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EFFECTS OF DENSITY AND ENVIRONMENTAL STIMULI ON AGGREGATIVE BEHAVIOR AND GROWTH IN *BUFO AMERICANUS* TADPOLES

By

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ABSTRACT

EFFECTS OF DENSITY AND ENVIRONMENTAL STIMULI ON AGGREGATIVE BEHAVIOR AND GROWTH IN *BUFO AMERICANUS* TADPOLES

By

D. Conrad Griffith

Light, heat, and food were tested as possible environmental stimuli which attract *Bufo americanus* tadpoles to aggregations. Numbers of tadpoles in different sectors of an artificial pond were recorded with one environmental stimulus present, and compared with a no-stimulus control. Food, light combined with heat, heat alone, and light alone were observed to attract tadpoles, their relative efficacies being in the order presented.

Tadpoles isolated from early cleavage were tested for aggregational tendency when introduced to a group at different developmental states. Much variabilty was observed in both isolates' and pool-raised tadpoles' reactions to conspecifics; no trends were observed.

Length and developmental state were measured for tadpoles in isolation, a pool environment, and tanks with one individual (semiisolate) separated by a screen partition from a group (cohorts). Both isolates and semiisolates developed faster than either cohorts or pool-raised tadpoles.

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I wish to thank all the people who have helped me with this study. I am indebted to Dr. Robert Robbins for his guidance. His knowledge of everything from statistics to grammar helped me bring order out of chaos. I would also like to thank the other members of my graduate committee, Drs. John King and Hiram Fitzgerald. Their input was much appreciated. A very special thank-you goes to Bruce Jayne who helped me with all stages of the study, from collection of specimens to the kind use of his word processor. To all the other individuals who provided helpful comments and support, I extend my sincere thanks. Finally, to my parents, David and Mary Jane Griffith, I would like to say that I couldn't have done it without your support from the home front.

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INTRODUCTION

The larvae of anuran amphibians exhibit many specialized adaptations to an aquatic existance. Behavioral interactions among tadpoles manifest themselves in several ways, among the most overt and well documented being the formation of aggregations. While the majority of anuran species' tadpoles do not aggregate, numerous examples of aggregation are documented in the families Pelobatidae, Ranidae, Bufonidae and Rhinophrinidae.

Several different types of aggregations have been described, and almost as many systems of aggregate classifications have been suggested. For clarity, I will employ the classification system of Bragg (1954, 1968). He defines, for the spadefoot toad Scaphiopus hurterii, two major types of aggregations: (1) those based on communal feeding, and (2) those based on metamorphosis. Feeding aggregations are further broken down into asocial aggregations, in which individuals cluster only around a food source, and social aggregations, that may involve feeding, but do not require the presence of food (Table 1). Feeding aggregations can contain anywhere from three to thousands of individuals (Beiswenger, 1975) as long as there is an abrupt end to the group at its margins. Aggregations can be

Table 1. Types of aggregations.

Feeding	social	aggregate even when food is absent
	asocial	aggregate only when food is present
Metamorphic	social	aggregate in the stages just before metamorphosis is complete
School	social	involves coordinated swimming, parallel orientation

Table 2. Aggregational classification. Degrees of contact and percent area in which tadpoles are found determine classification.

D1 - not aggregating

D2 - 1-5 cm apart, 20-50% of area covered by tadpoles

D3 - mostly in contact, 51-84% of area covered by tadpoles

D4 - all tadpoles in contact, one layer thick, cover 90-99% of area

D5 - all tadpoles in contact, multiple layers thick, cover 100% of area

- based on Beiswenger (1975)

stationary or moving, demonstrating coordinated swimming and/or head butting (Wassersug and Hessler, 1971; Beiswenger, 1975). Beiswenger has further classified tadpole aggregations based on number of individuals present, amount of contact beween individuals and percent area they cover (Table 2).

Tadpoles come from far-reaching locations in a pond to a given area in which they aggregate. The tadpoles are believed to use specific environmental stimuli to converge on the same general location. They may simply exhibit this asocial aggregation, using the environmental stimulus as a focus of attraction, or they may move close enough together that they can interact socially (Beiswenger, 1977). Environmental factors which elicit this taxic behavior to a given area of the pond include heat, light, stream currents (rheotaxis), oxygen concentration, substrate pattern and presence of food (Wassersug, 1973). Details on what is known about each of these environmental cues can be found in the Literature Review section of this thesis.

Bufo americanus tadpoles typically form dense, conspicuous aggregations containing hundreds to thousands of individuals (Wassersug, 1973; Beiswenger, 1975, 1977; Waldman and Adler, 1979; Waldman, 1980). These tadpoles remain relatively active on the bottom of the pond throughout the larval period. Social aggregations are first formed by tadpoles of Gosner stage 25 (1960), about the time the opercular flaps cover the gills (Beiswenger and Test, 1966; Beiswenger, 1977).

One of the objectives of the current study was to evaluate quantitatively the ability of *B. americanus* tadpoles to respond to external environmental stimuli. I wished to determine if heat, light, and the presence of food can attract *Bufo* tadpoles. Due to time constraints and a limited number of test animals, my experimental apparatus necessitated that I pit one environmental stimulus against another on the same test. This had the added advantage of allowing me to compare the relative ability of the various stimuli to attract tadpoles.

A second objective of this study was to determine whether isolated *B. americanus* tadpoles will exhibit aggregational behavior. There is a great deal of population variability in aggregational behavior (Wassersug, 1973). Previous studies on aggregating tendency of isolated tadpoles have involved tadpoles from very socially-aggregating populations. I wished to determine if less socially-inclined tadpoles would also exhibit such a strong tendency to aggregate after isolation, if a latency period occurs, or if no aggregation occurs. No study to date has tested isolated tadpoles from different age classes for their tendency to aggregate, so I wished to determine if age differences in aggregational tendencies exist.

Tadpole growth rate is negatively correlated with density; the more tadpoles per unit area, the slower on average they grow. This phenomenon may be called the crowding effect. A notable exception to this occurs in

R. dorsalis which grow poorly in low density situations (Foster and McDiarmid, 1982). Rose (1960) demonstrated that while the mean growth rate is suppressed during crowding of *Rana pipiens* tadpoles, a few individuals grow at an accelerated rate. The rate of development of these few tadpoles is independant of density. Two distinct size classes are observed in *R. dorsalis* tadpoles (Stuart, 1961).

The third purpose of this study was to look into the problem of differential growth in *Bufo americanus* tadpoles. I wished to test the merit of Wassersug's (1973) statement that *B. americanus* tadpoles are not very susceptible to the crowding effect. I also wished to provide some insight into the mechanism(s) by which aggregating tadpoles inhibit each others' growth. Chemo/physical substances, psychological stress, and exploitation competition have all been suggested as possible mechanisms for this phenomenon.

LITERATURE REVIEW

I. Environmental Stimuli

There is no one particular cause for tadpole aggregations. Among the most cited causes is the enhancement of food gathering capabilities by a group. In bottom-dwelling detritis feeders, a group may disturb the bottom more than an individual, thus exposing more food. This has been been demonstrated with *Scaphiopus hurterii* (Bragg, 1965, 1968). When temporary breeding ponds begin to dry up, food may become patchy and scarce, further leading to the likelihood of aggregational behavior (Bragg, 1954, 1965, 1968).

Predation may be another reason for aggregational behavior. Certain anuran species' tadpoles have epidermal poison glands, notably *Bufo* tadpoles, which secrete bufonin toxins. Experiments have shown that bluegill sunfish (*Lepomis macrochirus*) prefer tadpoles without poison glands, and in fact, learn to avoid the conspicuous dark mat of aggregating *Bufo americanus* tadpoles (Voris and Bacon, 1966). Epidermal glands may also be a source of chemical alarm signals (Pfeiffer, 1966) which would most benefit members of a group. The fright reaction in tadpoles is strong. A dead or dying tadpole causes the whole aggregation to scatter

(Wassersug, 1973). Finally, Carpenter (1953) hypothesized that *Rana pretiosa* tadpoles may be aggregating to avoid attachment of parasitic leeches (a problem for tadpoles in mountain lakes) and to dislodge those leeches which have already attached themselves to the tadpoles.

Bufo boreas tadpoles are highly pigmented. A group of such tadpoles will absorb more heat than an individual, thereby raising the temperature of the surrounding water several degrees Celsius (Brattstrom, 1962). This allows the poikilothermic tadpole to increase its metabolic rate, causing development to occur faster, a highly advantageous trait in a temporary pond.

Many species' tadpoles prefer the warmest areas in the pond, in the 25-30°C range, including *Bufo boreas* (Brattstrom, 1962), *Bufo canorus* (Mullally, 1853), *Bufo punctatus* (Tevis, 1966), and *Bufo americanus* (Beiswenger and Test, 1966; Beiswenger, 1977). Numerous other species from the genera *Bufo*, *Hyla*, *Rana*, *Pseudacris*, and *Scaphiopus* exhibit this behavior (Rugh, 1962; Wassersug, 1973). Some species' tadpoles do not prefer the warmest areas, especially those species which live in mountain streams. For example, *Ascaphus truei* (deVlaming and Bury, 1970) prefers water below 22°C.

Bufo americanus tadpoles are positively phototaxic (Beiswenger, 1977). In the morning, they move to the shallowest areas of the pond in response to light. *Hyla versicolor* tadpoles are also positively phototaxic when disturbed (Noble, 1954). Franz (1913) has described a number of other species whose tadpoles exhibit the same results. Wassersug (1973) claims the phototaxic response varies greatly between species.

Rheotaxis has been described as a most visible factor affecting asocial aggregation (Wassersug, 1973). Those tadpoles which develop in streams are regularly observed to head upstream. Tactile (lateral line) and visual cues are believed to be involved in this response.

In oxygen-deficient water, tadpoles come to the surface to gulp air (Bragg, 1954; Stuart, 1961; Wassersug, 1973). Shallow water may be sought by individuals in oxygen-deficient situations, as there is more dissolved oxygen in shallow areas (Wassersug, 1973).

Wiens (1970) experimentally demonstrated a species-specific selection for certain substrate patterns in Rana aurora and Rana cascadae tadpoles. A similar phenomenon has been suggested for Bufo americanus (O'Hara, 1974). In nature, such a phenomenon would be difficult to detect.

Presence of food appears to be an almost universal stimulus to form (typically asocial) aggregations. Risser, in his studies of Bufo americanus (1914), was one of the first to document the fact that tadpoles use olfactory cues to locate sources of food. Bragg (1954, 1968) has suggested that food may be the single most important stimulus to aggregate.

Once tadpoles are sufficiently close together, they may begin to aggregate socially (in the absence of food) (Beiswenger, 1977). Visual and/or lateral line stimuli, which are useful only at close range, may be the cues used by social aggregates (Wassersug, 1973). _Xenopus_ _laevis_ tadpoles can aggregate using only visual cues (Wassersug and Hessler, 1971), but the addition of lateral line stimuli allows for parallel orientation (Katz et al., 1981; Lum et al., 1982). _Bufo_ _boreas_ may need mechanical/tactile stimulation (i.e., actual physical contact) as well as visual cues to aggregate socially (Wassersug, 1973).

II. Isolate/Aggregation

Studies have shown that within a social aggregation,the position of individual tadpoles may not be random. Allee (1931) suggested "similar" tadpoles tend to remain together in unusual surroundings to minimize the "disturbing effects" of the strange environment. _Bufo_ _woodhousei_ tadpoles tend to associate with individuals in the same size class within the aggregation (Brenden et al, 1982), although this is not a strong tendency. A most intriguing discovery is that both _Bufo_ _americanus_ and _Rana_ _cascadae_ tadpoles, when given a choice, prefer to associate with siblings over non-sibs (Waldman and Adler, 1979; Waldman, 1980; Blaustein and O'Hara, 1981; O'Hara and Blaustein, 1981). _R_. _cascadae_ tadpoles reared with sibs or a mixed sib/non-sib group preferred to associate with sibs (O'Hara and Blaustein, 1981). Those isolated since Gosner (1960) stage 12 (yolk plug) also preferred siblings when given a choice. Those reared with sibs preferred unfamiliar sibs over familiar non-sibs (Blaustein and O'Hara, 1981). _B_. _americanus_ tadpoles within an aggregation tend to be closer to sibs than non-sibs. In some cases, two aggregations formed, one for each sib group (Waldman and Adler, 1979). *B. americanus* tadpoles reared with sibs or in isolation preferred associating with sibs over non-sibs. Those reared in a mixed group showed no tendency to prefer siblings (Waldman, 1980, 1981). These observations have been taken one step further. Tadpoles can discriminate half sibs from full sibs. *R. cascadae* tadpoles prefer full sibs over half sibs, and half sibs over non-sibs. It has been reported that they can even distinguish between maternal and paternal half sibs, and prefer maternal ones (Blaustein and O'Hara, 1982). *B. americanus* tadpoles raised in isolation can also distinguish paternal half sibs from full sibs (prefer full sibs), but they cannot discriminate between maternal half sibs and full sibs (Waldman, 1981). The above results are summarized in Table 3.

The most commonly cited explanation for these behaviors is a kin selection argument. *B. americanus* tadpoles, being darkly pigmented, form a conspicuous black mat in the pond. Since they are unpalatable, a predator would soon learn to associate their conspicuousness with their distastefulness. By remaining in close proximity to kin, the chances of a tadpole being eaten are reduced, as one or a few individuals act as altruists, giving their lives (anthropomorphically speaking) so that their relatives may be spared (Waldman and Adler, 1979; Waldman, 1982). The same kin selection argument is applied to *R. cascadae* tadpoles, even though they are not

Table 3. Summary of experiments on sibling recognition.

Species	Reared With	Options	Prefer
R. cascadae	mixed sibs/non-sibs	sibs/non-sibs	sibs
	non-sibs	unfamiliar sibs/familiar non-sibs	unfamiliar sibs
	isolation	sibs/non-sibs	sibs
	mixed group	full-sibs/half-sibs	full-sibs
	mixed group	half-sibs/non-sibs	half-sibs
	mixed group	maternal half-sibs/paternal half-sibs	maternal half-sibs
B. americanus	mixed sibs/non-sibs	sibs/non-sibs	no preference
	isolation	sibs/non-sibs	sibs
	isolation	paternal half-sibs/full-sibs	full-sibs
	isolation	maternal half-sibs/full-sibs	no preference

distasteful to predators. Tadpoles may be able to warn each other of a predator's presence, and by being close to kin, a tadpole is most likely to receive an inclusive-fitness benefit by warning its neighbors (Blaustein and O'Hara, 1981).

It is currently unknown if sibling recognition is genetically based (Blaustein and O'Hara, 1981; O'Hara and Blaustein, 1981), learned (Waldman and Adler, 1979; Waldman, 1982) or a combination of the two (Blaustein and O'Hara, 1982). The mechanism of recognition appears to be chemo-olfactory (Blaustein and O'Hara, 1982), possibly pertaining to some factor in the jelly mass which surrounds a clutch of sibling eggs (Waldman and Adler, 1979; Waldman, 1982).

Similar studies performed on Rhinophrynus dorsalis tadpoles produced very different results. Isolates introduced to a mixed group of about 200, or a small aggregation of 10 siblings actively avoided the others for the first ten minutes. They became more tolerant of the group over the next few hours, but were never observed to join the aggregation (Foster and McDiarmid, 1982). It is unknown whether these isolates would continue to refuse to aggregate, or if they were going through a latency period, much like that exhibited by schooling fish reared in isolation (Shaw, 1960).

III. Growth Study

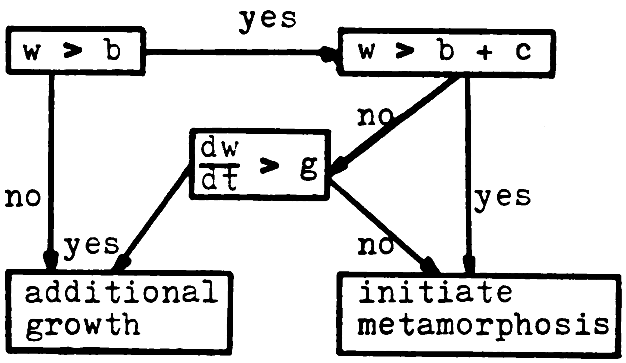
Rose found that water conditioned by the larger tadpoles was enough to inhibit growth of other tadpoles. It was hypothesized that algal-like cells (perhaps sloughed epithelial cells) found in the intestinal tract and feces of tadpoles was responsible for this growth inhibition (Richards, 1958, 1962). It is currently unknown exactly what these cells are, but they appear to resemble unicellular green algae. Licht (1967) found the algal-like cells in the feces of _Bufo valliceps_, _B_. _speciosus_ and _B_. _woodhousei_, and observed the same crowding effect Rose and Richards did.

Gromko et al. (1973) demonstrated the crowding effect in _R_. _pipiens_ in the absence of the algal-like cells. They suggested the phenomenon is a behavioral response to social stress, causing adrenocortical hormone secretion. This stress response may be due to increased contact with other tadpoles (Bilski, 1921; John and Fenster, 1975). Shvarts and Pyastolova (1970b) argue that if the inhibitor is a chemical substance, it would probably be a cellular metabolite which reduces the activity of the genes responsible for a particular stage of development of tissues or organs. Growth inhibition is such a widespread phenomenon in plants and animals, it would be difficult to explain the evolution of a special purpose growth inhibitor so many times.

Wilbur (1977) proposed yet another explanation for the crowding effect. He hypothesized that larger tadpoles were the ones better able to exploit resources. If food were a limiting resource, larger tadpoles could monopolize it, preventing the smaller ones from growing as quickly (Brockelman, 1969; Wilbur, 1977). Wilbur and Collins (1973) proposed a simple model which diagrams how an exploitation scheme might work (Figure 1).

High predation rates may prevent the crowding effect in nature. It may only be an artifact of laboratory conditions that we see this reduced growth rate and delayed metamorphosis (Brockelman, 1969). Regardless of the cause of the crowding effect, it is highly specific in its effects on *Rana arvalis* (Shvarts and Pyastolova, 1970a), with the greatest effect on the most closely related tadpoles. Shvarts and Pyastolova believe the crowding effect is a mechanism which ensures genetic diversity. They claim that growth inhibition is greatest among close relatives. By only permitting a few individuals from each family to survive (the ones which can suppress the growth rate of the others), it is more likely that more families will be represented in the next generation. This mechanism, of course, necessitates a group selection argument for its existence, as family-specific growth inhibition would not normally be expected to occur due to the advantages of kin selection.

Shvarts and Pyastolova (1970b) found that the presence of a group of small *R. arvalis* tadpoles actually accelerates the growth rate of the larger ones at the same time it inhibits the growth of the smaller ones. This gets the larger ones out of the ponds quickly, allowing the smaller ones a chance to utilize the remaining resources in the



w = weight of tadpole
b = minimum size necessary to metamorphose
b + c = maximum size at which metamorphosis must occur
g = growth rate below which it is advantageous to initiate metamorphosis

- from Wilbur and Collins (1973)

Figure 1. Potential size difference model. Tadpole continues to grow if its growth rate is "good" enough, but will metamorphose if it is not.

temporary pond on the outside chance that they may metamorphose before it dries up.

Wassersug claims some species' tadpoles, such as *Bufo americanus*, are less susceptible to growth inhibition due to selection against it. Constant selection pressure for individuals which are not affected by growth inhibition may have created a species-wide immunity to the crowding effect. Other species may deal with the problem by metamorphosing at a smaller size.

GENERAL METHODS

I collected two pairs of Bufo americanus toads in amplexus on the night of May 18, 1984. One pair was obtained on Bennett Road (on the road itself) one mile south of the Michigan State University campus. The other pair was found in a small temporary pond, about 500 meters south of Bennett Road. Each pair was transferred to a 3.5-liter plastic bucket where oviposition occurred. Fertilized eggs were collected on the morning of May 19th, within 12 hours of ovulation.

Seven-hundred tadpoles were raised under laboratory conditions from Gosner stage 9 (late cleavage) to stages 21-26 in a circular plastic wading pool, 91.5-cm diameter, filled 1.5-cm deep with dechlorinated tap water. Air and water temperature remained a nearly constant 22°C. Light was provided by overhead fluorescent fixtures set on a 12L, 12D cycle. The bottom of the pool was divided into 24 sectors, each with an area of 273.7 cm^2, by strips of black electrical tape affixed to the inside of the pool (Figure 2). Tadpoles were fed boiled lettuce ad libitum. Water in the pond was changed at three-day intervals, using dechlorinated tap water.

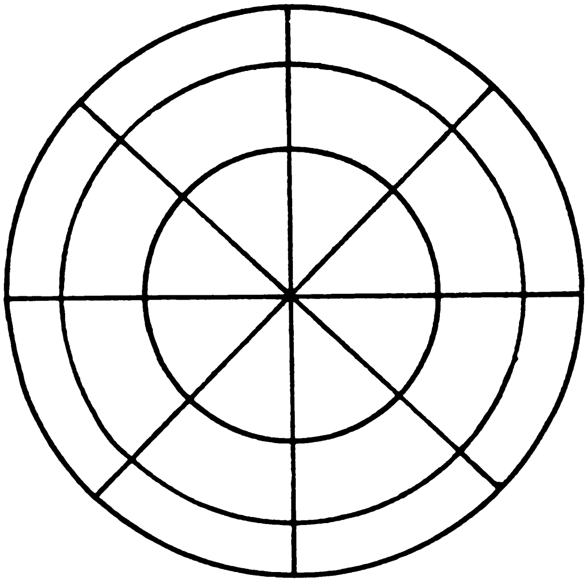


Figure 2. Test pool. Pool is divided into 24 sectors of equal area.

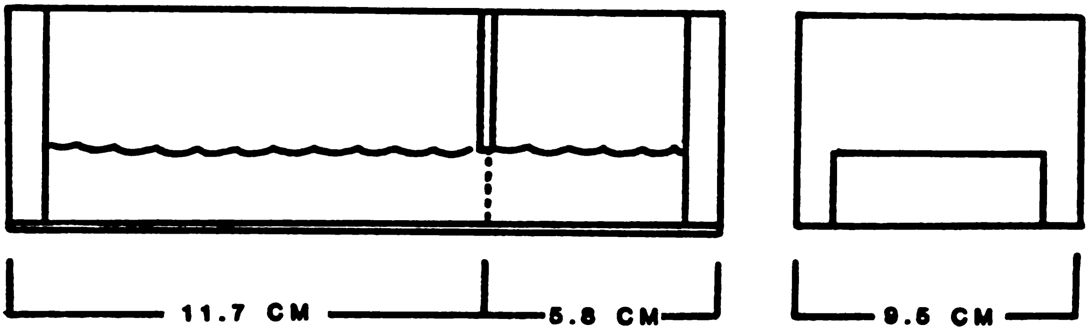


Figure 3. Special tank. On left is front view of tank as seen through clear plastic front. On right is clear plastic divider with screen.

Fifty tadpoles were raised in isolation in one-pint opaque plastic cups, 9-cm diameter, filled 1.5-cm deep with dechlorinated tap water. In addition, 24 special tanks (17.5 cm X 9.5 cm) were constructed of plywood and white pine (Figure 3) and painted gloss white inside. A screened partition made of 1-mm^2 mesh nylon window screen glued to a clear Plexiglas frame was positioned to isolate one third of the tank. The tanks were lined with clear plastic film. I was careful to expose the tadpoles only to inert plastic materials and silicone glue to prevent chemical or metal ion contamination of the water. A single tadpole was raised on the side with the larger area (two-thirds of the tank), and a group of 11-19 tadpoles was raised on the side with the smaller area (one-third of the tank). Care and feeding of tadpoles in the pint cups and special tanks was the same as for those in the pool.

ENVIRONMENTAL-STIMULUS EXPERIMENTS

A. Overall Introduction

I wished to test three environmental stimuli in their ability to attract B. americanus tadpoles: heat, light, and presence of food. Due to time constraints and a limited number of test animals, I chose to look at two stimuli at once, one stimulus on each side of the pool. This had the added advantage of allowing comparisons of the relative abilities of the three stimuli to attract tadpoles.

B. americanus tadpoles in the pool were raised to stages 24-27 before testing began. Tadpoles are mobile at stage 21, so they had been free to move about for several days. A removable cardboard partition was fashioned to prevent light from the fluorescent fixtures from reaching one side of the pool. This partition went down to the water's edge, yet allowed the tadpoles to move freely between sides. Two incandescent lamps, one with a 15-watt bulb and the other with a 50-watt bulb were affixed on the side opposite that with the fluorescent light. The intensities of the incandescent and fluorescent light sources were adjusted so as to be nearly equal. The incandescent light's intensity was 93.4% that of the fluorescent light, as measured at the water surface by a Photovolt model 502M photovoltmeter.

Due to time and equipment constraints, not all combinations of the three environmental stimuli were tested. I looked at those which were possible to set up within the time framework I had to work with, and complied with the physical constraints of my laboratory. A summary of these combinations is found in Table 4.

In each test, the starting positions of the tadpoles were initially randomized by thoroughly mixing the water. This also reduced the likelihood of the formation of an oxygen-gradient. The environmental cues were established in either side of the pool and the tadpoles were allowed to move about freely for 15-20 minutes, after which I counted the number tadpoles on each side. Four such trials were made for each test (exception: those with heat as the stimulus on one side - see Table 4).

Table 4. Combinations of stimuli tested.

Stimulus 1	vs.	Stimulus 2	No. Trials
heat		darkness	1
light, heat		light	4
food		light	4
light, heat		food	4
light, heat		light, food	4
darkness		darkness	4
light in center		darkness	3

B. Pilot Study - darkness control

Introduction

My first objective was to get some baseline data concerning what tadpoles do in the absence of any external stimuli. The possibility exists that tadpoles continue to aggregate without external stimuli, or a random orientation may occur, although a behavior intermediate to the two could also be possible.

Procedure

As previously outlined, initial tadpole orientation was randomized. I then turned off the lights which resulted in complete darkness. There was no source of food, nor any known thermal gradients in the pool. The tadpoles were allowed to acclimate for 15-20 minutes, after which a red, low-wattage overhead lamp was switched on. I then quickly recorded the number of tadpoles in each sector. Because *B. americanus* tadpoles are rather lethargic, very few changed position during the counting, which was done with a thumb-activated hand counter. Four such trials were made, each on a different day.

Results

I analyzed these four darkness trials in two ways. First, I looked at the number of tadpoles in the outside, middle, and inner rings, of eight sectors each. Note that results are reported only for analysis on the four trials combined. Results of analyses on the four separate trials were the same as the combined results. The outside ring contained 1074 tadpoles, the middle ring 855, and the inner ring 906 tadpoles. I compared two rings at a time using chi-square analysis (Table 5). My expectation was an equal number in each ring, since each sector and hence, each ring was of equal area. Next, I analyzed the data by "pie wedge", adding the outer, middle and inner sectors for each wedge together (Figure 4). Here, each of the four trials was analyzed separately (Table 6).

The results of the first analysis indicate that there is a preference for the outside ring of the pool. There appears to be no significant difference between the middle and inside rings. Results of the second analysis are more difficult to interpret. In every trial, there is a significant deviation from random, but the wedges with high and low counts are not the same in each trial (Kruskal-Wallis test, $H = 12.95$, $\underline{p} \leq .10$). These data suggest that even in darkness, there appears to be a tendency to aggregate, usually toward the outside, but not necessarily in the same location around the pool each time.

In order to provide accurate values for the expectations in future analyses, I needed a way to take this natural aggregating tendency into account. To do this, I ranked the sectors in each darkness trial according to the number of tadpoles per sector. I calculated the proportion of the total number of tadpoles in each sector. I then averaged the

Table 5. Results of ring analysis, darkness test. Outside, middle and inner rings of 8 sectors were compared for number of tadpoles present by chi-square analysis.

Comparison*	X^2	Significance	Preference*
o - m	24.9	$p \leq .005$	o
m - i	1.5	(n.s.)	-
o - i	14.3	$p \leq .005$	o

* o = outside ring
m = middle ring
i = inside ring

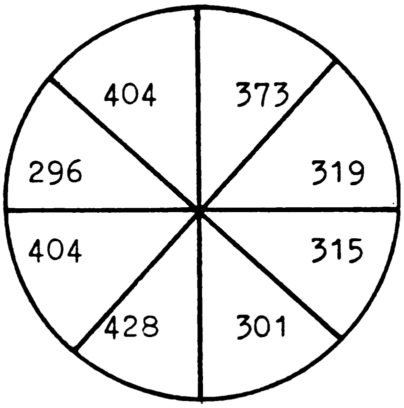


Figure 4. Number of tadpoles in "pie wedges". Total number of tadpoles in all four darkness trials are shown.

Table 6. Results of "pie wedge" analysis, darkness test. All eight "pie wedges" were compared by chi-square analysis for four trials.

Trial	X^2	Significance
1	28.6	$p \leq .005$
2	34.9	$p \leq .005$
3	41.6	$p \leq .005$
4	16.8	$p \leq .025$
total	55.6	$p \leq .005$

proportions for each rank for all four trials. For instance, each trial had a sector of rank 1 (fewest number of tadpoles). The mean proportion was calculated for the four sectors with rank 1, one sector from each of the four trials. The result was 24 mean proportions (Table 7). These were used as expectations in experimental tests. In the following analyses, I ranked the sectors in each trial as described above, and compared the observed proportion of the total number of tadpoles in a given sector with the corresponding expected proportion for that same ranked sector. I used these observed and expected proportions to do chi-square analyses. In actual practice, I converted expected proportions to expected numbers and compared these with observed numbers.

This method of analysis tests against the "worst case" for the expectation. In the worst case, the tadpoles naturally aggregate to the degree described in the pilot study. Any observed aggregation must be significant above and beyond this natural aggregation. In this respect, the test is quite good at preventing type II error in analysis, because any aggregation significant in this worst-case situation would also be so in anything less than the worst case.

Conclusions

It appears that *B. americanus* tadpoles will aggregate loosely even in the absence of any known environmental stimuli, specifically light, heat, an oxygen gradient, or

Table 7. Mean proportions for 24 ranked sectors, darkness test.

$p_1 = .017$
$p_2 = .022$
$p_3 = .027$
$p_4 = .029$
$p_5 = .031$
$p_6 = .032$
$p_7 = .034$
$p_8 = .035$
$p_9 = .036$
$p_{10} = .037$
$p_{14} = .044$
$p_{12} = .039$
$p_{13} = .043$
$p_{14} = .044$
$p_{15} = .045$
$p_{16} = .046$
$p_{17} = .049$
$p_{18} = .050$
$p_{19} = .051$
$p_{20} = .053$
$p_{21} = .055$
$p_{22} = .058$
$p_{23} = .062$
$p_{24} = .068$

food. As the statistics show, tadpoles tended toward the outside, but not toward any one particular area in the pool. My subjective observations confirm this; the aggregations were in the D1 to D2 range (Beiswenger, 1975), but no single area or substrate pattern was preferred. The water was slightly shallower toward the very edge of the pool, a consequence of its sloped sides. Perhaps tadpoles seek shallow water under the "assumption" that this is where food or some other important resource is located.

In any event, the data generated from this study were quite useful in looking at the rest of the experiments, as chi-square expectations could now be modified to reflect more accurately a null (no stimulus) expectation.

C. Food as a Stimulus

Introduction

Food was tested as a possible cue for aggregation. Food in vernal ponds is typically found in patches around the edges. Tadpoles may use some chemo-olfactory cue from the food to home in on it, hence forming asocial aggregations.

Procedure

Boiled lettuce leaves served as the source of food. When used as an experimental attractor, the food was not randomly scattered about the one side of the pool, but was confined to four sectors, randomly chosen for each trial. This more closely resembles the patchiness of the distribution of high quality food that is found in *B. americanus*' natural environment.

The tadpoles were not fed for at least a few hours before this test was run to ensure they were hungry. Tadpole starting location was randomized and the lettuce was added. I waited for 15-20 minutes, then counted the number of tadpoles in the four food-containing sectors and the eight other sectors on the same side of the pool for each of the four trials.

Results

In comparing the four food-containing sectors with the others on the same side of the pool (and in all subsequent tests), I used chi-square analysis with the expectations calculated earlier (see pilot study). A total of eight trials were looked at from two different tests, food versus fluorescent light, and food versus light and heat. In all cases, the food sectors were significantly preferred over the non-food sectors on the same side (Table 8).

Conclusions

Tadpoles grow as quickly as possible in order to metamorphose. As pointed out in the literature review, feeding rate and growth rate are directly correlated. It is therefore highly advantagous for a tadpole to be able to find sources of food. Since tadpole eyes are poorly developed, it may be that a chemo-olfactory stimulus is being detected by the hungry tadpole. Feeding aggregations are the most commonly described type of asocial aggregation (Bragg, 1954), and my results indicate that *B. americanus* tadpoles are quite capable of finding isolated sources of food, forming dense asocial aggregations about them.

Table 8. Food as a stimulus. First light, then light and heat were pitted against food. Number of tadpoles in food sectors was compared with number in non-food sectors on the same side of the pool using chi-square analysis.

- Food vs. light

Trial	X^2	Significance
1	75	$p \leq .005$
2	21	$p \leq .005$
3	84	$p \leq .005$
4	86	$p \leq .005$

- Food vs. light, heat

Trial	X^2	Significance
1	35	$p \leq .005$
2	290	$p \leq .005$
3	180	$p \leq .005$
4	20	$p \leq .005$

D. Hot Spots as Stimuli

Introduction

"Hot spots" are areas of increased light intensity and warmer temperature. This combination of two stimuli was looked at as a potential environmental cue for tadpole aggregation.

Procedure

When light and heat were to be used in combination, the incandescent lamps were used. The bulbs were approximately 25 cm from the water surface. They provided virtually the same light output as the fluorescent light, and were capable of raising the temperature of the water below them by 2°C.

Light intensity and temperature were not uniform in the incandescent light experiments, nor was the light from the fluorescent bulb of uniform intensity at all points on the pool. I measured light intensity and temperature in all 24 sectors with the the incandescent bulbs set up on one side and the fluorescent light on the other (partitioned by the cardbord divider). Light readings were made with a Gossen Luna-pro light meter at the water surface. Light meter readings ranged from 117 to 613 lux; temperature ranged from 19.4 to 21.1°C. Both stimuli were rescaled from 1 to 100 to give them equal ranges. It is not known whether the tadpole adds the two stimuli together or multiplies them in its perception of the total stimulus, so both methods are represented in Figure 5. In both cases, there were four sectors on the incandescent (light, heat) side whose light intensities and temperatures exceeded the others. These were the ones directly under the bulb. I have called these four sectors the hot spots; they are pictured in Figure 5. On the fluorescent (light, no heat) side, there were six sectors with light intensities greater than the others, and temperature was a constant 19.4°C on that side of the pool.

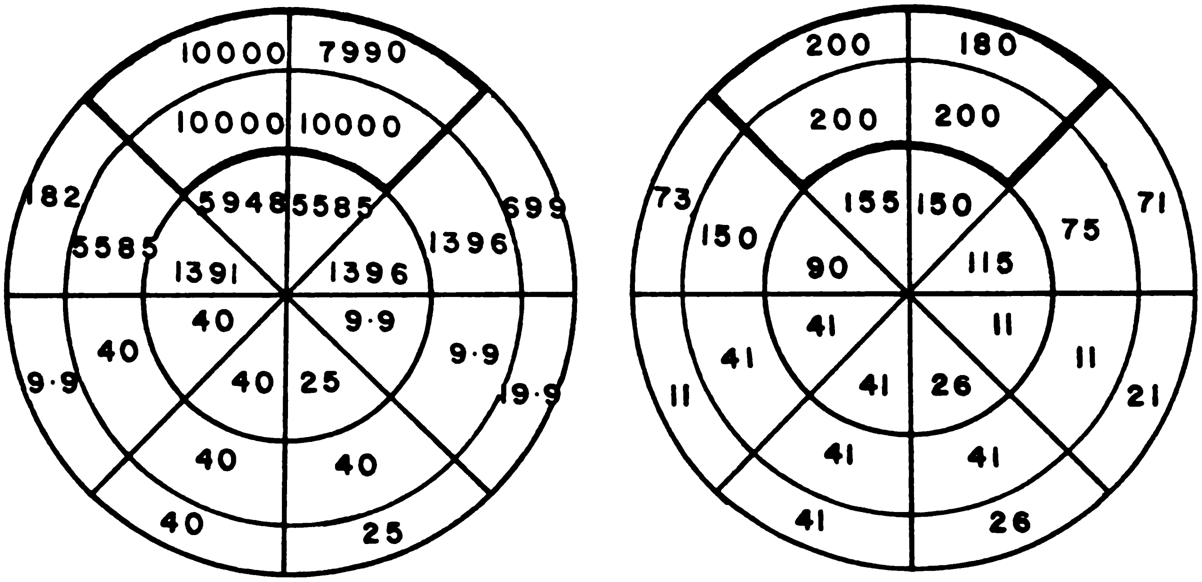
Counts of the number of tadpoles in each of the four hot spot sectors and the other eight sectors on that side of the pool were made.

Results

Hot spots were compared with non-hot spots on the same side of the pool. In this case, eight trials from the two tests of light and heat versus light, and light and of heat versus food were used. In seven of the eight trials, the hot spot sectors were significantly preferred over the non-hot-spot sectors (Table 9).

Conclusions

In nature, heat generally accompanies light and vice-versa. The pool's hot spots were therefore probably not an artificial anomaly. The edges of a pond are the most likely places for hot spots to occur, where the water is shallow enough that temperature can be quickly raised, and



LIGHT X TEMPERATURE **LIGHT + TEMPERATURE**

Figure 5. Sector light intensities and temperatures. Both stimuli are scaled from 1 to 100 and multiplied (left) or added (right) together, resulting in four hot-spot sectors, within heavy borders.

Table 9. Hot spots as a stimulus. First light, then food were pitted against light and heat (hot spots). Number of tadpoles in hot-spot sectors was compared with number in non-hot-spot sectors on the same side of the pool using chi-square analysis.

- Light, heat vs. light

Trial	X^2	Significance
1	18.9	$p \leq .005$
2	3.4	$p \leq .10$*
3	37.8	$p \leq .005$
4	5.9	$p \leq .025$

* not significant

- Light, heat vs. food

Trial	X^2	Significance
1	8.3	$p \leq .005$
2	23.0	$p \leq .005$
3	35.0	$p \leq .005$
4	120.0	$p \leq .005$

light easily penetrates to the bottom (where the benthic tadpoles are found).

Clearly, hot spots, as they are defined in this experiment, are attractors for tadpoles. The hot spots were strongly preferred over non-hot spots in all the trials but one, and in that case, they were preferred, but not significantly so. Any attractor of tadpoles is a candidate for a stimulus to aggregate. If, indeed, hot spots occur in nature, they would be ideal environmental stimuli for asocial aggregation.

E. Light as a Stimulus

Introduction

Light has often been cited as a stimulus for attacting tadpoles, as the literature review indicates. Whether or not it could act as a focus for aggregation in my experimental apparatus was tested next.

Procedure

The source of light by itself (with no heat) was the overhead fluorescent light under which the tadpoles had been raised. I counted the number of tadpoles in each of the twelve sectors on the fluorescent light side of the pool for each of the four trials. Recall from Section D (hot spots as stimuli) that six of the fluorescently-lit sectors had greater light intensities than the other six.

Results

I compared the six fluorescently-lit sectors with the highest light intensities with the six others on the same side, using the data from the experimental tests of light versus light and heat, and light versus food. In six of the eight trials, there were no significant deviations from the expectation (Table 10). In the two cases which did deviate, there were significantly more tadpoles in the less-intensely lit sectors than was expected.

Table 10. Light as a stimulus. First light and heat, then food were pitted against light. Number of tadpoles in the six most intensely-lit sectors was compared with the number in the six least intensely-lit sectors on the same side of the pool using chi-square analysis.

- Light vs. light, heat

Trial	X^2	Significance
1	.09	$p \leq .80$*
2	1.09	$p \leq .30$*
3	4.86	$p \leq .05$
4	1.23	$p \leq .30$*

* not significant

- Light vs. food

Trial	X^2	Significance
1	.46	$p \leq .50$*
2	.76	$p \leq .50$*
3	7.40	$p \leq .01$
4	.11	$p \leq .80$*

* not significant

Conclusions

In most cases, tadpoles were not preferentially aggregating in the sectors with the higher light intensities; in the two cases which did show a preference, it was toward the less-intensely lit sectors. These results indicate that fluorescent light itself may not be a good stimulus for aggregation.

The quality of the light may be very important for proper attraction of *B. americanus* tadpoles. Perhaps tadpoles are more sensitive to certain wavelengths of light than others. Since incandescent light has the added factor of heat, it may be that both stimuli are necessary to obtain a response. This hypothesis would seem logical, since in the vernal pond, one does not usually find light without heat. Future investigations could look into the ability of tadpoles to detect and respond to different wavelengths of light, all in the absence of heat.

F. Heat as a Stimulus

Introduction

It may seem like an unlikely situation where there is a source of heat in a pond but no light, but one purpose of this study was to attempt to dissect out the important component(s) of a complex stimulus-filled environment which the tadpole uses to aggregate. For this reason, heat alone was looked at as a potential environmental cue for aggregation.

Procedure

Heat was provided by a submersible aquarium water heater which was capable of raising the water temperature from 22°C nominal to 28°C. As with the food tests, I quickly counted the number of tadpoles in each sector on the heat side (and also on the other side of the pool) under a red, low-wattage lamp suspended from the ceiling.

Results

Unfortunately, only one trial of heat versus darkness was performed before the aquarium heater malfunctioned. The four warmest sectors (which were the same as the four hot-spot sectors) had a temperature of 28°C. They were compared with the other eight on that side (with temperatures ranging from 22-26°C), and no significant deviation from expected was observed ($X^2 = 2.84$, $\underline{p} \leq 0.10$). These four warmest sectors did, however, have significantly more tadpoles than expected when compared with the twelve sectors on the other side of the pool, each being 22°C ($X^2 = 5.34$, $p \leq 0.025$).

Conclusions

Heat, it appears, is a good attractor stimulus over a broad area, as witnessed by the significant deviation from expected number of tadpoles in the four warmest sectors versus the twelve on the other side. No significant difference from expectation was found when the four warmest sectors on the heat side were compared to the other eight on that side because temperature gradually went from 28° to 22° on that side; there was no sudden change in temperature between sectors.

Since the light-as-a-stimulus experiments showed that light (at least fluorescent light) is not a very good stimulus for attraction, and since the current study demonstrates that heat is a good "broad" area attractor, I suggest that it was the heat in the hot-spot sectors which attracted tadpoles to the general area, and the more intense light that brought them specifically to the four hot spot sectors.

G. Light in Pool Center

Introduction

As the pilot study indicated, there is a tendency for tadpoles to aggregate, even in darkness, toward the edge of the pool. I wished to determine if a light source in the center of the pool could draw the tadpoles away from edges, or if the edge preference took precedence. In addition, any bias toward one side or the other due to location of the light sources or other unforeseen reasons, could be checked this way.

Procedure

A 50-watt incandescent bulb was suspended 38 cm from the water surface with a 6-cm diameter cardboard tube (29 cm long) around it, acting to restrict the light into a 9-cm spot in the center of the pool. The light was far enough away that it did not affect water temperature below it. After randomizing initial location by mixing the water, I allowed the tadpoles 15-20 minutes to become accustomed to the stimulus, then counted the number of tadpoles in each sector. This procedure was repeated three times, each trial on a different day.

Results

I compared the outside, middle and inside rings of sectors, in pairwise combinations, using chi-square analysis.

As Table 11 shows, there were more tadpoles in the middle and inner rings than expected in darkness; there were significantly more in the middle than in the inner ring. There were significantly fewer tadpoles in the outer ring than expected.

Conclusions

Results of this experiment indicate that light appears to be a stronger attractant than the edges of the pool. This result is interesting when one considers that fluorescent light does not appear to be a good stimulus. Perhaps this difference has something to do with the quality of the light after all, as suggested by the hot spots and heat-as-stimuli experiments. In any event, this experiment demonstrates that at least one stimulus, incandescent light, is a greater attractor for asocial aggregation than the innate tendency to aggregate toward the pool edge. My subjective observations tend to confirm this. The densest aggregations were right around the 9-cm spot of light at the pool's center; density dropped off markedly outside this small area.

Table 11. Results of test with bulb at center of pool. A spot of light at pool center was used to test tadpole preference for outside, middle and inner rings of sectors.

Comparison	Preferred	X^2	Significance
out vs. mid	mid	8.7	$p \leq .005$
mid vs. in	mid	32.7	$p \leq .005$
out vs. in	in	18.5	$p \leq .005$

out - outside ring
mid - middle ring
in - inside ring

H. Stimulus Comparisons

Introduction

While the main purpose of this study was to determine if light, heat and food are used by tadpoles as environmental stimuli to aggregate, the experimental design and method of analysis were conducive to making comparisons between stimuli, assessing their relative importance to tadpoles. The following analyses were done with this in mind.

Procedure

Comparisons were possible due to the pairwise combination of stimuli presented in the pool, with the cardboard divider isolating the stimulus on each side. The following pairwise combinations were looked at: hot spots versus food, food versus light, and hot spots versus light. I counted the number of tadpoles in all sectors in each of the four trials for each of the above three combinations.

Results

I compared the four hot spot sectors with the four food sectors in the light and heat versus food test, using chi-square analysis with the expectations calculated in the pilot study. In three of the trials, food was significantly preferred over hot spots, but in the fourth, the hot spots were preferred (Table 12).

Table 12. Food and hot spots compared as stimuli. Sources of food were compared with hot spots (heat and light) in ability to attract tadpoles. The six most intensely-lit sectors were compared with the hot-spot sectors using chi-square analysis.

Trial	X^2	Significance
1	6.9	$p \leq .005$*
2	16.3	$p \leq .005$*
3	12.6	$p \leq .005$*
4	40.0	$p \leq .005$**

* food preferred
** hot spots preferred

Table 13. Food and light compared as stimuli. Sources of food were compared with light in ability to attract tadpoles. Food-containing sectors were compared with the six most intensely-lit sectors using chi-square analysis.

Trial	X^2	Significance
1	40.0	$p \leq .005$*
2	15.0	$p \leq .005$*
3	35.0	$p \leq .005$*
4	65.0	$p \leq .005$*

* food preferred

The four food-containing sectors were compared with all twelve fluorescently-lit sectors since there was no observable difference between the six with highest intensity and the six with lowest intensity. In all four trials, food was significantly preferred over light (Table 13).

The four hot-spot sectors were compared with all twelve fluorescently-lit sectors, for the same reason described above. In this case, three of the four trials showed no significant deviation from the expectation (Table 14). In one case, there were significantly more tadpoles on the hot-spot side than expected.

Conclusions

Any conclusions drawn from these limited tests are purely speculative, but are worth exploring. My results seem to point to food as the most effective environmental stimulus to aggregate. Since it was significantly preferred over hot spots in three of the four trials, and significantly preferred over light in all four trials, it appears to be a strong stimulus indeed. This makes intuitive sense, since a tadpole must eat as much as possible in order to grow as quickly as possible. This ensures it will get out of the vernal pond before it dries up.

As a second-best environmental stimulus, I would have to choose hot spots. They were significantly preferred over food in one trial (food was preferred in the other three),

Table 14. Light and hot spots compared as stimuli. Light was compared with hot spots (heat and light) in ability to attract tadpoles. The six most intensely-lit sectors were compared with the hot-spot sectors using chi-square analysis.

Trial	X^2	Significance
1	1.19	$p \leq .30$*
2	.23	$p \leq .70$*
3	13.40	$p \leq .005$**
4	.04	$p \leq .90$*

* not significant
** hot spots preferred

and over light in one trial (no difference in the other three). In any event, my results indicate that it is not nearly as good as food as an attractor. Clearly, more tests need to be done to determine if hot spots are superior to light as a stimulus to aggregate.

ISOLATE/AGGREGATION EXPERIMENTS

Introduction

In their aggregation behavior, the tadpoles used in my experiments were very unlike those described by other authors. Most *B. americanus* tadpoles have been described as forming tight-clustering social aggregations, D4-D5 on Beiswenger's (1975) scale (Table 2). My tadpoles seemed to form only loose, D2-D3 aggregations, and only in response to external environmental stimuli. They did not appear to be socially aggregating.

I wished to determine: (a) if individual *B. americanus* tadpoles, isolated since an early stage, would join an aggregation immediately, (b) if there was a latency period (see literature review), or (c) if they refused to join the aggregation. Such experiments have already been done with *B. americanus*, but to my knowlege, all previous work has been done with individuals taken from populations exhibiting tight-clustering social aggregation. I wished to determine if the possibility exists for intraspecific variation in joining an aggregation, both for the isolated individual and for individuals previously exposed to other tadpoles.

Procedure

To test the tendency for tadpoles raised in isolation to join an aggregation, I needed an apparatus which would "force" some of my asocially-inclined tadpoles into a tight cluster. I glued a 4-cm diameter screen tube (5-cm high) constructed of 1-mm^2 mesh nylon window screen to a thin square of clear Plexiglas. This apparatus will hereafter be known as the "corral". This was placed in the bottom of a 3.5-liter white plastic bucket with a 51-cm diameter (Figure 6).

I filled the bucket to a depth of 2.5 cm with dechlorinated tap water, and positioned the bucket directly below the fluorescent lamp fixture so that light was of uniform intensity. Between 20 and 30 pool-raised tadpoles, age class 25-30, were introduced in the corral and allowed to acclimate for 15 minutes. They were crowded enough to resemble a D5 aggregation. A single isolated tadpole, age class 29-38, was released in the bucket at a random location outside the corral and allowed five minutes to acclimate. Presumably, any visual, chemical or lateral line cues from the "aggregation" would pass through the screen tube. I observed tadpole feces easily passing through the screen, due to the turbulence caused by the tadpoles' tails beating.

The isolated tadpole's position was recorded every 30 seconds for five minutes (10 recordings). This was repeated 15 minutes later, and again 15 minutes after that for a total of 30 position recordings in a 45-minute period. The tadpole's age class was also noted. The water was changed and a new group of pool-raised tadpoles was placed into the corral before another isolate was tested. Each isolate was returned to its plastic cup when finished. I tested 15 tadpoles in this manner.

As a control, five of the isolates were retested in the same manner, except no pool-raised tadpoles were placed in the corral. This was to see if there was a tendency for the tadpoles to prefer one area or the other in the absence of any stimuli. As a further control, I tested 4 pool-raised tadpoles in the manner described above, first testing them with other tadpoles in the corral, then without. This was done to see if any difference existed between the behavioral response of the isolates and that of tadpoles raised in a group.

Of the 50 isolated individuals, all but 15 died en mass in unusual circumstances one time when I added water to their cups. I am not sure of the specific cause of this, but it severely limited the possibilities for study. Only five of the fifteen remaining individuals were tested both with and without tadpoles in the "forced school" corral. Due to time constraints, the other ten were only tested with others in the corral and four pool-raised tadpoles were tested both with and without tadpoles in the corral.

Results

Results of this set of experiments are summarized in Table 15. The location of a tadpole in the plastic bucket

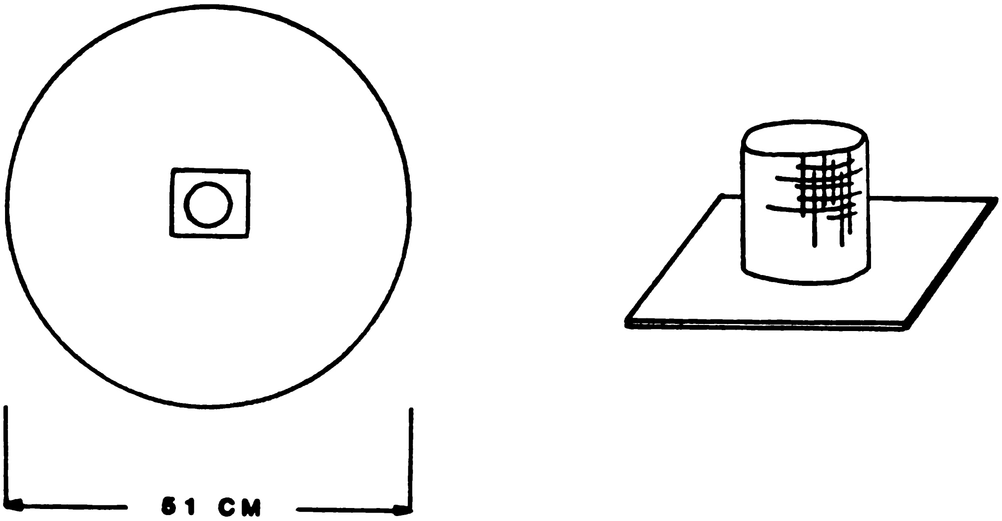


Figure 6. Sociality apparatus. At left is top view of 3.5-liter bucket with corral insert. At right is side view of screen-tube corral.

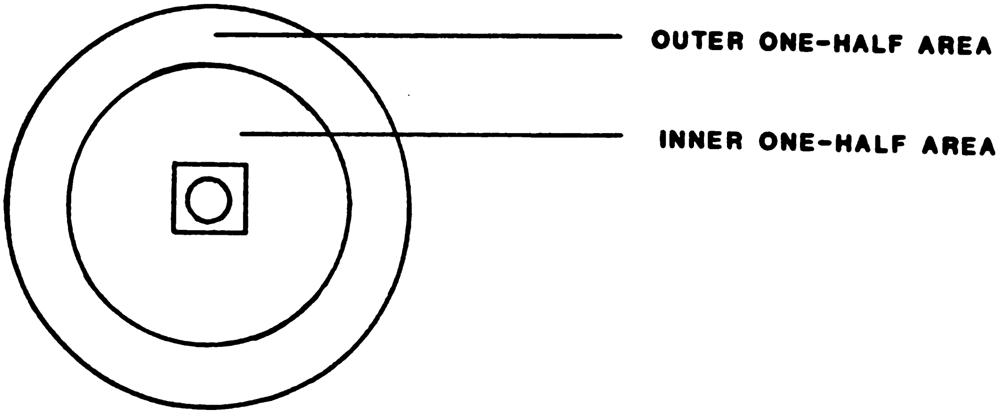


Figure 7. Inner and outer one-half areas of bucket.

had been recorded during the experiment. A count was made of the number of times a tadpole was in the inner one-half of the area of the bucket and the number of times it was in the outer half (Figure 7). The number of times the individual was in the inner half is recorded in Table 15.

I found no significant difference between the number of "in" scores (in the inner one half of the bucket) for the two groups: a) with and b) without others in the corral, when subjected to a Kruskal Wallis test ($\underline{p} \geq 0.25$). I obtained the same results using the matched-pair t-test ($\underline{p} \leq 0.10$). This indicates that there does not seem to be any more or less time spent socially, toward the center, when tadpoles are present in the corral. Similar comparisons were made with the pool-raised tadpoles, and there was no difference in the number of "in" scores between these and the isolated individuals (Kruskal Wallis test, $\underline{p} \geq 0.25$).

There did, however, appear to be some individual variation. Since only five isolates were tested both with and without tadpoles in the corral, it is impossible to draw any conclusions, but there were some marked changes in behavior in two of the individuals. As noted in Table 15, all five of these tadpoles had 10 or fewer "in" scores with no tadpoles in the corral. While three individuals exhibited the same behavior (10 or fewer "in" scores) after tadpole introduction to the corral (matched-pair t-test, $\underline{p} \geq 0.25$), two individuals, numbers 1 and 2 in Table 15, changed their behavioral pattern so that each had 29 "in" scores. This is a significant change (matched-pair $\underline{t}$-test, $\underline{p} \leq 0.025$).

Table 15. Results of isolate aggregation experiment. Number of tadpoles on inside half-area of bucket was counted, before and after addition of tadpoles to corral.

Tadpole	Age Class	No. In Before	No. In After	Significantly Different*
1	29	9	29	yes
2	36	7	29	yes
3	38	7	0	no
4	38	0	8	no
5	36	10	1	no
6**	28	10	8	no
7**	30	5	19	no
8**	30	9	6	no
9**	30	7	12	no

* matched-pair t-test, $\underline{p} \leq .025$
** pool-raised tadpoles

Pool-raised individuals were also tested for any change in behavior after the addition of tadpoles to the corral, but no change was noted (matched-pair *t*-test, $p \geq 0.25$).

I performed a correlation analysis on the five isolates to determine if there was a correlation between the age class of a tadpole and its relative behavior; its ability, desire, etc., to switch from asocial to social behavior. I found no correlation ($r = 0.009$). I then included the pool-raised sample in my analysis, but again, there was no correlation between age class and sociality ($r = 3.7 \times 10^{-6}$).

Conclusions

I cannot draw any firm conclusions from this study due to a lack of adequate number of test subjects. Some interesting trends were noted, however, which should be looked into further in future research. There was a great deal of variability in isolate response to the addition of tadpoles to the corral, even with only five isolates tested. Two individuals had a marked change in behavior, suggesting that some tadpoles are more inclined to aggregate socially than others.

Remember that this population of tadpoles was very non-social to begin with. I have no explanation for this, as all other studies I have read describe very tightly-knit social aggregation in *B. americanus*. Perhaps there is geographic variability in social inclinations. My test subjects came from two clutches which presumably would have been deposited in the same general location, quite probably in the same pond. In noting other tadpoles in that same area, both myself and a colleague have commented on the lack of distinct aggregations. Perhaps the genetic requirements for social aggregation have been lost in the local *B. americanus* population.

GROWTH STUDIES

Introduction

Previous studies have looked at differential growth and the crowding effect in many species' tadpoles, including *B. americanus* (see literature review). As noted previously, three hypotheses have been suggested for the crowding effect: (1) a physical or chemical substance, (2) an adreno-cortical response to stress, and (3) exploitation competition.

This experiment attempted to fill in some of the gaps created by previous research on the subject of growth inhibition. It was confined to a series of observations on tadpoles in the special tanks described in the general methods section, the tadpoles kept in isolation, and a sample of pool-raised individuals. I wished to compare the growth rate of aggregating individuals with that of isolates. In addition, the special tanks provided a unique opportunity to observe tadpoles in a partially isolated state.

I wished to investigate Wassersug's (1973) claim that *B. americanus* tadpoles may be less susceptible to the crowding effect than other species' tadpoles. Although this study did not involve any experimental manipulations, I also hoped to provide some insight into the mechanism(s) involved in the crowding effect in *B. americanus*.

Procedure

Tadpoles were assessed for differential growth in two ways. The total length (snout to end of tail) was measured after transferring a tadpole to a watch glass using a large-bore 20-cc syringe. This minimized the effects of handling, an important factor if psychological stress is involved in the crowding effect. When completely straight (i.e. no flex in the tail), tadpoles were quickly measured to within one millimeter with a small plastic ruler and assessed for Gosner age class. While age class is a somewhat subjective value, I estimate that I can accurately determine the age class of a tadpole to within one stage out of the 46 total (a 2.2% error). Tadpoles were immediately returned to their respective containers.

Individuals which had been isolated since Gosner stage 9 (late cleavage) were introduced to the special tanks at three different times. Three individuals were introduced at stage 12 (late gastrula), five were introduced at stage 17 (tail bud) and four were introduced at stage 24 (opercular flaps nearly complete). Between 11 and 19 tadpoles of mixed parentage had been raised together in the small-area side of the tank since stage 9. I call the introduced isolates in the large-area side of the tank "semiisolates" because they are no longer in complete isolation. I term the group in the small-area side the "cohorts". Originally, 24 such special tanks were set up, eight for each stage of introduction, but in 12 of them, the grouped tadpoles in the small-area side were able to get under the screen partition which separated them from the lone individual. I was subsequently unable to determine which one was the original isolate. Data from these were discarded.

Total length and age class were assessed for all semiisolates and cohorts on three occasions, 16 days after the experiment began (6-5-84), 10 days later (6-15-84), and 12 days after that (6-27-84). On the latter two occasions (6-15 and 6-27), I also assessed the total length and age class of 19 isolate tadpoles and a random sample of 20 pool-raised tadpoles, using the methods described earlier.

Results

The mean length and mean age class of the isolates, semiisolates, cohorts, and the pool-raised sample were compared with each other, two groups at a time, using Student's *t*-test. I treated the six comparisons of mean age class independently of comparisons of mean length. I also analyzed the data from June 15th independently of those from June 27th.

With *n* different treatment groups, by convention, *n* - 1 independent pairwise comparisons are acceptable, each with a significance level of $\alpha = .05$. In this case, $n - 1 = 4 - 1 = 3$ comparisons. At the .05 level, the experiment-wide protection against type I error is $1 - (.95)^{n-1}$ or $1 - (.95)^3 = 0.143$ in this experiment.

Because I did more than three comparisons, two problems arise: (1) inflated experiment-wide α (type I error), and (2) lack of independence. The problem of increased type I error can be remedied by adjusting the significance level of individual comparisons such that the experiment-wide probability of type I error is less than or equal to that with $\underline{n} - 1$ independent comparisons (= 0.143 in this experiment). Since I did six comparisons, they could not all be considered independent (remember, I actually looked at four groups of six comparisons each: June 15 age class, June 15 length, June 27 age class, and June 27 length).

The experiment-wide probability of type I error is the union of the probabilities of type I error for the individual comparisons (corollary 1 to the addition rule in Rohtogi, 1976). Furthermore, the probability of the union of any set of events is less than or equal to the sum of the individual probabilities of the separate events. Therefore, regardless of the dependence relationships among tests, the experiment-wide probability of type I error is always less than or equal to the sum of the individual probabilities of type I error. If an individual comparison's level of significance is selected such that the sum across all six comparisons equals 0.143, one can be sure the experiment-wide protection against type I error is as good or better than that which would occur with three independent comparisons at the 0.05 level.

This study employed six pairwise comparisons, all with identical levels of significance. Since the six comparisons' combined levels of significance must be less than or equal to 0.143, each must have a level of significance less than or equal to 0.024 (0.143/6 = 0.024). I will adopt the convention that a comparison is significant if $\alpha \leq 0.024$, or not significant if $\alpha > 0.024$. In addition, because of unequal variance, certain comparisons are done with Cochran's *t*'-test (Snedecor and Cochran, 1967) instead of Student's *t*-test.

From the June 15th data (Table 16), both the isolates and the semiisolates appear both older and larger than either the pool-raised sample or the cohorts. There were no significant differences in the mean age or size between the isolates and semiisolates; the cohorts and pool-raised tadpoles also did not differ significantly in size or age class. Note that while mean age class and length are treated independently, they both elicit the same results.

By June 27th, some changes in these relationships were observed (Table 17). Isolates and semiisolates remained indistinguishable in mean size or age class, as did cohorts and the pool-raised sample, but I also observed no significant difference in length between semiisolates and the pool sample, or the semiisolates and the cohorts. At the same time, the isolates were still significantly larger than the cohorts. The disparity in developmental state remained, as the semiisolates and isolates were both at a significantly more advanced age class than the cohorts or pool sample. Comparing Tables 16 and 17 for isolate length, one notes that length decreases over time. This is due to the metamorphosis of some of the isolates between June 15 and June 27, resulting in a smaller sample size for length studies.

Table 16. Results of growth study, June 15. Comparisons were made for the four groups independently for degree of development and length, using pairwise Student's *t*-tests or Cochran's *t'*-tests. Means for each class are in parentheses. Significance was restricted to the .024 level. A line connecting two or more groups implies no significant difference exists between them.

Age Class:	isolates (33.45)	semiisolates (32.67)	cohorts (28.20)	pool sample (27.35)
Length:	semiisolates (19.67)	isolates (19.50)	pool sample (14.25)	cohorts (13.60)

Table 17. Results of growth study, June 27. Comparisons were made for the four groups independently for degree of development and length, using pairwise Student's *t*-tests or Cochran's *t'*-tests. Means for each class are in parentheses. Significance was restricted to the .024 level. A line connecting two or more groups implies no significant difference exists between them.

Age Class:	isolates (39.43)	semiisolates (37.08)	cohorts (31.38)	pool sample (30.90)
Length:	isolates (21.65)	semiisolates (19.22)	cohorts (17.53)	pool sample (17.14)

Recall that the semiisolates were introduced to the special tanks at three distinct Gosner stages, 12, 17, and 24. Correlation analysis of the data from June 15 revealed that no significant correlation exists between the stage at which a semiisolate is introduced to the test tank and the difference between the mean length of the semiisolate and its cohorts (r = -0.15). There is also no correlation between the stage of semiisolate introduction and the difference between the mean age class of the cohorts and their semiisolate (r = 0.12). This suggests that growth inhibition may occur at any time during early development; it does not require that the tadpoles be in association from the start.

As an interesting aside, I calculate that there is a significant positive correlation (r = 0.74) between the size difference and age class difference of the semiisolates and cohorts. This correlation coefficient increases to 0.94 when only one data point is removed. This confirms that at least during early development, length corresponds fairly well with degree of development.

Conclusions

The results of the comparisons of the four groups suggest a few major points. First, isolates and semiisolates seem to grow at the same rate. They remained about the same size and age class throughout the experiment. The same can be said for the pool-raised sample and the cohorts. Notice that, while food was not a limiting factor, both the isolates and semiisolates had more space than the cohorts or pool sample. Isolates had 63.6 cm^2 each and semiisolates 166.3 cm^2, while pool-raised tadpoles had 9.4 cm^2 each and cohorts 4.0 to 6.9 cm^2 each. This rather large disparity seems to suggest that the adreno-cortical stress response may be acting in this situation.

A second point to note is that age-class differences remained unchanged between June 15th and June 27th, but length differences broke down. There is most likely a maximal size the tadpoles reach, at about stage 30-32. While isolates and semiisolates continued to develop in advance of the cohorts and pool sample, they were no longer at a size advantage. Length fails to be an accurate indicator of degree of development at that time.

The third point is that the crowding effect will occur regardless of the time during development in which the test tank is established, at least early on (stages 12-24). I got distinct age-class disparity when I introduced a semiisolate to the test tank at all three stages tested, 12, 17, and 24.

From these observations, it is clear that the crowding effect does occur, quite readily, in _B_. _americanus_. It is difficult to refute Wassersug's (1973) claim that _B_. _americanus_ tadpoles are less susceptible to growth inhibition than other species because of a lack of data to compare my results to. It seems unlikely, however, that other species could have a more pronounced effect than I observed, especially when one considers the lack of reports in the literature on such an obvious effect.

It is clear that exploitation competition is not necessary to cause a growth differential. Since tadpoles had unlimited access to food, this hypothesis seems unlikely. This is not to say, however, that it is impossible to induce the crowding effect via exploitation competition.

Semi-isolated individuals were exposed to the same environment as the cohorts, yet were able to develop significantly faster. Indeed, they developed as quickly as isolated individuals. Any chemical or physical substance responsible for the crowding effect could easily have passed through the screen partition. The probability that, by random chance, the semiisolate (as opposed to one of the cohorts) is always the fast-growing individual which secretes this substance is approximately 3.6×10^{-15}. In eleven of the twelve experimental test tanks, the semiisolate was indeed the fastest-growing tadpole. It is extremely unlikely that this is due to random chance ($p = 5.7 \times 10^{-14}$). If an alleged chemo/physical substance worked over a very short range (say, less than 5 mm), such results would be possible, otherwise, my results suggest that the presence of a chemo/physical substance in unlikely.

As pure speculation, I suggest that the initial disparity in growth rate could have been caused by a stress reaction. There must be a mechanism by which certain tadpoles gain an initial growth rate advantage. The cohorts, being much more crowded than the semiisolates, would be under such stress. In the pool, I observed various tadpoles in a wide range of developmental states at one time. Obviously, not all individuals were developing at the same rate, yet all were exposed to the same conditions. This implies that the stress reaction is not the only factor which could effect growth rate, assuming that all individuals are equally susceptible to the stress reaction (which may not be true). I suggest that secondarily, exploitation competition may be employed by some tadpoles to gain a growth advantage, possibly supplemented by a short-ranging chemo/physical substance.

Another possible explanation for this phenomenon pertains to energy utilization. Tadpoles in crowded conditions may not be under stress per se, but because they are in close proximity to each other, they are constantly bumping into each other and continually moving. When disturbed, tadpoles tend to swim away from the disturbance. This keeps crowded tadpoles in nearly constant motion, while the uncrowded tadpoles remain quiescent. It is a well documented fact that anuran growth rate is positively correlated with net energy gain. The semiisolates and isolates, because of their relative quiecence, may have simply had a larger net energy gain, and hence, grew at an accelerated rate relative to that of the cohorts or pool sample.

GENERAL DISCUSSION

This study looked at three aspects of aggregational behavior in B. americanus tadpoles. In the first experiment, I investigated three environmental factors which may lead to asocial aggregation. I observed that B. americanus tadpoles have a tendency to aggregate in the absence of these three stimuli (light, heat and food), particularly toward the shallow edges of the pool. I also observed that some stimuli are better than others in attracting tadpoles. Food is a very good asocial aggregational stimulus, as is incandescent light (light and heat). Fluorescent light, however, does not appear to be a good attractor. Tadpoles do not seem to be able to discriminate well between various intensities of florescent light. Heat alone is a good "broad area" attractor.

Shallow water seems to be an important common denominator for these stimuli of attraction. The warmest water in a pond duing the daytime is toward the edge, in the shallows, where the sun's rays can heat it quickly. Food is also very abundant in shallow water, where light intensity is strongest (personal observations). All this suggests that a tadpole programmed to seek cues corresponding to shallow, food-rich water would be at a great advantage. Natural selection would act strongly on such tadpoles. My studies indicate that tadpoles are able to find food in and of itself in a small pool, but in a large pond, where food might be more widely-scattered, other cues such as a temperature gradient or water depth could become very important in bringing a tadpole close enough to a food source that the tadpole could locate the food by chemo-olfactory cues, or by whatever other means it uses.

Optimal-foraging theory predicts that individuals will prefer to live in groups if food is spatially arranged in high quality patches (Pulliam and Caraco, 1984). It is easier to locate these food patches as a group (by whatever means). A group-living individual has a higher net energy gain than if it remains on its own under such circumstances. Natural selection would therefore be expected to favor individuals which exhibited social tendencies in this situation. I believe this is why we see many anuran species' tadpoles forming social aggregations.

Not all species' tadpoles are social aggregators, and the possiblity exists for variation in aggregating ability in some species, as suggested by my second experiment. While only observing a limited number of replications, I did observe a wide range of social aggregations in tadpoles tested, from both isolated and pool-raised samples. While I realize that many more replicates need to be done before any substantial claims can be made, I hypothesize that a single species' tadpoles may exhibit variation in social behavior.

Recall that my tadpoles came from ponds where tadpoles did not form the tight social clusters described by other authors. While it is possible that food does not occur in high quality patches in these ponds, thereby nullifying an assumption necessary to explain social optimal foraging groups, I see no reason why this should be the case. Something, however, is different here. Perhaps some genetic anomaly has gotten into the local B. americanus population. Since American toads may return to the same area, possibly even the same pond to breed each year (depending on local availability of bodies of water), and since their offspring are not likely to travel very far away, it is quite possible for a genetic condition such as this to develop on a local basis. Lack of sociality may be a genetic drift phenomenon, due to chance.

On average, the growth rate of tadpoles in an aggregation is reduced over that of an isolated or semi-isolated individual (as defined by my experiments). An important goal for a tadpole is to get to metamorphic size and get out of the water as quickly as possible. Thus, there seems to be a conflict of interests here. On the one hand, joining a group causes a reduction in growth rate, but on the other hand, it may increase the individual's feeding efficiency, assuming optimal-foraging theory is correctly applied here. It can be assumed that aggregation, if a genetic phenomenon, would not occur if there wasn't an overall advantage to it. If one considers the other advantages to being in an aggregation; (1) increased heat due to formation of a large black mat in the water, (2) reduced predation due to the conspicuous nature of the black mass of tadpoles in association with distastefulness, and (3) the possibility for increased fitness due to kin selection in the group, it is quite conceivable that the advantages outweigh the disadvantages.

In any event, the crowding effect does occur in *B. americanus*, apparently regardless of whether the aggregation is social or asocial. Many experimenters have shown that some tadpoles grow quite normally while the growth rate of the others is inhibited (Richards, 1958; Licht, 1967; Steinwascher, 1978). This is an inhibition phenomenon, not an accelerated growth phenomenon as some have hypothesized (Rose, 1960; Shvarts and Pystolova, 1970b).

My experiments seem to support the idea that the crowding effect is either an adreno-cortical response to stress caused by physical contact with other tadpoles, or the result of a greater net energy gain due to quiscence in less-crowded conditions. Those individuals which are not being severely affected may be producing some physical or chemical substance which further reduces the growth rate of their fellow tadpoles while not affecting their own. Having a size advantage would allow such tadpoles increased access to food via exploitation competition, implying they could further increase their growth advantage. In a positive feedback loop such as that outlined in Figure 1, this system could go on until the larger tadpoles metamorphosed, leaving the pond and all its resources to a few individuals.

This study attemped to fill in some of the voids left by previous research on tadpole aggregative behavior. While attempting to answer some questions, it raised others which must be addressed by future researchers. More work needs to be done on exploring population differences in aggregation. Observations and tests need to be done comparing the local East Lansing _B_. _americanus_ tadpoles with those from surrounding regions, using standardized tests to determine if there are differences in degree of sociality between populations.

To my knowledge, no one before has suggested that aggregating tadpoles are optimally foraging. This is a fascinating idea which undoubtedly should be researched further. Quantitative measurements of the food base of vernal ponds in different areas should be taken to confirm that food exists in high quality patches.

It may be that a single population can exhibit social or asocial aggregation depending on prevailing conditions. Such a hypothesis could be explored by varying conditions under which groups of tadpoles from a single clutch are raised.

Finally, more work needs to be done on the problem of differential growth and the crowding effect. Specifically, the question of whether a chemo-physical substance or a stress reaction being primarily responsible for this phenomenon needs to be addressed in more detail. The notion that it could be primarily a stress reaction supplemented by a chemo-physical substance should be explored further.

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