg and Toft (2000) did not explicitly test this question. Given the conflicting  
84 evidence between studies on different animal groups, it is notable that no research is yet  
85 available on many groups commonly maintained in captivity, such as amphibians.

86 Axolotls (*Ambystoma mexicanum*) are neotenic salamanders kept in large numbers  
87 in captivity, including in the pet trade, zoos, aquariums, museums, and in laboratories. They  
88 are listed as critically endangered in the International Union for Conservation of Nature Red  
89 List of Threatened Species since 2006 as they occupy an area of approximately 10km<sup>2</sup> or  
90 less and are threatened by habitat degradation (IUCN, 2008). Previous conservation efforts  
91 have ranged from habitat restoration to reintroductions, and axolotls have been used as a  
92 flagship species due to their status as a charismatic species that may engage members of  
93 the public to support their conservation (Simberloff, 1998; Caro and O'Doherty, 1999).  
94 However, populations have continued to decline to the extent that they may be extinct in the  
95 wild and the species may be heavily reliant on the captive population to ensure its survival.  
96 Amphibians have suffered global population declines (Stuart et al., 2004; Beebee and  
97 Griffiths, 2005) and managed captive breeding programs have been recognized as an  
98 important conservation tool (Griffiths and Pavajeau, 2008). Therefore, research aimed at  
99 improving husbandry for axolotls and other amphibians is important both for the welfare of

100 the vast number of individuals in captivity and for the conservation of threatened species.  
101 Nutrition is an important facet of husbandry for these aims (Oftedal and Allen, 1996).

102 We fed axolotls on diets consisting of either one of two prey species (bloodworm or  
103 *Daphnia*) or a mixed diet consisting of both prey types to investigate whether a mixed diet  
104 was beneficial. We measured both morphology and behavior to assess the effect of diet on  
105 growth, development, and welfare (using behavior as a proxy). We predicted that, if mixed  
106 diets are beneficial, axolotls in this experimental treatment would grow faster, reach a larger  
107 size, and exhibit more activity such as locomotion than axolotls fed either invariant diet.

108

## 109 **Materials and Methods**

### 110 *Study animals and general husbandry*

111

112 We acquired 24 axolotls from a local breeder. All individuals were siblings and hatched in  
113 April 2013. Axolotls were randomly (using a random number generator) assigned to one of  
114 six separate and identical tanks, ensuring only that each tank was assigned four individuals.  
115 Dechlorinated water, a filter, shelters for hiding (in the form of a perforated building brick),  
116 and an aerating stone were provided in each tank. Cleaning was carried out once per week,  
117 including an approximately one-third water change. Axolotls were housed in a laboratory  
118 setting at Liverpool John Moores University.

119 All axolotls were left to acclimate for one week before the experiments, during which  
120 time they were fed on a mixed diet of two frozen/thawed prey species: bloodworm and  
121 *Daphnia*. These two prey species are commonly used for captive axolotls and therefore  
122 maintain the realism and applicability of our experiments to a practical setting. Thereafter, for  
123 the 15 week duration of the experiment, two tanks each were assigned to one of three  
124 separate diets: two invariant diets (bloodworm only or *Daphnia* only) and a mixed diet  
125 (alternating between bloodworm and *Daphnia* on subsequent feeding days). All axolotls

126 were fed three times per week (Monday, Wednesday, and Friday). Total quantity of food was  
127 increased over the course of the experiment to account for increasing size of the animals  
128 (initially 1.5g, increasing by 0.25g every two weeks until a maximum of 2.5g per tank), but  
129 food quantities were identical across diet treatments.

130 We used digital photographs of natural tail markings to identify individual axolotls, a  
131 common, non-invasive, and reliable method for amphibians (Caorsi et al., 2012). We first  
132 verified that we could accurately identify each individual from these photographs and then, in  
133 order to ensure that reliability did not decline with growth, they were regularly updated during  
134 the course of our experiment.

135

#### 136 *Morphological data*

137

138 Body mass (g) was measured once per week by placing each axolotl in a petri dish and  
139 using a laboratory balance with an accuracy of 0.01g. Each measurement was taken three  
140 times and the mean was recorded as our measure of body mass.

141 Snout-vent length (cm), torso width (cm) and head width (cm) were recorded each  
142 week using digital photographs taken from above. A tripod was used to standardize the  
143 distance and angle between the camera and axolotl. These photographs included a sheet of  
144 graph paper to enable us to calibrate the scale and our three measures were calculated  
145 using ImageJ version 1.41 (Rasband, 1997-2014).

146

#### 147 *Behavioral data*

148

149 Behavioral observations were made using instantaneous sampling (*sensu* Altmann, 1974) of  
150 each individual at 10 second intervals for one minute (including time 0, giving 7 observations



151 per individual per sampling period). Sampling of every individual was conducted on two days  
152 each week: one on a feeding day ('food present'), and one on a non-feeding day ('food  
153 absent'). On feeding days, observations were made five-ten minutes after introducing food to  
154 the tank. Prior to the start of the experiment pilot observations were made to assess which  
155 behaviors were performed by the axolotls, and these were used to create an ethogram  
156 (Table 1). Of these behaviors (feeding, locomotion, resting, spitting, and time out), spitting  
157 was too rare to allow meaningful analysis and time out was of limited value to interpretation.  
158 Therefore analyses of behavioral data were conducted on the other behaviors separately as  
159 the proportion of samples in which they were recorded in each observation period. Because  
160 the axolotls could not be observed during time out behavior (by definition, see Table 1),  
161 these were excluded such that the proportions were calculated based on samples when the  
162 individual was visible. We should also clarify that despite our terminology of 'food present'  
163 versus 'food absent', feeding was possible even on non-feeding days as some food was  
164 typically left over from the previous feeding day. Nevertheless, there was usually little food  
165 left over and this was often partially decomposed, so although possible, feeding  
166 opportunities were far more limited on non-feeding compared to feeding days.

167

### 168 *Data analysis*

169

170 In order to control for individual differences in growth and behavior, all analyses were  
171 conducted using generalized linear mixed models (GLMMs) performed in the lme4 package  
172 version 1.0-4 (Bates et al., 2013) in R version 3.0.1 (R Core Team, 2013). Model fitting  
173 started with a 'full model', containing all explanatory variables and their two-way interactions.  
174 The final, or 'best', model was selected using stepwise model selection wherein the simpler  
175 model at each stage was accepted if it did not provide a significantly poorer fit to the data  
176 based on analysis of deviance (a standard means of comparing nested models, see Thomas  
177 et al., 2013).

178 Morphological variables were modelled with a Gaussian error structure, and residuals  
179 of all models were visualized to check for normality. GLMMs were fit for each response  
180 variable (body mass, snout-vent length, torso width and head width) using diet treatment,  
181 time (as week of the experiment), and their interaction as explanatory variables and with  
182 individual as a random effect in the full model.

183 Behavioral variables were converted to proportions of total events (excluding time out)  
184 per sampling period using the cbind function in R and then modelled with a binomial error  
185 structure. GLMMs were fit for each response variable (proportion of samples feeding,  
186 locomotion, and resting) using 'food present/absent', diet treatment, time (as week of the  
187 experiment), and their two-way interactions as explanatory variables and individual as a  
188 random effect in the full model.

189

## 190 **Results**

191 All of our morphological variables showed the same structure in our best models (Table 2).  
192 There was a significant interaction between growth (body size as a function of time) and diet,  
193 such that axolotls fed an invariant bloodworm diet grew significantly faster than those on a  
194 mixed diet, which in turn grew significantly faster than those fed an invariant *Daphnia* diet  
195 (Table 2; Figure 1). The effect of diet treatment on growth was slightly less pronounced in  
196 torso width compared to body mass, snout-vent length, and head width (Figure 1), but  
197 significant in all cases (Table 2).

198 In contrast, only the 'presence of food' (feeding versus non-feeding days) influenced  
199 our behavior traits according to our best models (Table 3). During feeding days, axolotls  
200 exhibited more feeding and locomotion behavior and less resting behavior compared to non-  
201 feeding days (Figure 2). The particular diet treatment had no significant effect on behavior  
202 and we did not find that behavior changed over the course of our experiment.

203

204 **Discussion**

205 This study aimed to assess whether mixed diets are inherently better than invariant diets for  
206 the welfare of captive animals, as is often assumed. We looked for the influence of feeding  
207 regime on growth (in four morphological traits: body mass, snout-vent length, torso width,  
208 and head width) and behavior in axolotls. We found that bloodworm-only diets produced  
209 higher growth rates than a mixed diet (or a *Daphnia*-only diet), and that these three  
210 treatments had no influence on the behaviors recorded herein. Because increased activity  
211 and other such behavior is frequently used as a proxy for welfare and successful enrichment  
212 (Newberry, 1995; Hosey et al., 2009), we suggest that mixed diets are not necessarily better  
213 for the growth or welfare of captive axolotls.

214 The higher growth rates in bloodworm-fed axolotls compared to those fed mixed or  
215 *Daphnia* diets is likely due to the higher protein and fat content of bloodworm versus  
216 *Daphnia* (5% versus 2.4% protein, 1% versus 0.7% fat). Therefore the additional nutritional  
217 resources available from bloodworm confer the ability to grow quicker than when fed  
218 *Daphnia*, or in a mixed diet where the nutrient content of bloodworm is 'diluted' with that of  
219 *Daphnia*. Since the two prey species in the mixed diet differ in nutrient composition, it is  
220 possible that the impacts on growth in this study are a result of lower nutrition and not that  
221 the diet was mixed *per se*. However, in practice, a mixed diet rarely consists of nutritionally-  
222 matched prey, and so a claim that mixed diets are better must stand up to differences in  
223 nutritional quality between prey items. Since the prey items we chose are commonly used in  
224 axolotl husbandry, our experiments assess such claims in a realistic way that is applicable to  
225 actual captive care regimes. Nevertheless, we acknowledge that a similar experiment with  
226 prey items matched for nutritional value would provide further insights into the perceived  
227 benefit of mixed diets.

228 In contrast to our results, Aquilino et. al. (2012) found that the turban snail  
229 (*Chlorostoma funebris*) and the lined shore crab (*Pachygrapsus crassipes*) displayed a  
230 higher growth when fed a variety of algal species compared to single algal species. However,

231 it is possible that differences in nutrient composition amongst plant or fungal species are  
232 greater than that amongst animal species due to differential micronutrient uptake of primary  
233 producers. If this is the case then we might expect herbivores to react differently to mixed  
234 diets than carnivores. Indeed, amongst captive exotic animals, many carnivores are typically  
235 considered to do well on a single prey item, whereas herbivores may be more likely to have  
236 problems such as refusal to feed on such diets (Funk, 2006; Arbuckle, 2010). In any case,  
237 axolotls appear to have higher growth rates when fed on a nutritionally-rich (rather than a  
238 varied) diet. Since feeding behaviors did not show a decrease with time (Table 3), we also  
239 present evidence that axolotls do not refuse to feed when fed an invariant diet, at least over  
240 a 15 week period, arguing against the type of issues noted in some other species (Funk,  
241 2006).

242         Although our finding of increased activity (both feeding and locomotion) and  
243 decreased resting when food is present is unsurprising, we failed to find any effect of diet  
244 treatment on behavior. We initially predicted that a mixed diet may be enriching and provide  
245 benefits to welfare as manifest through an increased activity, either via motivation effects of  
246 a varied diet or by requiring greater movement to capture different types of prey. This  
247 prediction was in line with the common folklore husbandry claim that varied diet are in some  
248 way 'better' than invariant diets. Our data provide no evidence to support this and suggest  
249 that, similar to Mehrparvar et al.'s (2013) findings in aphid predators, mixed diets are not  
250 necessarily a better choice when feeding animals.

251         We urge caution when using our results because we only investigated the effects of  
252 mixed diets on behavior and morphology. It is possible that dietary factors influence  
253 physiological function such as immune response (Kelly & Tawes, 2013), and mixed diets  
254 could have benefits here that we were unable to measure in our study. Specifically, Kelly &  
255 Tawes (2013) found that female crickets fed a lower quality diet actually had better immune  
256 function, presumably due to preferential investment of resources, although male crickets  
257 showed no such effect. Therefore under this scenario the lower quality *Daphnia* diet may

258 improve immune function and a mixed diet could provide a compromise between a better  
259 immune response and more nutritional resources in axolotls. However, this may not be  
260 generalizable since Venesky et al. (2012) found the opposite result in an amphibian – that  
261 higher quality diets conferred higher resistance to the pathogenic chytrid fungus.  
262 Consequently, the influence of a mixed diet on aspects of health and welfare other than  
263 those considered here remain unknown in axolotls, although our study still provides  
264 evidence from a morphological/developmental and behavioral perspective.

265 We would also like to stress that we are not recommending an overly general  
266 interpretation of our results to say that invariant diets are beneficial for captive animals as a  
267 whole. Different species are likely to respond in different ways to diet variability and the  
268 nutrient content of captive diets is also likely to vary between classes of food items (e.g.  
269 herbivorous versus carnivorous diets, vertebrate versus invertebrate feeders). Nevertheless,  
270 we show that mixed diets have no discernable impact on behavior of axolotls and result in a  
271 slower growth rate than a bloodworm-only diet. For this common laboratory and pet species,  
272 and perhaps other amphibians or aquatic carnivores, it seems that an invariant but good  
273 quality diet is a better option. At the very least, our results highlight that the dogma of mixed  
274 diets being best is not universally true.

275 This paper contributes to the growing literature addressing examples of folklore  
276 husbandry (e.g. Schwitzer et al., 2008; Arbuckle, 2009, 2010; Ferguson et al., 2010; Rosier  
277 & Langkilde, 2011). Testing such claims is an important step towards improving our  
278 husbandry regimes and potentially allows us to achieve better success in captive breeding,  
279 increase welfare standards, and perhaps reduce time and financial costs (Arbuckle, 2013).  
280 Furthermore, in the case of the axolotl, which is not only commonly held in captivity but also  
281 threatened in the wild, amassing evidence to inform husbandry can improve conservation  
282 programmes. This is particularly important considering the recognized importance of *ex situ*  
283 approaches to amphibian conservation (Griffiths and Pavajeau, 2008), for which good quality  
284 husbandry conditions are vital to the success of any strategy.

285

**286 Conclusions**

287 We found no advantage to a mixed diet over a high quality single-prey-species diet for the  
288 growth or behavior of axolotls. Diet variability had no influence on behavior and, in the case  
289 of growth, bloodworm-only diets performed significantly better than a mixed diet. We suggest  
290 that for this species, and possibly other amphibians or aquatic carnivores, a good-quality  
291 invariant diet is a better strategy than a mixed diet. More generally, this paper adds to the  
292 growing literature aimed at providing a platform for evidence-based husbandry (*sensu*  
293 Arbuckle, 2013). Continued research in this vein is required if we are to promote good  
294 captive management practices, improve welfare standards, and inform conservation efforts  
295 for amphibians and other species.

296

**297 Acknowledgements**

298 The authors thank B. McGrath for sourcing and obtaining the study animals for the  
299 experiments. We dedicate this study to 'Tiny', a very charismatic axolotl.

300

**301 Ethical Statement**

302 The work described in this article was approved by Liverpool John Moores University.  
303 Furthermore, the procedures were non-invasive, experimental conditions were non-stressful,  
304 and the husbandry regime was designed to incorporate accepted standards of welfare for  
305 axolotls. The work was carried out in a manner consistent with the Association for the Study  
306 of Animal Behaviour's 'guidelines for the treatment of animals in behavioural research and  
307 teaching'.

308

**309 Conflict of Interest Statement**

310 None of the authors have any conflicts of interests that could be deemed to influence the  
311 objectivity of this work.

312

### 313 **Author Contributions**

314 The idea for the paper was conceived by DS, HJN, and KA. The experiments were designed  
315 by DS, HJN, and KA. The experiments were performed by DS. The data were analyzed by  
316 DS and KA. The paper was written by DS, KA and HJN.

317

### 318 **References**

319 Altmann, J., 1974. Observational study of behavior: sampling methods. *Behavior* 49, 227-  
320 267.

321 Anderson, U.S., Kelling, A.S., Maple, T.L., 2008. Twenty-five years of Zoo Biology: a  
322 publication analysis. *Zoo Biol.* 27, 444–457.

323 Aquilino, K., Stachowicz, J., Coulbourne, M., 2012. Mixed species diets enhance the growth  
324 of two rocky intertidal herbivores. *Mar. Ecol. - Prog. Ser.* 468, 179-189.

325 Arbuckle, K., 2009. Influence of diet on mineral composition of crickets used as prey for  
326 captive amphibians, specifically Hylidae. Unpub. MSci diss. University of Glasgow. 51  
327 pp.

328 Arbuckle, K., 2010. Suitability of day-old chicks as food for captive snakes. *J. Anim. Physiol.*  
329 *Anim. Nutr.* 94, e296-e307.

330 Arbuckle, K., 2013. Folklore husbandry and a philosophical model for the design of captive  
331 management regimes. *Herpetol. Rev.* 44, 448–452.

332 Barrie, M.T., 1999. Chameleon medicine. In: Fowler, M.E., Miller, R.E. (Eds), *Zoo and Wild*  
333 *Animal Medicine: Current Therapy 4*. W.B. Saunders Company, Philadelphia, pp.  
334 200-205.

- 335 Barten, S.L., 2006. Lizards. In: Mader, D.R. (Ed), Reptile Medicine and Surgery, 2<sup>nd</sup> edition.  
336 Saunders Elsevier, Missouri, pp. 59-77.
- 337 Bates, D., Maechler, M., Bolker, B., Walker, S., 2013. lme4: linear mixed-effects models  
338 using Eigen and S4. R package version 1.0-4. Available from: [http://CRAN.R-](http://CRAN.R-project.org/package=lme4)  
339 [project.org/package=lme4](http://CRAN.R-project.org/package=lme4).
- 340 Beebee, T., Griffiths, R., 2005. The amphibian decline crisis: a watershed for conservation  
341 biology? Biol. Conserv. 125, 271-285.
- 342 Bonnet, X., Shine, R., Lourda, O., 2002. Taxonomic chauvinism. Trends Ecol. Evol. 17, 1–  
343 3.
- 344 Borg, C., Toft, S., 2000. Importance of insect prey quality for grey partridge chicks *Perdix*  
345 *perdix*: a self-selection experiment. J. Appl. Ecol. 37, 557-563.
- 346 Calvert, I., 2004. Nutrition. In: Girling, S.J., Raiti, P. (Eds), BSAVA Manual of Reptiles, 2<sup>nd</sup>  
347 edition. British Small Animal Veterinary Association, Gloucester, pp. 18-39.
- 348 Caorsi, V.Z., Santos, R.R., Grant, T., 2012. Clip or snap? An evaluation of toe-clipping and  
349 photo-identification methods for identifying individual southern red-bellied toads,  
350 *Melanophryniscus cambaraensis*. S. Amer. J. Herpetol. 7, 79-84.
- 351 Caro, T., O'Doherty, G., 1999. On the use of surrogate species in conservation biology.  
352 Conserv. Biol. 13, 805-814.
- 353 Diaz-Figueroa, O., 2008. Basic husbandry and nutrition of snakes. NAVC Conf. Vet. Proc.  
354 22, 1743-1745.
- 355 Donoghue, S., 2006. Nutrition. In: Mader, D.R. (Ed), Reptile Medicine and Surgery, 2<sup>nd</sup>  
356 edition. Saunders Elsevier, Missouri, pp. 251-298.
- 357 Ferguson, G.W., Brinker, A.M., Gehrman, W.H., Bucklin, S.E., Baines, F.M., Mackin, S.J.,  
358 2010. Voluntary exposure of some Western-Hemisphere snake and lizard species to



- 359 ultraviolet-B radiation in the field: how much ultraviolet-B should a lizard or snake  
360 receive in captivity? *Zoo Biol.* 29, 317-334.
- 361 Funk, R.S., 2006. Anorexia. In: Mader, D.R. (Ed), *Reptile Medicine and Surgery*, 2<sup>nd</sup> edition.  
362 Saunders Elsevier, Missouri, pp. 739-741.
- 363 Greene, M.J., Nichols, D.K., Hoyt, R.F., Mason, R.T., 1997. The brown tree snake (*Boiga*  
364 *irregularis*) as a laboratory animal. *Lab Anim.* 26, 28-31.
- 365 Griffiths, R.A., Pavajeau, L., 2008. Captive breeding, reintroduction, and the conservation of  
366 amphibians. *Conserv. Biol.* 22, 852-861.
- 367 Hadfield, C., Clayton, L., Barnett, S., 2006. Nutritional support of amphibians. *J. Exot. Pet*  
368 *Med.* 15, 255-263.
- 369 Hosey, G., Melfi, V., Pankhurst, S., 2009. *Zoo Animals: Behavior, Management, and Welfare*.  
370 Oxford University Press, Oxford.
- 371 IUCN, 2008. IUCN Red List of Threatened Species. Version 2008.1. Available from:  
372 [www.iucnredlist.org](http://www.iucnredlist.org). Accessed 28/01/2014.
- 373 Kelly, C., Tawes, B., 2013. Sex-specific effect of juvenile diet on adult disease resistance in  
374 a field cricket. *PLoS ONE* 8, e61301.
- 375 Mehrparvar, M., Arab, N., Weisser, W., 2013. Diet-mediated effects of specialized tansy  
376 aphids on survival and development of their predators: is there any benefit of dietary  
377 mixing? *Biol. Control* 65, 142-146.
- 378 Newberry, R.C., 1995. Environmental enrichment: increasing the biological relevance of  
379 captive environments. *Appl. Anim. Behav. Sci.* 44, 229-243.
- 380 Oftedal, O.T., Allen, M.E., 1996. Nutrition as a major facet of reptile conservation. *Zoo Biol.*  
381 15, 491-497.

- 382 Preece, D.J., 1998. The captive management and breeding of poison-dart frogs, family  
383 Dendrobatidae, at Jersey Wildlife Preservation Trust. Dodo, J. Wildl. Preserv. Trusts  
384 34, 103-114.
- 385 R Core Team, 2013. R: A language and environment for statistical computing. R Foundation  
386 for Statistical Computing, Vienna, Austria. Available from: <http://www.R-project.org/>.
- 387 Rasband, W.S., 1997-2014. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland.  
388 Available from: <http://imagej.nih.gov/ij/>.
- 389 Rosenthal, K.L., Mader, D.R., 2006. Bacterial diseases. In: Mader, D.R. (Ed), Reptile  
390 Medicine and Surgery, 2<sup>nd</sup> edition. Saunders Elsevier, Missouri, pp. 227-238.
- 391 Rosier, R.L., Langkilde, T., 2011. Does environmental enrichment really matter? A case  
392 study using the eastern fence lizard, *Sceloporus undulatus*. Appl. Anim. Behav. Sci.  
393 131, 71-76.
- 394 Schwitzer, C., Polowinsky, S.Y., Solman, C., 2008. Fruits as foods – common  
395 misconceptions about frugivory. 5th European Zoo Nutrition Conference. Chester,  
396 U.K. p. 18.
- 397 Simberloff, D., 1998. Flagships, umbrellas, and key-stones: is single-species management  
398 passé in the land-scape era? Biol. Conserv. 83, 247–25.
- 399 Stuart, S., Chanson, J., Cox, N., Young, B., Rodrigues, A., Fischman, D., Waller, R., 2004.  
400 Status and trends of amphibian declines and extinctions worldwide. Science 306,  
401 1783-1786.
- 402 Thomas, R., Vaughan, I., Lello, J., 2013. Data Analysis with R Statistical Software: A  
403 Guidebook for Scientists. Eco-explore, Cardiff.
- 404 Venesky, M.D., Wilcoxon, T.E., Rensel, M.A., Rollins-Smith, L. Kerby, J.L., Parris, M.J., 2012.  
405 Dietary protein restriction impairs growth, immunity, and disease resistance in  
406 southern leopard frog tadpoles. Oecologia 169, 23-31.

407

408 **Table 1** - Ethogram for behaviors recorded in this study

409

<b>Behavior</b>	<b>Description</b>
Feeding	Ingestion of foodstuffs
Locomotion	Movement without other accompanying behaviors
Resting	No movement or display of other behaviors
Spitting	The forceful expulsion of items (e.g. food) from the mouth
Time out	Out of view of observer

410

411

412 **Table 2** – Results from the best model for each morphological variable. All models are  
 413 GLMMs controlling for individual as a random effect. For all morphological variables the best  
 414 model includes a significant interaction between diet and time, indicating that diet influenced  
 415 growth over the course of the experiment. Effects of diet treatments were estimated as  
 416 contrasts to the mixed diet. N=359.

417

Response variable	Explanatory variable(s)	$\beta \pm SE$	t	P
Body mass	Constant	2.126 $\pm$ 0.510	4.167	<0.001
	Bloodworm	-0.513 $\pm$ 0.567	-0.905	0.36
	<i>Daphnia</i>	0.006 $\pm$ 0.794	0.008	0.99
	Time	0.369 $\pm$ 0.014	24.803	<0.001
	Bloodworm x time	0.111 $\pm$ 0.021	5.284	<0.001
	<i>Daphnia</i> x time	-0.145 $\pm$ 0.021	-6.952	<0.001
Snout-vent length	Constant	3.576 $\pm$ 0.151	23.551	<0.001
	Bloodworm	0.077 $\pm$ 0.151	0.511	0.60
	<i>Daphnia</i>	0.078 $\pm$ 0.241	0.325	0.74
	Time	0.104 $\pm$ 0.003	28.576	<0.001
	Bloodworm x time	0.030 $\pm$ 0.005	5.792	<0.001
	<i>Daphnia</i> x time	-0.032 $\pm$ 0.005	-6.119	<0.001
Torso width	Constant	0.701 $\pm$ 0.038	18.091	<0.001
	Bloodworm	-0.03 $\pm$ 0.045	-0.665	0.50
	<i>Daphnia</i>	-0.014 $\pm$ 0.059	-0.250	0.80
	Time	0.028 $\pm$ 0.001	22.923	<0.001
	Bloodworm x time	0.004 $\pm$ 0.001	2.314	0.02
	<i>Daphnia</i> x time	-0.003 $\pm$ 0.001	-1.955	0.05
Head width	Constant	1.165 $\pm$ 0.042	27.623	<0.001
	Bloodworm	-0.012 $\pm$ 0.045	-0.269	0.78
	<i>Daphnia</i>	0.009 $\pm$ 0.065	0.145	0.88
	Time	0.031 $\pm$ 0.001	26.287	<0.001
	Bloodworm x time	0.007 $\pm$ 0.001	4.473	<0.001
	<i>Daphnia</i> x time	-0.008 $\pm$ 0.001	-5.387	<0.001

418

419

420 **Table 3** - Results from the best model for each behavior of interest. All models are GLMMs  
 421 controlling for individual as a random effect. All behaviors were influenced only by the  
 422 presence of food. There was no significant effect of diet treatment nor was there a change in  
 423 any behavior over the course of the experiment. N=718.

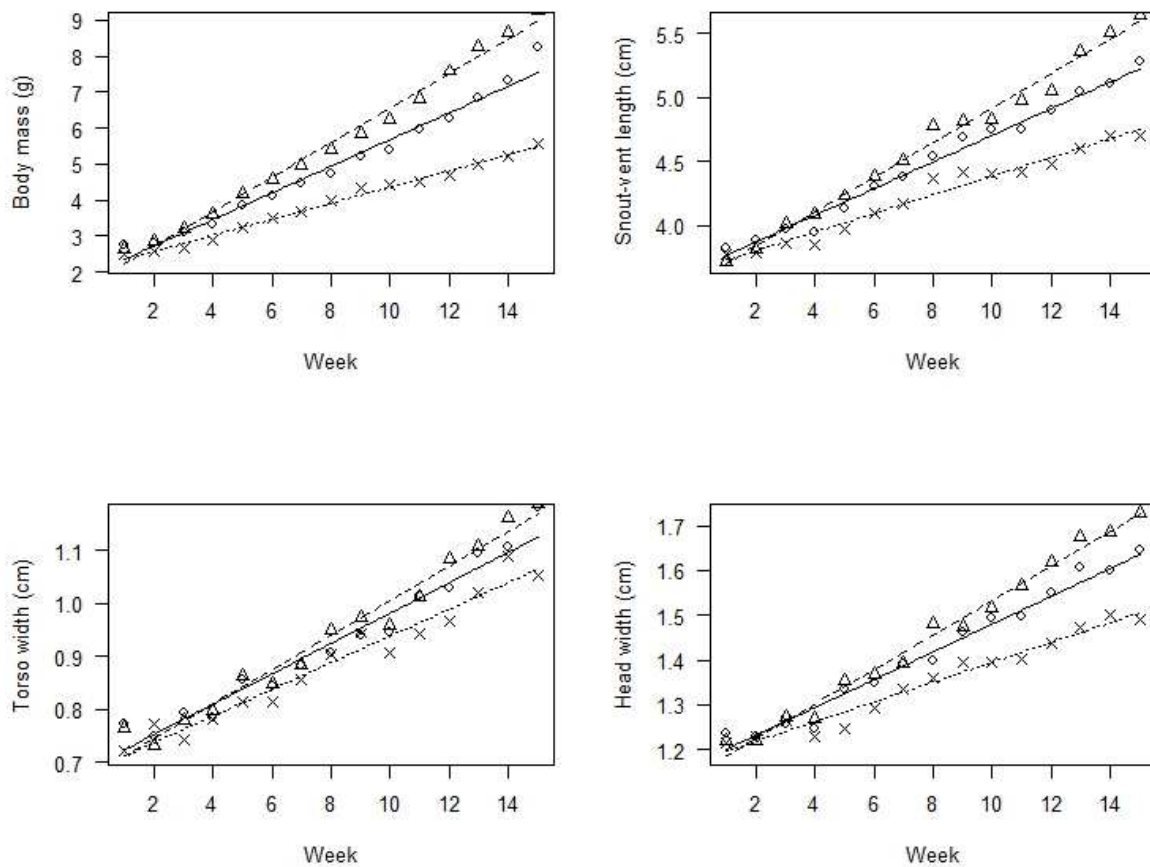
424

Response variable	Explanatory variable(s)	$\beta \pm SE$	z	P
Feeding	Constant	-5.431 $\pm$ 0.302	-17.98	<0.001
	Food present	4.300 $\pm$ 0.304	14.11	<0.001
Locomotion	Constant	-2.422 $\pm$ 0.070	-34.66	<0.001
	Food present	1.300 $\pm$ 0.081	16.12	<0.001
Resting	Constant	-0.195 $\pm$ 0.030	-6.58	<0.001
	Food present	-1.315 $\pm$ 0.056	-23.69	<0.001

425

426 **Figure 1** – Growth (increase in size over the duration of the experiment) varies with diet in  
 427 all four measures of size used herein. Lines are the predictions from our GLMMs, and points  
 428 are mean values for each diet treatment in each week. Dashed lines and triangles represent  
 429 a bloodworm diet, solid lines and circles represent a mixed diet, solid lines and crosses  
 430 represent a *Daphnia* diet. Axolotls fed an invariant bloodworm diet grew fastest, followed by  
 431 those fed a mixed diet, and *Daphnia*-fed individuals grew slowest.

432

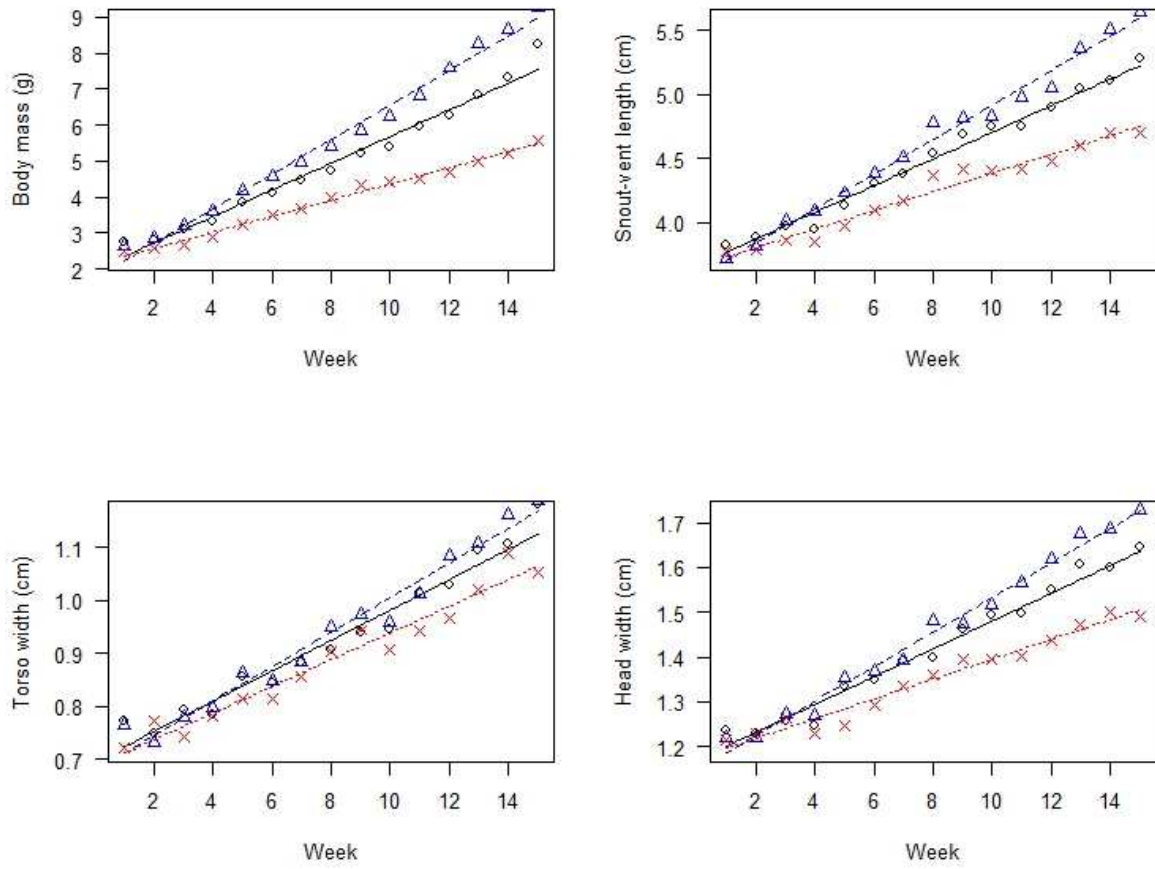


433

434

435 GREYSCALE VERSION FOR PRINT PUBLICATION (FIGURE 1)

436



437

438

439 COLOR VERSION FOR ONLINE PUBLICATION (FIGURE 1)

440 **Figure 2** – Behavior was only influenced by the presence of food, not diet treatment.

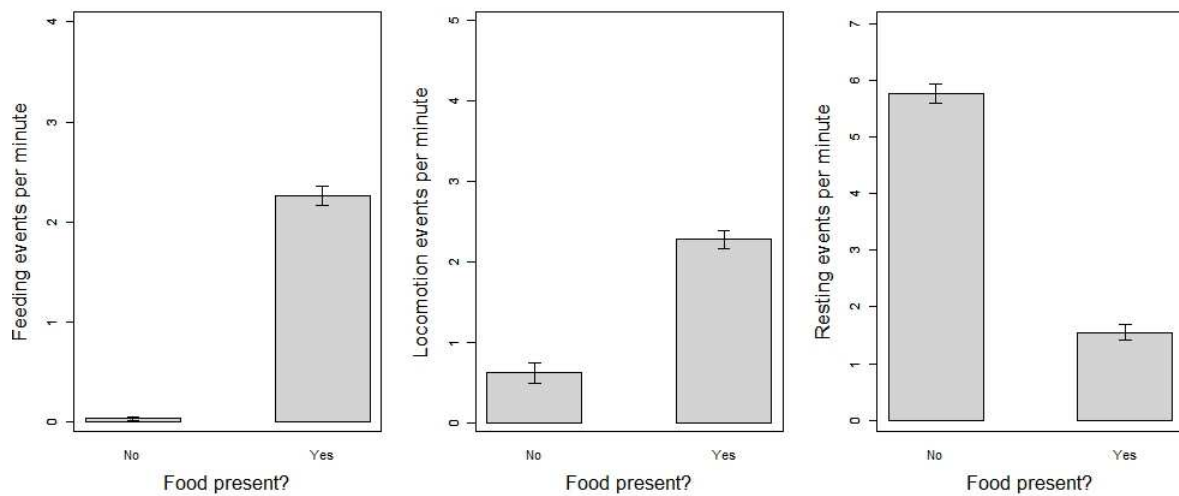
441 Feeding and locomotion behaviors increased and resting decreased on feeding days

442 compared to non-feeding days. Error bars are 95% confidence intervals. Behavioral events

443 per minute are based on scan samples taken at 10 second intervals over one minute per

444 individual (i.e. 7 samples per minute).

445



446



- Groups of axolotls were fed bloodworm, *Daphnia*, or a mixed diet.
- Morphometric and behavioural measurements over time were recorded.
- Axolotls grew best on an invariant bloodworm diet.
- Bloodworm-fed animals were more active than others, though a mixed diet may temporarily increase activity.
- Despite common perceptions, mixed diets do not necessarily provide improved welfare compared to invariant diets.

ACCEPTED MANUSCRIPT