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Evolution of coprophagy and nutrient absorption in a Cave Salamander

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Abstract

The transition from carnivory to omnivory is poorly understood. The ability to feed at more than one trophic level theoretically increases an animal's fitness in a novel environment. Because of the absence of light and photosynthesis, most subterranean ecosystems are characterized by very few trophic levels, such that food scarcity is a challenge in many subterranean habitats. One strategy against starvation is to expand diet breadth. Grotto Salamanders (*Eurycea spelaea* (Stejneger, 1892)) are known to ingest bat guano deliberately, challenging the general understanding that salamanders are strictly carnivorous. Here we tested the hypothesis that grotto salamanders have broadened their diet related to cave adaptation and found that, although coprophagous behavior is present, salamanders are unable to acquire sufficient nutrition from bat guano alone. Our results suggest that the coprophagic behavior has emerged prior to physiological or gut biome adaptations.

Keywords

Guano feeding, salamander, evolution, physiology, behavior

Introduction

Coprophagy is a feeding strategy commonly found in invertebrates (Weiss 2006), but much less so in vertebrates. Coprophagy sometimes exists in mammals such as rodents and lagomorphs, and to a lesser degree in pigs, horses, dogs and nonhuman primates (Thacker and Brandt 1955; Soave and Brand 1991; Marinier and Alexander 1995; Aitken 2003; Krief and Hladik 2004). In amphibians, coprophagy is rare but when present may influence larval development of some species with herbivorous larvae. For example, herbivorous tadpoles regularly feed on feces of conspecifics in captivity (Gromko et al. 1973; Steinwascher 1978; Pryor and Bjorndal 2005), even when other food sources are available ad libitum (Pryor and Bjorndal 2005). Herbivorous tadpoles have digestive morphologies and physiologies similar to other herbivorous vertebrates that rely on hindgut fermentative digestion (Pryor and Bjorndal 2005) and ingest feces to inoculate their digestive tracts with beneficial microbes (Steinwascher 1978; Beebee 1991; Beebee and Wong 1992). Growth rates are slower when feces are removed from the diet (Steinwascher 1978) suggesting that herbivorous tadpoles benefit nutritionally from coprophagy even though feces are lower in energy (Gromko et al. 1973).

The literature is scarce when it comes to coprophagy in predatory amphibians (Fenolio et al. 2006). However, faces consist of a readily available food resource for animals living in energy-limited environments, such as caves. Food and nutritional resources in caves are derived from surface inputs and can be limited both temporarily and spatially within these systems (Culver and Pipan 2014). Likewise, foraging in aphotic habitats of caves presents significant challenges for animals that potentially may go weeks to months between feeding bouts. Guano produced by seasonally roosting bats represents an important food source for both terrestrial and aquatic invertebrates (Howarth 1983; Poulson and Lavoie 2000), which in turn are prey for fishes and salamanders (Poulson and Lavoie 2000; Graening 2005; Niemiller and Poulson 2010; Fenolio et al. 2006, 2014). Salamanders have been known to be strictly carnivorous but Fenolio et al. (2006) showed that obligate cave-dwelling Grotto Salamander larvae (*Eurycea spelaea* (Stejneger, 1892), Fig. 1) ingests bat guano. This behavior is not incidental to the capture of aquatic invertebrate prey. Stable isotope signatures suggest nutrients from bat guano could be incorporated into salamander tissues, and nutritional analyses revealed that bat guano is comparable to potential prey items in nutritional and energy content, suggesting that bat guano could be a viable alternative food source in some energy-poor cave systems. Since the relative importance of guano in the diet of subterranean salamanders is unknown, the aim of this study was to determine whether subterranean salamander larvae could persist on an exclusive guano diet compared to the typical carnivorous diet of salamanders.

Materials and methods

All experiments were conducted under the approval of animal protocol #15022 by the Rutgers Newark Institutional Animal Care and Use Committee that handles NJIT re-

Figure 1. *Eurycea spelaea* showing troglobitic characters, lack of pigmentation and microphthalmy. Scale bar: 0.5 cm.

search. We collected 46 specimens of *Eurycea spelaea* from January-Stansbury Cave located in the Ozark Plateau National Wildlife Refuge in Delaware County, Oklahoma. Salamanders were housed individually in mesocosms submerged in the cave stream. Each mesocosm consisted of a 500 ml plastic bottle with small holes so that the salamanders had continuous access to fresh cave water. All lids were connected to a central line via a short string. Salamanders were collected June 6th but the study did not until June 22nd, salamanders were fed amphipods until the start of the study. During the study, salamanders were fed every four days either a strict diet of live amphipods, bat guano, or nothing. We collected amphipods and bat guano fresh on the day of feeding from the cave. The cave is inhabited by a maternity colony (ca. 15,000 individuals) of federally endangered Gray bats (*Myotis grisescens* A.H. Howell, 1909) from late April through early November (Fenolio et al. 2006, 2014). Salamanders were randomly assigned to a negative control group, or one of two prey types and one of four feeding treatments based on percentage of initial body mass: 0% (control) 2.5%, 5%, and 10%. Salamanders were massed before feeding to track body mass loss or gain and fed

the corresponding percentage of initial body mass of amphipods or guano. We used a milligram- accuracy scale (Ohus, Parsippany, NJ, USA). Care was taken to remove any food remnants before the next feeding, although in most cases salamander ate all the food. Salamanders that lost ≥30% of initial body mass were removed the study. We used ANCOVA to compare body mass of the different treatments in MatLab with an alpha level of 0.05. Normality of ranked data was verified via the Kolmogorov-Smirnov test. Salamanders were released back into the cave after the study per permitting regulations.

All data associated with this study are available from the figshare digital repository: https://doi.org/10.6084/m9.figshare.4805656

Results

Loss of body mass in treatment groups: All treatment groups lost some body mass during the study (34 days; Fig. 2). Animals in the control group (n = 10) were removed from the study earlier (27 days) than the other groups (34 days) due to body mass loss. Salamanders in the control group experienced the steepest loss of body mass. In general, salamanders fed guano lost more body mass than salamanders fed amphipods, and at 34 days, most guano-fed salamanders had reached the 30% loss limit. Body mass was more variable in amphipod-fed groups with both gains and losses. Salamanders fed 2.5% of initial body mass (IBM) lost an average of 31.3% (±14.3%) body mass eating guano compared to an average of 9.6% (±36.0%) eating amphipods. Salamanders fed 5% IBM lost 30.2% (±4.8%) body mass when eating guano compared to 7.9% (±28.1%) eating amphipods. Body mass loss was least for salamanders fed 10% IBM, and guano-fed salamanders lost 28.3% (±4.4%) body mass compared to 7.6% (±11.5%) for the amphipod group.

Comparisons of weight loss: For salamanders fed 2.5% IBM, body mass loss rates for guano-fed and amphipod-fed groups were slower than the control group (Guano-fed: $F = 6.82$, $P = 0.01$; Amphipod-fed: $F = 12.14$, $P = 0.0007$) but not different from each other ($F = 2.86$, $P = 0.09$). For salamanders fed 5% IBM, guano-fed animals lost body mass at a slower rate than amphipod-fed animals ($F = 11.05$, $P = 0.0012$) and control animals ($F = 14.75$, $P = 0.0002$), while amphipod-fed animals lost body mass similarly to control animals ($F = 0.07$, $P = 0.795$). For salamanders fed 10% IBM, the amphipod-fed group lost body mass slower than the guano-fed group ($F = 6.4$, $P = 0.131$) and control group ($F = 26.26$, $P = 1.09e\text{-}6$), while the guano-fed group was similar to the control group ($F = 8.02$, $P = 0.005$).

Discussion

All amphipod groups had individuals that lost and gained weight within the period of the study. The variability in body mass was smaller in the 10% amphipod IBM com-

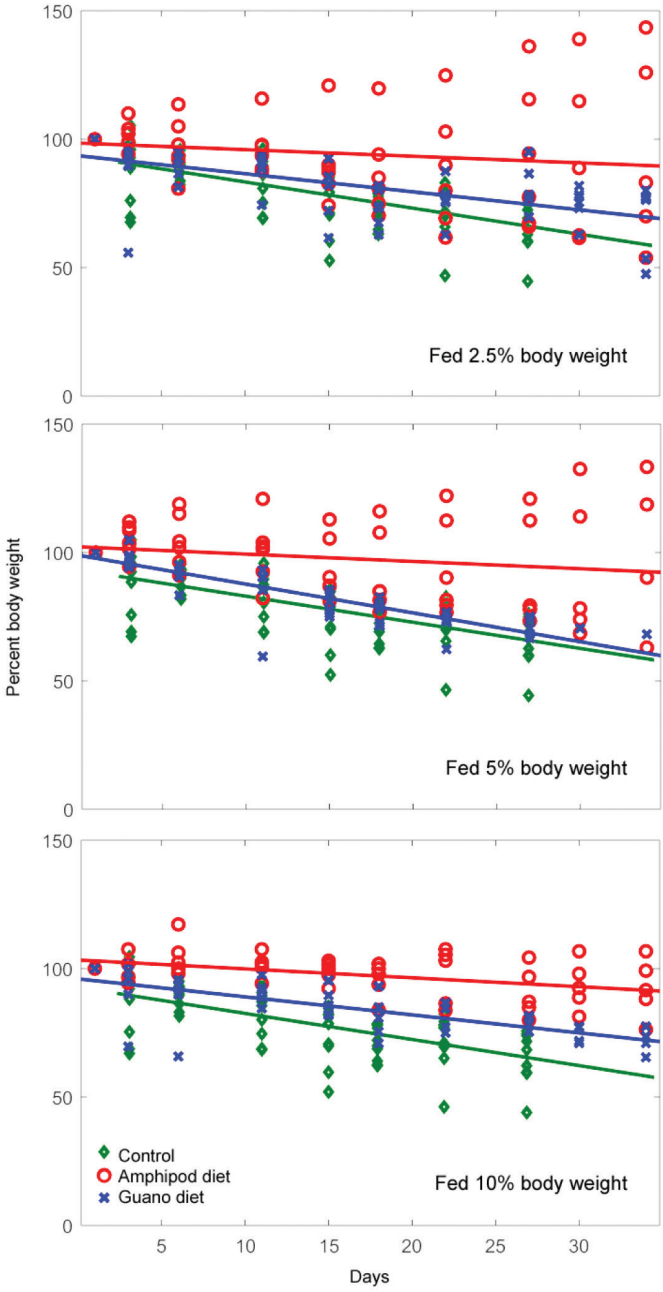

Figure 2. Regression lines based on body mass loss of different diet types and amounts. Salamanders were fed nothing (green), live amphipods (red) or guano (blue). Groups were fed every four days based on their initial body weight, with 2.5% (**A**), 5% (**B**) or 10% (**C**). The calculated regression lines were as follows: Control -1.16x+96.01 R^2 = 0.54, n = 10; $2.5\%_{amphipod}$ -0.26x+98.49, R^2 = 0.39, n = 6; $2.5\%_{guano}$ -0.70x+93.58, R^2 = 0.02, n = 6; $5\%_{amphipod}$ -0.28x+102.22, R^2 = 0.03, n = 6; $5\%_{guano}$ -1.12x+98.89, R^2 = 0.77, n = 6; $10\%_{amphipod}$ -0.35x+103.36, R^2 = 0.21, n = 6; $10\%_{guano}$ -0.70x+96.01, R^2 = 0.53, n = 6.

pared to the 5% and 2.5%, but a few individuals lost weight making the overall average mass at the end of the study slightly less than the IBM. The large variability in the 5% and 2.5% amphipod groups suggests that individuals may be behaving differently or some individuals may be stressed in the mesocosm. Nonetheless, since guano groups resemble more closely control groups we conclude that amphipods are a better food source for the salamanders.

Shifts in habitat are often linked with dietary shifts, as environmental changes frequently cause organisms to alter foraging behaviors (Rosalino et al. 2005; McMeans et al 2015). The transition from surface to subterranean habitats involves dramatic morphological, physiological, and behavioral changes associated with life in complete darkness and often limited energy resources, including a predicted increase in dietary breadth (Culver 1982, 1994; Holyoak and Sachdev 1998; Fenolio et al. 2006). In subterranean salamanders, the evolution of coprophagy may be an unusual foraging strategy to exploit a nutritious and seasonally abundant resource (i.e., bat guano) in an otherwise food-limited environment. While it has been demonstrated that Grotto salamander larvae will regularly employ coprophagy of calorically-rich bat guano (Fenolio et al. 2006), our study suggests that Grotto salamander larvae are unable to thrive on a guano-exclusive diet for a prolonged period. So in this case the coprophagous behavior has emerged in evolution prior to the necessary physiological changes to gain nutrition from it.

The apparent disagreement between coprophagous behavior in Grotto salamanders and the lack of apparent absorption may have several possible explanations. First, Grotto salamander larvae, and salamanders in general, do not possess the morphological and physiological digestive traits necessary to exploit guano as a food resource. Salamanders in general are strict carnivores with short digestive tracts and have buccal enzymes with low amylolytic activity (Stevens and Hume 2004). In contrast, coprophagy is most often associated with herbivory, which predominately utilize postgastric (hindgut) fermentation and the consumption of feces increases the absorption of nutrients and inoculate the hind gut with microbes (Clauss et al. 2007). The selective consumption of predigested material is a form of omnivory. We know relatively little about the adaptive advantages of and the selective drivers that favor omnivory, and by proxy coprophagy, in vertebrates (but see Diehl 2003). Coprophagy requires the evolution of not only a coprophagous behavior but also the evolution of morphological and physiological digestive traits to process feces. It is unknown whether these traits are linked, but theoretically behavioral evolution can precede physiological and morphological evolution. Second, since Grotto salamanders are ingesting feces with high protein content (54%; Fenolio et al. 2006) of insectivores (bats) rather than feces from herbivores, a vastly different gut microbiome is needed to efficiently digest feces. So in addition to lacking the morphological and physiological traits, Grotto salamanders may not possess the necessary gut flora to digest and fully process the contents of bat guano. Ley et al. (2009) found that diet can impact gut microbiome diversity in mammals, which increases with evolution from carnivory to omnivory. Digestive

evolution in amphibians, as well as their gut biomes and the gut's propensity for evolution, is yet to be examined in detail. Finally, coprophagy may reflect mistaken identity due to an innate feeding response for moving prey. In subterranean habitats, aquatic salamanders and cavefishes rely heavily on mechanosensation to detect and capture moving prey. Guano falling into a pool and settling on the substrate may elicit a similar feeding response as crustaceans and other aquatic invertebrates. Guano may not be immediately rejected but ingested instead because of the high protein and fat content of the insectivorous guano. Alternatively, guano may possess a micronutrient, vitamin or mineral otherwise scarce in the subterranean habitat (see Fenolio et al. 2006). While guano may not prevent a loss in mass, it may still offer some nutritional benefit.

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References

Aitken MM (2003) Coprophagy in dogs. Veterinary record 153(3): 96–96.

Beebee TJC (1991) Purification of an agent causing growth inhibition in anuran larvae and its identification as a unicellular unpigmented alga. Canadian Journal of Zoology 69: 2146–2153. https://doi.org/10.1139/z91-300

Beebee TJC, Wong ALC (1992) Prototheca-mediated interference competition between anuran larvae operates by resource diversion. Physical Zoology 65: 815–831. https://doi.org/10.1086/physzool.65.4.30158541

Belgrad BA, Griffen BD (2016) The Influence of Diet Composition on Fitness of the Blue Crab, *Callinectes sapidus*. PLoS ONE 11(1): 1–15. https://doi.org/10.1371/journal.pone.0145481

Clauss M, Schwarm A, Ortmann S, Streich WJ, Hummel J (2007) A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 148(2): 249–65. https://doi.org/10.1016/j.cbpa.2007.05.024

Cohen AC (1996) Plant Feeding by Predatory Heteroptera: Evolutionary and Adaptational Aspects of Trophic Switching. In: Alomar O, Wiedenmann RN (Eds) Zoophytophagus Heteroptera: Implications for Life History and Integrated Pest Management. Entomological Society of America. Lanham, Maryland, USA, 1–7.

Culver DC, Pipan T (2014) Shallow Subterranean Habitats: Ecology, Evolution, and Conservation (1st edn). Oxford University press, New York, USA. https://doi.org/10.1093/acprof:oso/9780199646173.001.0001

Diehl S (2003) The Evolution and Maintenance of Omnivory: Dynamic Constraints and the Role of Food Quality. Ecology 84(10): 2557–2567. https://doi.org/10.1890/02-0399

Eubanks MD, Denno RF (1999) The Ecological Consequences of Variation in Plants and Prey for an Omnivorous Insect. Ecology 80: 1253–66. https://doi.org/10.1890/0012-9658(1999)080[1253:TECOVI]2.0.CO;2

Eubanks MD, Styrsky JD, Denno RF (2003) The Evolution of Omnivory in Heteropteran Insects. Ecology 84: 2549–2446. https://doi.org/10.1890/02-0396

Fenolio DB, Graening GO, Collier BA, Stout JF (2006) Coprophagy in a Cave-Adapted Salamander; the Importance of Bat Guano Examined through Nutritional and Stable Isotope Analyses. Proceedings of the Royal Society of London B: Biological Sciences 273(1585): 439–43. https://doi.org/10.1098/rspb.2005.3341

Fenolio DB, Niemiller ML, Bonett RM, Graening GO, Collier BA, Stout JF (2014) Life history, demography, and the influence of cave-roosting bats on a population of the Grotto salamander (*Eurycea spelaea*) from the Ozark Plateaus of Oklahoma (Caudata: Plethodontidae). Herpetological Conservation and Biology 9: 394–405.

Graening GO (2005) Trophic structure of Ozark cave streams containing endangered species. Journal of Oceanological and Hydrobiological Studies 34: 3–17.

Gromko MH, Mason FS, Smith-Gill SJ (1973) Analysis of the crowding effect in *Rana pipiens* tadpoles. Journal of Experimental Zoology 186: 63–72. https://doi.org/10.1002/jez.1401860109

Howarth FG (1983) Ecology of cave arthropods. Annual Review of Entomology 28: 365–389. https://doi.org/10.1146/annurev.en.28.010183.002053

Kitaysky AS, Kitaiskaia EV, Piatt JF, Wingfield JC (2006) A Mechanistic Link between Chick Diet and Decline in Seabirds? Proceedings. Biological Sciences/The Royal Society 273(1585): 445–50. https://doi.org/10.1098/rspb.2005.3351

Krief S, Jamart A, Hladik CM (2004) On the possible adaptive value of coprophagy in free-ranging chimpanzees. Primates 45(2): 141–145. https://doi.org/10.1007/s10329-003-0074-4

Marinier SL, Alexander AJ (1995) Coprophagy as an avenue for foals of the domestic horse to learn food preferences from their dams. Journal of Theoretical Biology 173(2): 121–124. https://doi.org/10.1006/jtbi.1995.0049

Martin SD, Shepard DB, Steffen MA, Phillips JG, Bonett RM (2016) Biogeography and Colonization History of Plethodontid Salamanders from the Interior Highlands of Eastern North America 12625: 410–22. https://doi.org/10.1111/jbi.12625

McMeans BC, McCann KS, Humphries M, Rooney N, Fisk AT (2015) Food Web Structure in Temporally-Forced Ecosystems. Trends in Ecology & Evolution 30(11): 662–72. https://doi.org/10.1016/j.tree.2015.09.001

Nalepa CA, Bignell DE, Bandi C (2001) Detritivory, Coprophagy, and the Evolution of Digestive Mutualisms in Dictyoptera. Insectes Sociaux 48(3): 194–201. https://doi.org/10.1007/PL00001767

Niemiller ML, Poulson TL (2010) Subterranean fishes of North America: Amblyopsidae. In: Trajano E, Bichuette ME, Kapoor BG (Eds) The Biology of Subterranean Fishes. Science Publishers, Enfield, New Hampshire, USA, 169–280. https://doi.org/10.1201/EBK1578086702-c7

Poulson TL, Lavoie KH (2000) The trophic basis of subsurface ecosystems. In: Wilkins H, Culver DC, Humphreys WF (Eds) Ecosystems of the World: Subterranean Ecosystems. Elsevier, Amsterdam, 231–249.

Pryor GS, Bjorndal KA (2005) Symbiotic fermentation, digesta passage, and gastrointestinal morphology in bullfrog tadpoles. Physiology and Biochemical Zoology 78: 201–215. https://doi.org/10.1086/427050

Rabosky DL, Lovette IJ (2008) "Explosive Evolutionary Radiations: Decreasing Speciation or Increasing Extinction Through Time?" Evolution 62(8): 1866–75. https://doi.org/10.1111/j.1558-5646.2008.00409.x

Rosalino LM, Loureiro F, Reis MS, Macdonald DW, Santos-Reis M (2005) Dietary Shifts of the Badger (*Meles meles*) in Mediterranean Woodlands: An Opportunistic Forager with Seasonal Specialisms. Mammalian Biology 70(1): 12–23. https://doi.org/10.1078/1616-5047-00172

Sakamaki T (2010) Coprophagy in wild bonobos (*Pan paniscus*) at Wamba in the Democratic Republic of the Congo: a possibly adaptive strategy? Primates 51(1): 87. https://doi.org/10.1007/s10329-009-0167-9

Soave O, Brand CD (1991) Coprophagy in animals: a review. The Cornell Veterinarian 81(4): 357–364.

Steinwascher K (1978) The effect of coprophagy on the growth of *Rana catesbeiana* tadpoles. Copeia 1978: 130–134. https://doi.org/10.2307/1443833

Stevens CE, Hume ID (2004) Comparative Physiology of the Vertebrate Digestive System (2nd edn). Cambridge University Press, Cambridge, 400 pp.

Thacker EJ, Brandt CS (1955) Coprophagy in the rabbit. Journal of Nutrition 65: 375–385.

Weiss MR (2006) Defecation behavior and ecology of insects. Annual Review of Entomology 51: 635–661 https://doi.org/10.1146/annurev.ento.49.061802.123212

Whitman DW, Blum MS, Slansky Jr F (1994) Carnivory in Phytophagous Insects. In: Anantha krishnan NT (Ed.) Functional Dynamics of Phytophagous Insects. Science Publications. Lebanon, New Hampshire, 161–205.

Yoshizawa M, Gorički Š, Soares D, Jeffery WR (2010) Evolution of a Behavioral Shift Mediated by Superficial Neuromasts Helps Cavefish Find Food in Darkness. Current Biology 20(18): 1631–36. https://doi.org/10.1016/j.cub.2010.07.017