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Reproductive Ecology of *Ambystoma opacum* and the
Construction of a Guide for Larval Identification

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Departmental Honors, Senior Honors Project
Abstract

Distinguishing between the three ambystomatid larvae that can be found in this region can be difficult. These include the marbled salamander (Ambystoma opacum), spotted salamander (Ambystoma maculatum), and Jefferson salamander (Ambystoma jeffersonianum). The goal of this research was ultimately to determine defining features in marbled salamander larvae in order to facilitate in identification of the species at this stage of development. Field surveys were conducted in the fall during breeding time in order to gauge the size of the population and the amount of males vs. females (~67:9). Eggs and larvae were collected and raised in order to document development through photographs. Additional collections were taken weekly for the same purpose. Total length size comparisons can effectively be used when collecting during the spring to distinguish between species, as marbled salamanders are much larger (4.9 cm vs. 1.7 cm). Additionally, some common features have been found between the specimens as markers for identification. These include lateral spots, head morphology, coloration, and tail patterning.

Introduction

Identification

Ambystoma opacum is a medium-sized member of the Ambystoma genus having a black body with white or light gray crossbands across the head, back, and tail (Trauth and Richards 1988). Adults often measure between 77 and 127 mm TL. Males and females are sexually dimorphic with males having markings more silvery white in comparison to silvery gray of females (Petranka 1998). Sexually active males are also easily distinguishable from females by their swollen cloacal glands (Petranka 1998).

Hatchlings are blackish with a pond-type morphology, having bushy gills and dorsal fins that extend almost to the front limbs (Petranka 1998). On average, they measure 10-14 mm TL.
(Kaplan 1980). Embryos that hatch later may produce hatchlings of greater size, reaching 19 mm (Bishop 1941; Noble and Brady 1933). Larvae are “drab brown” or blackish with ventrolateral light spots formed in a line below the level of limb insertion (Anderson 1967; Trauth et al. 1989). As they age, larvae develop varying degrees of light yellowish green coloration and mottling (Trauth et al. 1989). Dark speckling is present on the throat while there are some scattered melanophores on the venter (Petranka 1998). Because this species breeds in the fall, unlike most salamander species, larvae can be distinguished by others in the spring by their relatively larger size (Petranka 1998). Juveniles that have recently transformed are brown or black with light flecks, which become more pronounced 1-3 weeks after metamorphosis. After 1-2 months, they begin developing adult patterning (Bishop 1941).

**Distribution & Habitat**

This species can be found from southern New England to Northern Florida, westward to tallgrass prairie in eastern deciduous forest habitat (Petranka 1998).

**Reproduction & Courtship**

*Ambystoma opacum* is one of two *Ambystoma* species that mates and oviposits on land (Petranka 1998). Females nest in dried beds of temporary ponds or along margins of reduced ponds. Adults begin migrating to their breeding sites in late summer or autumn and move at night during rainy weather (Petranka 1998). 31-49% of females that are intercepted while migrating will lay fertilized eggs in captivity. This indicates that males often court females before arriving at breeding sites (Krenz and Scott 1994). Northern populations generally breed before Southern ones (Anderson and Williamson 1973). Nests can be found in Pennsylvania beginning in September (Pawling 1939). Males will arrive at breeding locations anywhere from a few days to 2 weeks before females (Noble and Brandy 1933). At a site in South Carolina, 64% of males arrived before first female (Krenz and Scott 1994). Females arrive 9 days later than males on average (Petranka 1998). In breeding surveys, the operational sex ratio varies from 6:1 to 85:1.
Accordingly, four times as many males as females enter a site in a breeding season. Adults will enter and exit breeding ponds in roughly the same location, using the same pathways. The mechanism used by these salamanders to detect sites that will later flood are not known (Petranka 1998).

Annual breeding pattern data is lacking. It is believed that females breed biennially (Petranka 1998). During dry years, the amount of breeding is not reduced. The number of males and females that reproduce annually is positively correlated with the amount of rainfall during the breeding season (Semlitsch et al. 1996). There is also a positive correlation between the number of breeding adults and the number of metamorphs produced in previous years (Petranka 1998).

**Courtship in the Field and Lab**

The male initiates courtship when rapidly moving about, nudging and lifting other males or females, particularly near tail or cloaca (Bishop 1941; Noble and Brady 1933). Other males proceed to join in. Females often respond by nudging the cloacal region of males in return. Once paired, male and female move in a circular fashion while mutually nudging cloacal regions. The male then moves forward along the body of the female while undulating his tail and raising his body. He will then deposit a spermatophore. A responsive female will then position her chin and body over the spermatophore while being led forward by the male. The female ultimately positions her cloaca over sperm cap and picks up seminal fluid from the top of the spermatophore. Sometimes the female is not led by the male, but instead noses around until she encounters a spermatophore (Bishop 1941; Noble and Brady 1933).

**Spermatophores**

Spermatophores may be single or multiple (Petranka 1998). A multiple spermatophore is when a male deposits one on top of an existing spermatophore. This serves as competition
between males (Arnold 1976). Spermatophores usually measure 4-5.5 mm tall, 6 mm wide at the base, and 2 mm wide at the top. The top has 4 quadrangularly spaced, raised knobs. This creates a slightly concave receptacle that holds the seminal fluid (Noble and Brady 1933).

**Ova**

The ova range from 1.9-2.6 mm in diameter when freshly laid and increase in size as development proceeds (Kaplan 1979). They have a tough, sticky outer membrane which often appears black from the soil and debris stuck to it (Petranka 1998). Egg capsules may shrink or swell depending on the state of hydration. On average, they have a diameter of 5.4 mm (Trauth et al. 1989). The placement of eggs with respect to depth determines the time of hatching (Petranka and Petranka 1981). The eggs at the bottom of a pond hatch first, but can be killed if a lack of rain causes the pond to dry. Eggs laid at margins of the pond hatch last, are susceptible to freeze damage, and may not be inundated (Petranka 1998). They is also a competitive disadvantage for hatching later. These larvae will be smaller than conspecifics and have less time to exploit food resources before the pond dries in summer.

**Substrate Conditions**

When gravid females were exposed to three substrate types including leaves, wood, and grass and two moisture regimes in outside experimental chambers, almost all females laid their eggs beneath the grass clumps, and only 4% oviposited in dry conditions. This result suggests that there is a minimum moisture level required to stimulate egg laying (Figiel and Semlitsch 1995). When given the option of various soil moisture gradients, females did not show a trend in which level of moisture they preferred to lay their eggs in (Marangio and Anderson 1977).

**Nests**
A female selects a suitable site in the dried or partially dried bed of a temporary pond or ditch and constructs a shallow nest (Petranka 1998). Nest construction occurs as follows: The female burrows ovoid to oblong cavities in the soil surface immediately below leaf litter or surface cover (Noble and Brady 1933). Eggs are laid singly within this depression. The female coils around her clutch, and occasionally moves to turn the eggs (Bishop 1941; Noble and Brady 1933; Petranka and Petranka 1981). Nests are usually single, but 2-7 clutches have been observed in communal nests (Petranka and Petranka 1981, 1990). Clutch size typically increases with female SVL (Petranka 1990, Walls and Altig 1986). Depending on the region, clutch size ranges from 37-232 eggs. Greater numbers of eggs are often found in nests attended by females than in abandoned ones (Petranka 1990). Possible locations for nests include bare soil or rodent burrows immediately below leaf litter, at the base of grass clumps, in moss mats near bases of trees, or beneath bark, logs, and stones (Petranka 1998). Nest site selection by females appears to vary by region. Also, it is possible that as many as 44% of nests may not flood in dry years as shown by a study in South Carolina (Jackson et al. 1989).

**Brooding**

Although most females brood their nests until flooding, nests without females are frequently found as well (Bishop 1941). Females often brood nests where all eggs are dead and leave those in which eggs are viable (Petranka 1990). Nests where brooding is not being performed have a lower mean egg viability and clutch size (Petranka and Petranka 1981). A study in South Carolina revealed that the embryonic survival increases as female brooding time increases (Jackson et al. 1989). As a whole, the available data indicates that brooding enhances the survival of offspring (Petranka 1998),

Brooding most likely works effectively to protect eggs against predators, minimize fungal infections, and reduce desiccation (Petranka 1998). Depending on the pond filling pattern and
region, females may stay with their egg clutch from 2 weeks to several months (Petranka 1998). Further north where winters are more severe, the brooding period is short as females are only able to use ponds that fill in autumn or early winter (Bishop 1941). The opposite is true for southern locations where eggs are less likely to freeze. Females are able to brood longer because females can use ponds that don’t flood until late winter (Petranka 1998). For a nine-day period, there is no detectable energetic cost associated with brooding. However, brooding that takes place for many weeks or months most likely produces a significant cost (Kaplan and Crump 1978). Viable eggs that had not been flooded have been found as late as March (Noble and Brady 1933).

**Embryo Development**

Embryos develop to the hatchling stage between 9-15 days after oviposition of eggs. However, they will not hatch until inundation occurs (Kaplan and Crump 1978; King 1935). Hypoxia is what ultimately triggers hatching (Petranka et al. 1982). When the eggs become inundated with water, the embryos become oxygen stressed because their environment does not supply enough oxygen for their metabolic demands. Hatching glands on the snout of embryos are then stimulated to release digestive enzymes that dissolve the egg capsule and allow the embryo to escape. Since hatching is dependent on environmental conditions, the size of larvae at hatching varies depending on the stage they reach before egg flooding. According to one study, initial ovum size and development rate to hatching have no relationship. However, there is a strong positive correlation between the size of the ovum and hatchling. When compared at corresponding times in development, a larger larva will tend to reach the feeding stage sooner and are larger earlier on.

**Aquatic Ecology**
Embryos hatch within a few hours to 1-2 days after a nest is submerged (Petranka 1998). Larvae begin feeding on zooplankton very soon after hatching. They also feed on macrozooplankton, and larger larvae will consume amphibian larvae and eggs (Petranka 1998). The prey organisms consumed in the largest numbers include cladocerans, copepods, and ostracods (Branch and Altig 1981; Petranka and Petranka 1980; Stewart 1956). These are primarily consumed by small larvae. As they grow, they begin to incorporate larger prey items into their diet on top of consuming large amounts of zooplankton (Petranka 1998). Other organisms that larvae feed on are aquatic insects, isopods, mites, snails, and oligochaetes. Large larvae have sometimes been found to eat caterpillars (Petranka and Petranka 1980). In addition, the large larvae feed on toes and tails of conspecifics and *A. maculatum* larvae (Petranka 1998). Large larvae also eat *Pseudacris triseriata* tadpoles, *Lithobates sylvatica* embryos, *Ambystoma tigrinum* larvae, and freeze-damaged *Ambystoma jeffersonianum* eggs (Stine et al. 1954; Walters 1975).

During the day, small larvae and hatchlings can be found congregating in the leaf litter of warm, shallow water. They disperse throughout the ponds at night to feed in the water column (Petranka 1998). Larvae will also float at or near the surface of the water while feeding in a behavior known as stratification (Branch and Altig 1981; Petranka and Petranka 1980). This behavior is often exhibited by intermediately sized larvae on murky habitats during the day and on overcast days for clear water habitats (Anderson and Graham 1967; Petranka 1998). Hatchlings and pre-metamorphosis larvae spend more time on the bottom of the pond (Hassinger et al. 1970). There is evidence that larvae feed continuously throughout the day and night, while others single it out to nocturnal activity (Branch and Altig 1981, Petranka and Petranka 1980).

As the temperature of the water increases with the change of season from late winter to spring, larval growth occurs rapidly with larvae achieving a mass of 2 g or greater before
metamorphosing. Metamorphosis usually occurs in May and June in the Central and Northern areas of the range of this species (Petranka 1998). The larval stage is often prolonged in the North due to colder temperatures. For instance, it tends to last 8-9 months in many New York populations (Bishop 1941; Deckert 1916). In one study, metamorphs from New York ranged from 66-72 mm TL between two sites in late June (Bishop 1941).

It is often the case that larval growth, the size at metamorphosis, and survival are inversely correlated with larval density in a pond (Petranka 1998). Although larval density does not affect the biomass of available zooplankton in ponds, it is true that with an increased biomass of zooplankton, larval growth also increases (Petranka 1998). Larvae raised in lower densities tend to yield larger specimens at metamorphosis that breed at an earlier age and larger size (Scott 1994). At some point, most larvae receive tail damage from attacks by conspecifics, which is also positively correlated with larval density. In addition, when larvae of the same size are paired together, larvae are less aggressive towards siblings than non-siblings, indicating that they have an ability to recognize kin (Walls and Roudebush 1991).

Premetamorphic survival is typically low in natural populations as most individuals die before metamorphosing. In North Carolina, premetamorphic survival was found to be 8.8%, followed by near 0% in consecutive years (Petranka 1989). Larval survival was 24.2% followed by <1%. Hatching to the beginning of metamorphosis ranged from 1-44% and averaged 15% (Petranka 1989).

Little is known about the ecology of juveniles. After recently transforming, they can be found beneath leaf litter and debris around the margins of breeding ponds. Upon the event of rainy weather, the metamorphs will disperse themselves (Stenhouse 1987). Specimens have been found as deep as 1 m beneath the surface (Pike 1886). Throughout much of the year, both the juveniles and adults are fossorial. They become more active on the ground surface in rainy
weather of summer and fall. Adults live in rodent burrows or in self-constructed burrows enlarged from existing crevices which they defend and exhibit territoriality over (Semlitsch 1983). As adult females mature, their growth rate, lipid levels, and egg and clutch size are positively correlated with food intake (Scott and Fore 1995).

Proposed Research

The proposed research project aims to gather data about the reproductive ecology of the species *A. opacum* in a known breeding location in Dillsburg, Pennsylvania. Data will be collected on the number and presence of males and females in relation to weather conditions and time elapsed. This will produce a better understanding of what the population at this site is composed of. Eggs will then be collected and brought back to the lab. Gravid females and sexually active males will also be collected in an attempt to have them breed in the lab. The eggs from these two sources will be hatched and the hatchlings raised. Over the course of development, photos will be taken in order to ultimately construct a guide for identification of this species during its larval stages as the need for such a guide has been reported.

Materials and Methods

The preliminary stage of the project consisted of field work performed at Kimmel Pond in Dillsburg, Pennsylvania. Beginning in early September, surveys of breeding adults in the field were conducted on rainy nights for 10 days straight (9/5/11-9/15/11). Headlamps and spot-beams provided by the biological sciences department were used to illuminate the surroundings. A Rite in the Rain waterproof notebook was used to record field data. Field equipment for recording weather conditions included a Kestrel weather meter for recording relative humidity and temperature. Every third salamander found was sexed and measured for mass and snout vent length (SVL). A hanging scale and Ziploc bags were used to record the mass of the salamanders while a caliper was used to record the SVL. Sex was determined by
sexually dimorphic crossband variations (silvery gray in females while white in males) as well as the presence of a swollen cloaca in males. General observations concerning the progression of breeding, for example mating or courtship behaviors or the presence of females, were also recorded. Repeated trips into the field were made until data points for measurements reached 100. In addition, two females, each with two males, were brought back to the lab and encouraged to mate.

Additional trips were made to the field to return one set of female and males and to search for nests. Nests with females present were brought back to the lab. Measurements of the female were taken and the number of eggs was counted. The same was done once the female in the lab laid eggs. The eggs were washed in glass distilled water and placed in glass dishes with glass distilled water. The water was initially oxygenated through the use of air stones and pumps, but was later discontinued. Water changes were made every other day. Embryos were viewed under a microscope equipped with a camera, allowing for photographs to be taken of the embryos. Photos were taken intermittently until external gills and forearms became highly developed and embryos appeared ready to hatch. Embryos which appeared to have deceased due to fungal infection were discarded. Those that appeared infected but still alive were quarantined.

Once hatchlings emerged, they were placed in separate dishes in sets of five labeled with the female they came from, the date hatched, and the number of hatchlings in the dish. Brine shrimp were raised from a kit and subjected to a heat lamp in order to keep the water temperature between 26-30 °C. Brine shrimp were fed to salamander larvae every day or when otherwise available. Photographs of larvae were taken at various stages through development using a glass tank which tapers to a point at the bottom. Additionally, specimens were collected from the field in order to document growth and development in a natural setting on comparison to the lab. Specimens were then documented. All larval specimens in the lab deceased over
Christmas break (12/22/11) due to fungal problems. New collections were made once J-term began. 10 were collected the first week, and an additional 5 every following week. 15 photos were taken per week at 5 angles, resulting in a total of 50 specimens photographed.
The total length (TL) of *A. opacum* and *A. maculatum* larvae were taken on 4/7/12 as a backup for identification between these two species in case photos for comparison could not be obtained. Photos of *A. maculatum* and *A. jeffersonianum* were shared by Sarah Bartle from Shippensburg University. Photos were compared between species in order to assess differences between them to aid in identification. Lateral spots were compared between *A. opacum* and *A. maculatum* using lateral photos. Head morphology was compared by drawing a typical silhouette from a lateral and dorsal view for each species. Photoshop was used to increase the contrast to pure black and white of tail images to compare the distribution of pigmentation at both early and later stages in development in each species. Lastly, the eyedropper tool was used in Adobe Photoshop to select predominant colors in images of each species in order to create color palettes for base and pattern colorations.

**Results**

**Field Surveys**

Data was collected on the number and presence of males and females in relation to weather conditions and time elapsed. The mass and SVL were recorded for every third specimen collected. Measured specimens’ longest toe on the right hind limb were clipped in order to signify recapture in the future.

The weather conditions, and male/female survey data is displayed in the following tables:
Table 1: Weather Conditions

<table>
<thead>
<tr>
<th>Date</th>
<th>Time in Field (min)</th>
<th>Temp. (°C)</th>
<th>Humidity (%)</th>
<th>Dew Point</th>
<th>Barometric Pressure (in/Hg)</th>
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<tr>
<td>9/5/11</td>
<td>106</td>
<td>16.6</td>
<td>89.5</td>
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<td>80.8</td>
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<tr>
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<td>9/9/11</td>
<td>Total</td>
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<td>22.246</td>
<td>81.9</td>
<td>66.8</td>
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<tr>
<td>9/10/11</td>
<td>83</td>
<td>19.1</td>
<td>85.3</td>
<td>62.5</td>
<td>29.15</td>
</tr>
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<td>9/11/11</td>
<td>Lightning &amp; High Winds-Unsafe Weather Conditions</td>
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Table 2: Male/Female Specimen Counts

<table>
<thead>
<tr>
<th>Date</th>
<th>Males ♂</th>
<th>Females ♀</th>
<th>Recaptures</th>
<th>Total</th>
</tr>
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<td>3</td>
<td>0</td>
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<th>Recaptures</th>
<th>Total</th>
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<td>9/16/11</td>
<td>Total</td>
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<td>336</td>
<td>381</td>
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<td>9/17/11</td>
<td>83</td>
<td>19.1</td>
<td>85.3</td>
<td>62.5</td>
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<th>Barometric Pressure (in/Hg)</th>
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<tr>
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<td>19.1</td>
<td>85.3</td>
<td>62.5</td>
<td>29.15</td>
</tr>
</tbody>
</table>
As can be seen in the table above, there was a total of 381 unique specimens collected, consisting of 336 males and 46 females. There was only 1 recapture (female). The male: female ratio was found to be approximately 67:9.

**Table 3: Average Measurements by Sex**

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>Avg Mass (g)</th>
<th>Avg SVL (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male ♂</td>
<td>75</td>
<td>9.14</td>
<td>65.61</td>
</tr>
<tr>
<td>Female ♂</td>
<td>41</td>
<td>12.50</td>
<td>67.51</td>
</tr>
</tbody>
</table>

As can be seen in the table above, the average mass and SVL (12.50 g and 67.51 cm) were found to be larger in the females than the males (9.14 g and 65.61 cm), as expected.

**Larval Identification**

**Size Comparison**

In case the photo comparisons failed as a means for identification, backup TL measurements were taken. The size difference between *A. opacum* and *A. maculatum* and *A. jeffersonianum* is clear in the spring. Since *A. opacum* larvae hatch in the fall, they have months to grow much larger than the spring breeders. On 4/7/12, the average size of *A. opacum* was 4.9 cm while *A. maculatum* was 1.7 cm. *A. jeffersonianum* would be similar in size to *A.
*maculatum*. The following image shows the stark contrast in size:

![Image of A. opacum and A. maculatum with lateral spots](image)

**Lateral Spots**

Lateral spots can be found both in *A. opacum* and *A. maculatum*. However, they are more prominent in *A. opacum*, and exhibit central pigmentation whereas *A. maculatum* does not. There are between 8-10 spots on either side.
A. opacum

A. maculatum

Figure 3: Lateral spots on A. opacum (A) and A. maculatum (B). Spots are more prominent in A. opacum, and have centralized pigmentation. A. maculatum photo from http://www.virginiaherpetologicalsociety.com/amphibians/salamanders/spottedsalamander/spotted%20salamander%20lavae2.jpg

Head Morphology
Differences in the shape and size of the head were found between all three species. The following are summarized descriptions: 1) *A. opacum*: broad, angled, flattens at mouth, extends further beyond gills. 2) *A. maculatum*: rounded, short. 3) *A. jeffersonianum*: broad, angled, flattens at mouth, shorter.

**Figure 4**: Differences in head morphology between species. Curves drawn overtop photos from lateral (left) and dorsal (right) view.

**Figure 5**: Differences in head morphology between species. Curves drawn overtop photos from lateral and dorsal view.
Tail Patterning

Pigmentation on tails was edited in Adobe Photoshop in order to see differences in pigment distribution at early and late stages in development, and in comparison of one species to another. Differences were depicted as follows:
Figure 5: Tail of young (A) and more mature *A. opacum* (B). Tail of young (C) and more mature *A. maculatum* (D). Tail of young (E) and more mature *A. jeffersonianum* (F). Original photo “D” from http://www.virginiaherpetologicalsociety.com/amphibians/salamanders/spotted_salamander/spotted%20salamander%20larvae.jpg. Original photo “E” and “F” from Sarah Bartle.
Color Patterning

Differences were seen in the coloration of the species. The following is a summary of those differences: 1) *A. opacum*: gold-brown to brown 2) *A. maculatum*: ruddy- to blackish-brown 3) *A. jeffersonianum*: olive- to gray-brown.

*A. opacum*

![Color Palette](image)

Figure 6: Pattern coloration palette for each species is shown in the top row, while base coloration palette for each species is shown in the bottom row. Top left: *A. opacum*. Bottom left: *A. maculatum*. Right: *A. jeffersonianum*.

Discussion

With a total of 381 unique specimens found, an approximate male:female ratio of 67:9 was calculated. Population size can be estimated in the future by analyses with recapture data.
Key characteristics found to differ between species were lateral spots, head morphology, pattern coloration, and tail patterning. Size will likely be the most reliable difference to identify *A. opacum*. Better and more plentiful photographs of *A. maculatum* and *A. jeffersonianum* would be helpful for further comparison and confirmation of these results.

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**Works Cited**


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