

Sibling cannibalism in noncannibal morph *Ambystoma tigrinum* larvae and its correlation with high growth rates and early metamorphosis

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We describe here, for the first time, the existence of an *Ambystoma tigrinum tigrinum* larval morph characterized by fast growth rates and early metamorphosis and triggered by cannibalism. This new morph does not have the anatomical specializations of true *A. tigrinum* cannibal morphs, i.e., enlarged vomerine teeth and a wider head described previously by several workers. Functionally, however, this new morph and true cannibal morphs achieve the same end; high growth rates and early metamorphosis may facilitate survival in individuals inhabiting temporary and unpredictable wetlands.

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On trouvera ici la description inédite d'une forme larvaire d'*Ambystoma tigrinum tigrinum* caractérisée par un taux de croissance rapide et une métamorphose précoce et dont l'existence est déclenchée par le cannibalisme. Cette nouvelle forme n'a pas les spécialisations anatomiques des vraies formes cannibales d'*A. tigrinum*, décrites précédemment par plusieurs chercheurs, à savoir les dents vomériennes plus grandes et la tête élargie. Fonctionnellement cependant, la nouvelle forme et les formes cannibales classiques parviennent aux mêmes fins; les taux de croissance rapides et la métamorphose précoce facilitent probablement la survie chez les individus qui habitent les zones humides temporaires ou imprévisibles.

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Introduction

Salamanders of the species *Ambystoma tigrinum* (family Ambystomatidae) are morphologically diverse. In addition to paedomorphic adults (i.e., sexually mature animals that exhibit an otherwise larval phenotype; Gould 1977) there are at least three reported larval phenotypes (herein termed "morphs" *sensu* Huxley (1955), large and small typical morphs and cannibal morphs (Rose and Armentrout 1976). Cannibal morphs are distinguished from typical morphs by their larger size, wider heads, and hypertrophied vomerine teeth (e.g., Powers 1907; Rose and Armentrout 1976; Collins and Cheek 1983; Pierce et al. 1983; Lannoo and Bachmann 1984).

Cannibal morphs are rare; they occur in some populations of typical morphs, but they are never common (Powers 1907; Lannoo and Bachmann 1984). The occurrence of cannibal morphs is associated with high densities of typical morphs (Collins and Cheek 1983), which occur, for example, during early pond drying (Lannoo and Bachmann 1984). Cannibal morphs are not obligate cannibals, they tend to be macrophagous carnivores; typical morphs take a wider range of prey sizes and may be predominantly zooplanktivorous (Collins and Holomuzki 1984; Lannoo and Bachmann 1984).

Powers (1907) reports that cannibal morphs are slow to metamorphose. However, Lannoo and Bachmann (1984) argue that these fast-growing cannibal morphs will reach mini-

um metamorphic size during droughts and therefore should metamorphose earlier than typical morphs.

Using electrophoretic techniques, Pierce et al. (1981) found differences in allozyme frequencies between cannibal and small morphs inhabiting the same pond. Powers (1907) reports that most cannibal morphs are males.

We report here the existence of an *Ambystoma tigrinum* morph with characteristics intermediate between typical and cannibal morphs. This morph is characterized by (i) sibling cannibalism, at least in the laboratory; (ii) subsequent accelerated growth, even after only one cannibalistic event; and (iii) early metamorphosis. Additionally, this morph is genetically indistinguishable from typical individuals, using electrophoretic techniques, is present in males and females in approximately a 50:50 ratio, and metamorphoses at the same size as typical individuals. The fast-growing morph is similar to typical morphs in body proportions and in having small vomerine teeth. In addition to describing this morph and the conditions of its occurrence, we discuss the potential physiological and ecological mechanisms underlying this phenomenon.

Materials and methods

In April 1985, seven *Ambystoma tigrinum* egg masses were collected from Kelley's Island, located in the western basin of Lake Erie off northwest Ohio (42°N, 83°W), and brought into the laboratory. Eggs were allowed to hatch and siblings were raised together, but larvae from each clutch of eggs were reared separately. The larvae were maintained in a series of enamel pans (36 × 24 × 5.5 cm) at room temperature (21 ± 1°C) and fed brine shrimp (*Artemia salina*) and Tubifex worms *ad libitum*. A total of 268 larvae were cultured, at

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an initial density of 40 per pan. Animals from all seven clutches were closely monitored.

The initial goal of this culture technique was to raise some larvae through metamorphosis for electrophoretic analysis. However, once we began observing cannibalism, we immediately designed a set of observations to determine the outcome of cannibalism on growth rates and time to metamorphosis.

Early in larval development a few (one to seven) larvae in each clutch began eating siblings, despite being provided with brine shrimp *ad libitum*. Cannibals were identified by the presence of siblings in their buccal cavity or stomach; about 1 day is required to completely ingest a slightly smaller sibling, and thus cannibals were easily identified. Additionally, after cannibalizing, salamanders exhibit a growth spurt which distinguishes them from their siblings. Cannibals were isolated after the first cannibalistic event and raised individually in plastic boxes (18.5 × 13.5 × 4.0 cm) to metamorphosis. An equal number of noncannibal siblings from each clutch where cannibals were identified were also raised individually in an identical manner. All animals were fed *ad libitum* on the same prey items. After metamorphosis but before complete gill resorption, tissues of 27 cannibalistic and 21 typical animals were removed for electrophoretic analysis. Animals were then fixed in 10% neutral buffered formalin and preserved in 65% ethanol. Dates of cannibalistic events and the initiation of metamorphosis (defined by the 1st day of observable resorption of gill filaments) were recorded for each animal. Dates were converted to days from April 11, the average date of hatching (± 2 days), to achieve approximate larval ages.

Preserved animals were examined and measured. Animals were sexed by the presence of ovaries or testes. Inside the mouth, vomerine teeth were examined to determine whether they most closely resembled the pattern found in typical morphs or the hypertrophied condition of cannibal morphs. (These differences are obvious; see figure in Lannoo and Bachmann (1984).)

The following measurements were taken for morphometric analysis and were based on measurements that differentiate cannibal and typical morphs (Pierce et al. 1983): snout-vent length (SVL), dorsal head length (DHL), head width at angle of jaw (HWA), head width at posterior mouth margin (HWM), interocular distance (EYD), and eye to snout distance (EYS). SVLs were not significantly different between cannibals and typicals at metamorphosis (Table 1), permitting comparisons between absolute values for these measurements. Comparisons between cannibals and typical individuals were made using pooled *t*-tests (Snedecor and Cochran 1967). We decided *a priori* to consider probability values of 0.05 or less as indicative of a significant difference.

For electrophoretic analysis, processing of salamanders, tissue extraction, separation and storage, and enzymes were identical to that described in Bogart et al. (1985). Procedures for horizontal starch gel electrophoresis likewise paralleled the methods of Bogart (1982) and Bogart et al. (1985). Buffers and stains were those employed by Selander et al. (1971), Shaw and Prasad (1970), and Clayton and Tretiak (1972) with some slight modifications.

Results

Twenty-seven out of 268 *Ambystoma tigrinum* were cannibalistic. Cannibalistic individuals grew faster and metamorphosed significantly earlier than larvae that were not cannibalistic. Figure 1 illustrates size differences among three siblings: a recently metamorphosed cannibal, a larval cannibal, and a noncannibal (or typical) individual. Cannibals metamorphosed at an average of 100 days posthatching, whereas typicals metamorphosed in an average of 135 days (Table 1A). Interestingly, there was no difference in size at metamorphosis between cannibal and typical individuals; both metamorphosed at about 56 mm SVL (Table 1B).

There were no electrophoretically detectable differences between cannibal and typical individuals in either allele com-

TABLE 1. Number of days to metamorphosis (A) and size at metamorphosis (B) for cannibalistic and noncannibalistic *Ambystoma tigrinum* larvae

Larval type	N	Mean \pm SE	t-value	P
(A) Days to metamorphosis				
Cannibals	27	100.3 \pm 6.9	3.8	<0.0005
Noncannibals	21	137.5 \pm 6.6		
(B) Size at metamorphosis (SVL, in mm)				
Cannibals	27	55.1 \pm 0.8	0.1	>0.4
Noncannibals	21	55.3 \pm 1.0		

NOTE: Means \pm SE, *t*-values, and probability levels are given. Note that while cannibals metamorphosed in fewer days, at metamorphosis both cannibals and noncannibals were the same size.

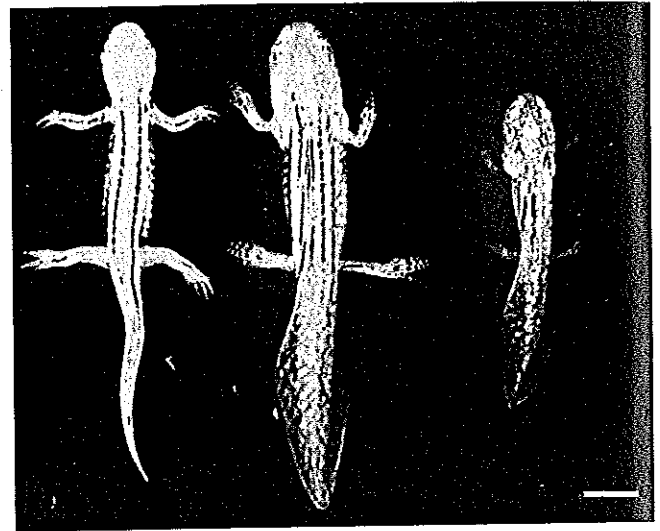


FIG. 1. Relative size differences among *A. t. tigrinum* siblings from Kelley's Island. From left to right: a recently metamorphosed cannibal; a larval cannibal; and a noncannibal larva. Scale bar, 2 cm.

position or frequency at the 35 enzyme loci examined (cf. Bogart et al. 1987). We also found no differences in body proportions between cannibal and typical individuals that had just undergone metamorphosis (Table 2), with one exception: cannibals had eyes set wider apart (Table 2D). Of the 25 cannibals that were sexed, 12 were male and 13 were female. Eight of 20 sexed typical animals were male and 12 were female. Vomerine teeth were not hypertrophied in any animal.

Discussion

Cannibalistic events clearly trigger accelerated growth and early metamorphosis in otherwise typical morph larvae in the Kelley's Island population of *A. t. tigrinum*. *Ambystoma t. tigrinum* from a population in Manitoba raised under identical laboratory conditions in 1987 did not cannibalize (L. Lowcock, personal observation). Three general questions emerge from this observation: (i) What are the potential mechanisms underlying this phenomenon? (ii) To what extent may this simply be a laboratory occurrence? (iii) Regardless of proximal factors governing its occurrence, what are the possible functional and ecological implications of this phenomenon?

At least two mechanisms may underly accelerated growth

TABLE 2. Morphometric comparisons between cannibal and typical *Ambystoma tigrinum* larvae

Larval type	N	Mean \pm SE (mm)	t-value	P
(A) Head width at widest portion				
Cannibals	27	13.9 \pm 0.2	1.4	>0.05
Noncannibals	21	13.4 \pm 0.3		
(B) Head width at mouth corners				
Cannibals	27	9.4 \pm 0.2	0.8	\geq 0.4
Noncannibals	21	9.6 \pm 0.2		
(C) Dorsal head length				
Cannibals	27	20.1 \pm 0.3	0.7	\geq 0.4
Noncannibals	21	20.5 \pm 0.3		
(D) Eye to eye distance				
Cannibals	27	6.1 \pm 0.1	2.7	<0.005
Noncannibals	21	5.8 \pm 0.1		
(E) Eye to snout distance				
Cannibals	27	4.6 \pm 0.1	0.9	\geq 0.4
Noncannibals	21	4.2 \pm 0.1		

NOTE: Parameters compared are head width at the widest point, head width at posterior mouth margins, dorsal head length, distance between the eyes, and eye to snout distance. Means \pm SE, t-values, and probability levels are given. Note that there were no differences between cannibals and noncannibals except that cannibals had eyes that were slightly farther apart.

after cannibalistic events. First, larvae may gain a growth advantage over their zooplanktivorous siblings simply by eating larger prey and thereby incorporating more energy. Fast-growing cannibalistic larvae might grow just as fast eating frog tadpoles, crayfish, dragonfly naiads, or other large prey. Within this strictly energetic argument, accelerated growth may be somewhat enhanced by eating amphibians if the anabolic--catabolic pathways, and therefore digestive energy expenses, were reduced. This argument cannot, however, account for some of our observations. In particular, noncannibal controls raised individually on the same prey items as identified cannibals ate the largest prey they could ingest, including rainbow trout fry, earthworms, and tadpoles, without showing signs of accelerated growth. Additionally, the noncannibal larvae that remained together in pans never attempted to ingest siblings at any size; cannibalism in the crowded pans was not observed after about 30 days of larval growth. There could, however, be a developmental window during which time eating larger prey results in accelerated growth. After this time, small larvae can never achieve the size of larger conspecifics, despite eating large prey.

Secondly, hormone, particularly thyroxine, levels may be enhanced in cannibalistic individuals by uptake from prey salamanders (Gorbman 1964). Cannibalistic individuals receive one extra dose of thyroxine when they ingest one conspecific, which may be responsible for accelerated growth. This idea originates from experiments by Gudernatsch (1912), who fed various amphibian organs to live amphibians and reported enhanced growth with thyroid gland ingestion. Preliminary results by D. Pfennig (personal communication) suggest that thyroxine may be involved in inducing the cannibal morph in *Scaphiopus*. However, while thyroxine surges may explain fast growth and early metamorphosis in the larvae described by us, we should point out that they cannot explain the fast growth and unusual morphology of true cannibal morphs (*sensu* Powers 1907). Collins and Cheek (1983) showed that cannibal

morphs may develop their morphology without actually being cannibalistic. Their data suggest that crowding *alone* induces this morph. It may be that *A. tigrinum* larvae release chemicals into the water that are taken up cutaneously, orally, or branchially and that only some larvae respond to these chemicals by developing the cannibal morphology (Collins and Cheek 1983).

The laboratory conditions under which the larvae described in this study were raised were characterized by abundant prey but relatively crowded conditions. These conditions are not unusual for salamander larvae that inhabit temporary wetlands (Semlitsch 1987). Crowding alone does not appear to induce these fast-growing morphs because (i) fast-growing and typical larvae were initially crowded equally; (ii) we observed that in every case the growth spurt was preceded by a cannibalistic event; subsequent isolation and release from competition for food was not a factor, because controls raised in isolation did not exhibit accelerated growth; and (iii) larval *A. tigrinum* from Manitoba raised under identical conditions in 1987 were not cannibalistic.

Because alternative prey were available, we presume that cannibalistic individuals were electing to take conspecifics. Whether this behavior is part of an adaptive strategy to grow faster and metamorphose quicker, or whether it simply reflects a preference for larger prey, we cannot say. However, the relatively large numbers of cannibals we observed (10% of our cultured larvae) suggest that salamanders are not reluctant to take conspecifics, even if they are siblings, and further suggest that cannibalism would occur in nature if conditions were conducive to it, as is likely the case on Kelley's Island (where the density of viable *Ambystoma* sp. eggs, and subsequently larvae, can be as high as several thousands per cubic metre; L. Lowcock, personal observation). The fact that cannibalism has been reported in other salamanders (e.g., in the family Hynobiidae; Kusano and Kusano 1985) and anurans (see Crump 1983 for references) indicates that this is not an uncommon phenomenon.

Crump (1983) has pointed out that cannibalism in anurans may be a built-in mechanism which allows certain individuals to survive in crowded and food-poor conditions. This advantage almost certainly holds for salamanders also, but the *A. tigrinum* group has carried this tendency further in two ways: (i) in the expression of a cannibalistic morph (as originally described by Powers (1907)) which despite being facultative, may physically predispose an individual towards cannibalism, and (ii) in accelerated growth after a cannibalistic event, evident both in true cannibalistic morphs and in our cannibals with normal morphology. One anuran, *Scaphiopus multiplicatus*, also exhibits accelerated growth and development in association with cannibalism (Pomeroy 1981). It is clear that both the mechanisms and, indeed, the entire phenomenon of cannibalism in *A. tigrinum* require further study.

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