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Centrolenella fleischmanni (Boettger),
with special reference
to its frogfly associates**

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University of Missouri — Kansas City**

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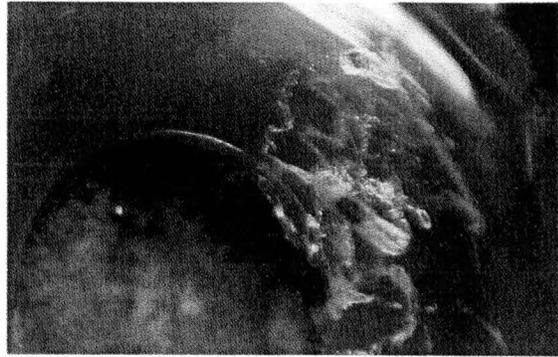
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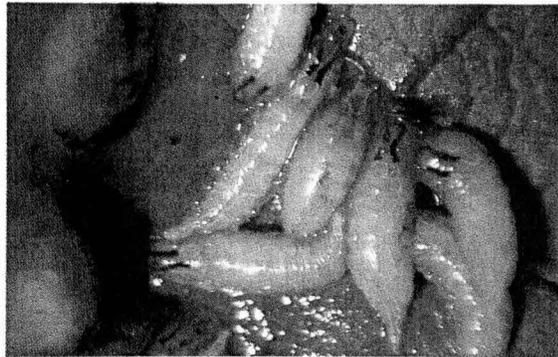
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FRONTISPIECE

Frogfly maggots feeding on differently-colored frog embryos. After artificial inoculation of hatching maggots, the embryo tissues can be seen passing through the maggots' digestive tract, and ultimately coloring the entire animal. (A) Normal feeding on light-green *Centrolenella fleischmanni* embryos. (B) On yellow and brown *Hyla ebraccata*. (C) On black embryos of *C. prosoblepon*.

ABSTRACT

Centrolenella fleischmanni is a small neotropical tree frog found in moist environments from southern Mexico through Central America to western Ecuador, on the Pacific slope, and through northern Colombia and Venezuela to Surinam on the Atlantic slope. It ranges in altitude from near sea level to about 1500 m. It is abundant through most of its range, most commonly found in the vegetation near moderate to fast-flowing streams, where it breeds. Breeding occurs mostly during the wet season (May to October). A few egg clutches may be found throughout the year in localities where the wet season is not well defined. Males call from upper or lower surfaces of leaves near streams, and show some signs of territoriality. Amplexing pairs oviposit usually on the underside of leaves above or very close to the streams. Either parent, but most frequently the male, accompanies the egg clutch, sitting directly on the eggs at night and sleeping near (but not on) them during the day. The number of eggs per clutch varies from locality to locality, and within a locality it varies throughout the rainy season. The mean number of eggs in the localities studied varied from 18 to 30.15. The tadpoles hatch in about 250-350 hrs, after the external gills disappear (Gosner's stage 25). Over 95% of the eggs that are not destroyed by biotic or abiotic factors hatch, but almost 80% of the clutches are destroyed. Upon hatching, tadpoles drop into the stream and quickly disappear into the mud or under rocks. Little is known about their later development in nature. Apparently they live in the mud or debris of the stream banks, often above water level and beyond the stream margins. Unlike tadpoles of other anurans, they are bright red in nature. Their color is not due to chromatophores but to blood showing through the nearly pigmentless skin, probably a respiratory adaptation to their fossorial life. In the laboratory tadpoles are difficult to raise and under standard conditions never attain their unusual red color. The duration of their larval stage is not known, but is estimated to last one, possibly two, years.

Flies of the drosophilid genus *Zygothrica* oviposit on viable egg masses of this frog and the resulting maggots kill and consume many or most of the eggs of the affected clutches. The incidence of fly infestation varies from locality to locality and throughout the rainy season, reaching 80-100%, in many cases, at the height of the breeding season, and then abruptly decreases. A correlation was found between the number of eggs laid by the frogs and the flies. There is evidence that flies tend to lay their eggs soon after the frogs lay theirs; otherwise the maggot's development is not successful. Laboratory experiments suggest that the fly's larval development is restricted to eggs of *C. fleischmanni* and a few other species. This appears to be the first documented case of an obligatory insect-frog relationship and of drosophilid larvae feeding on vertebrate embryos. The details of this relationship, and others involving frogs and flies, are reviewed.

INTRODUCTION

Many reports on *Centrolenella fleischmanni*, and other centrolenids, have been published (see review of literature by Lynch & Duellman, 1973, and add: Goin, 1971; Duellman, 1977; Villa, 1972, 1977, 1978, 1979b, 1980; Villa & Valerio, 1982; Starrett & Savage, 1973; McDiarmid, 1975, 1978; Greer & Wells, 1980; Wells & Schwartz, 1982). However, most of these deal with taxonomic* or zoogeographic aspects, and little has been published on the ecology, habits and reproduction of *C. fleischmanni*. Aspects of its biology (especially breeding and ecologic distribution) have been described by Duellman & Tulecke (1950), Starrett (1960), Villa (1972, 1977, 1978, 1980), Villa & Valerio (1982), Starrett & Savage (1973), McDiarmid (1975, 1978), McDiarmid & Adler (1974), Greer & Wells (1980), Wells & Schwartz (1982), and Hayes (1983).

During 1974-77 I undertook a field study of *C. fleischmanni* in Nicaragua and Costa Rica. Although my main interest was in the symbiotic association between *C. fleischmanni* egg masses and the drosophilid "frog-fly" (Villa 1977, 1980), I obtained information on other aspects of the biology of this frog. The observations made during the study, complemented by literature reports, are summarized herein.

MATERIALS & METHODS

General

Most of the materials used were freshly collected in Nicaragua and Costa Rica. An attempt was made to disturb the vegetation as little as possible, especially in those areas visited periodically. When a clutch was located and examined, the following information was immediately recorded: locality, hour, number and developmental stage of the embryos, presence or absence of frogfly eggs, maggot or pupae, and dehydration of jelly, if any. Usually the number of maggots was not counted because it rarely was possible to count them without disturbing them considerably. However, maggot counts were made when possible. Eggs, maggots and pupae were always counted in the laboratory using a binocular stereomicroscope. In 1974 all clutches were brought to the Managua

*Taxonomic Note. — If one accepts Lynch's (1981) conclusions, this species (and all other *Centrolenella*) should be placed in the genus *Hylopsis*. This genus was based on a frog (*Hylopsis platycephala*) meagerly described a century ago by Werner (1894), presumably collected in South America. The actual locality, and the whereabouts of the specimen, are not known. Lynch's detective work is admirable, but his conclusion that the frog is a *Centrolenella* must be taken with some reservation since even in the brief description of *H. platycephala* there are characters (i.e. outer metatarsals separated, amount of webbing between fingers) not consonant with what Lynch calls "*Hylopsis platycephala*". Even if we accept Lynch's allocation, I have strong reservations for replacing the genus name *Centrolenella* with *Hylopsis* even if the latter is an older name. Although *Centrolenella* is a much younger name (Noble, 1920) it is much better characterized, better known and practically universally used at least since Goin (1964). Centrolenids described since then, and most species described after Noble (1920) have been placed in *Centrolenella*, and the family Centrolenidae is based on the genus' name. Lynch (1981) briefly argues that replacing *Centrolenella* with *Hylopsis* "does not disrupt stability," a statement with which I do not concur. The genus has about 90 known species, most of which were described as *Centrolenella*, and all are now known by that name. Whether taxonomic stability is disrupted or not, it would be an exercise in futility to move them out of a genus that has been well established for at least twenty years, in order to place them in another which may or may not be its older synonym. Taxonomy would be much better served if the name *Hylopsis* is forgotten, or suppressed.

laboratory and kept under observation for at least 5 days to determine whether frogflies were present or not; surviving tadpoles were eventually returned to the stream where they would have hatched, or near it. Many frogflies were similarly released, but many were also preserved as voucher specimens.

After the frogfly eggs were recognized and the life cycle was better known, in 1975, it was not necessary to take all clutches to the laboratory, and most were left *in situ* after being examined. Many were marked with a bright-orange numbered tag affixed to the leaf some 10 cm away from the clutch (after the rains washed away many of the tags it was necessary to staple them to the leaves). Thus it was possible to study the development, growth and infestation of the clutches under natural conditions. In many cases, however, a microscope was necessary because of the small size of frogfly eggs and maggots. Clutches were then brought to the laboratory, observed, and placed above the stream nearest the laboratory, where hooks were installed to keep the leaves in place.

Many clutches, especially in 1974-75, were brought to the laboratory and kept there as long as necessary to observe them on a 24-hour basis. Details on the developmental stages of both the frog and the frogfly, the time necessary to complete each stage, and most experiments, were studied in this way. For this purpose, clutches (with a part of the leaf upon which they were laid) were placed in plastic cups or in jars containing 1 cm of water, and sealed to insure maximum humidity. In some cases Petri dishes were also used, but gave misleading results on some experiments, since the clutches were not actually suspended from the leaf where they were laid, as they are in nature, but lying flat on bottoms of the Petri dishes.

Inoculations

In order to answer some questions it was necessary to transfer frogfly eggs or maggots from one clutch to another (or to another medium). This was done under the appropriate magnification using fine-tipped forceps and a dissecting or teasing needle. Considerable care was taken not to injure the specimen. If it was thought that an injury had occurred, the specimen was discarded and another one was used. Although I had much assistance in the field and laboratory, I made all of the inoculations myself.

Because of the tenacity with which frogfly eggs are attached to the jelly, and because a good number of them (about 15-40% usually) would hatch too late, or not at all, newly-hatched maggots were preferred for inoculations in 1976. Although no significant difference was found in the results obtained using eggs or newly-hatched maggots (even if they had been feeding for a few hours; see, for example, Fig. 19), it assured that the time-consuming process of inoculation was not wasted with nonviable or late-hatching eggs.

An effort was made to transfer each specimen with as little of the original jelly as possible (although this was later found to be of little or no significance), and to remove the bits of jelly once the maggots had worked their way out of them. Transferring some jelly seemed undesirable because some of the original food, or any other factor, may also be transferred. However, the jelly protected the speci-

mens from possible injury by the transferring instruments; this was especially true when frogfly eggs or newly-hatched maggots were involved.

Sample Size

Although I was conscious of the need for large sample sizes in the inoculations (for purposes of statistical analyses of the results) this was not always possible for several reasons: (i) recently-laid frogfly eggs were not easy to find, so when available they were used sometimes in several experiments or replicates; (ii) the number of frogfly eggs laid is rather small (about 30), so usually no more than 10 could be used for the experimental, the control, and one experimental replicate, from the same source. In some cases even fewer had to be used (because of reduced viability); in any event, 6 was the lowest number used in inoculations; (iii) the actual process of inoculating frogfly eggs or maggots required a great amount of patience and, especially time. To inoculate a typical set of 30 eggs without harming them or destroying the clutch often required a whole morning under the best conditions, so concessions had to be made with regard to the ideal number of inoculated frogflies. Fortunately, however, inoculation results were usually clear-cut and repeating the experiment several times usually produced the same results.

Incidence Estimates

Incidence estimates were made to assess the impact of frogfly infestation upon the *C. fleischmanni* egg population. Clutches were located and either left *in situ* (marked with an identifying number), or taken to the laboratory for closer scrutiny. If no frogfly eggs, maggots, pupae or mouthparts were found at first inspection, the clutch or its residue was examined with a 2X magnifying lens or with a microscope. A clutch was considered to be "infested" if it contained one or more frogfly egg, maggot or pupa *and* signs of tadpole destruction if maggots or pupae were found*. If empty puparia were present, they were included in the incidence estimation only if some jelly was present. If jelly masses were found without tadpoles, or puparia, they were recorded "not infested" if also no mouthparts were present. Because evidence of puparia remains on the leaf much longer than does the jelly, to do otherwise would increase the apparent rate of infestation.

Only those clutches that were visible in the normal course of a walk were examined. Many were considerably beyond reach many meters above ground, and a few clutches were occasionally obtained from high up in trees. However, common sense dictated not to seek them on a regular basis because of the physical danger involved in climbing slippery trees in a cloud forest. Thus the incidence estimates may be radically different for the upper stories of the forest, but

*If infested and non-infested clutches were placed together in the same container on the way to the laboratory it would be possible for a maggot to move to an uninfested clutch, but there would be no evidence of embryonic destruction until 1-2 hr later.

because the samples were always taken in the same way, they can be compared meaningfully.

Incidence estimates were made (i) occasionally in December 1973 and June-August 1974 in Nicaragua; (ii) in June-December 1976 in Nicaragua and Costa Rica and (iii) periodically (at least once a week) throughout the rainy season (June-December) in Nicaragua in 1975.

Observation Periods

Observations were made on a full-time basis during part of the rainy season of 1974 (early June to early August); in laboratory clutches, observations would be as frequent as every 3-6 hours; this was later found to be unnecessary in most cases and in 1975-6 they were made once or twice a day, depending upon the nature of the observation or experiment. In some cases, for example, observations or checks were made almost continuously for 5 to 10 hours, but this was fortunately rare.

In 1975 research was carried out intensively throughout the rainy season. Since I could not be in the field Mondays and Tuesdays, routine observations and counts were made by a field assistant, Sr. Miguel Torres, who worked full-time on the project (July-December 1975). The design of the experiments and the most critical observations were scheduled from Wednesday to Sunday and were done by me. I returned to the USA in mid-October for one month, and again in November for two weeks, during which no fieldwork was possible. By then most of the critical aspects of the study were completed but although Torres obtained some data, the incidence of frogfly infestation was not determined as frequently as it had been in the preceding months.

At the end of the 1975 rainy season the Matagalpa field lab was closed, but the site was visited each weekend (when possible) to search for possible centrolenid breeding and alternate frogfly breeding sites.

In 1976 the Matagalpa site was visited once every two weeks and only necessary materials were taken to the Managua laboratory. Instead, more attention was devoted to Costa Rican frogflies. I visited Costa Rica every three to five weeks between May and November, when possible. In addition, clutch collections were made by Dr. Carlos E. Valerio every two weeks; collections were preserved in 10% formalin for later examination, and yielded data on the degree of infestation, number of frog and frogfly eggs, maggots, pupae, and their survival (by counting empty puparia). In some cases maggots left the clutch when it was placed in formalin, and counting the remaining number was considered unnecessary (because of this, and to avoid disturbing them, the number of maggots counted was always lower than the eggs or pupae used in statistical analyses).

One month was devoted to Costa Rican frogflies. Work was done on a full-time basis with the assistance of Mr. Allen Porter, and local help. Although several field trips were made in the first quarter of 1977 no positive results were obtained, because it was a dry period and centrolenids, and frogflies, would not be found.

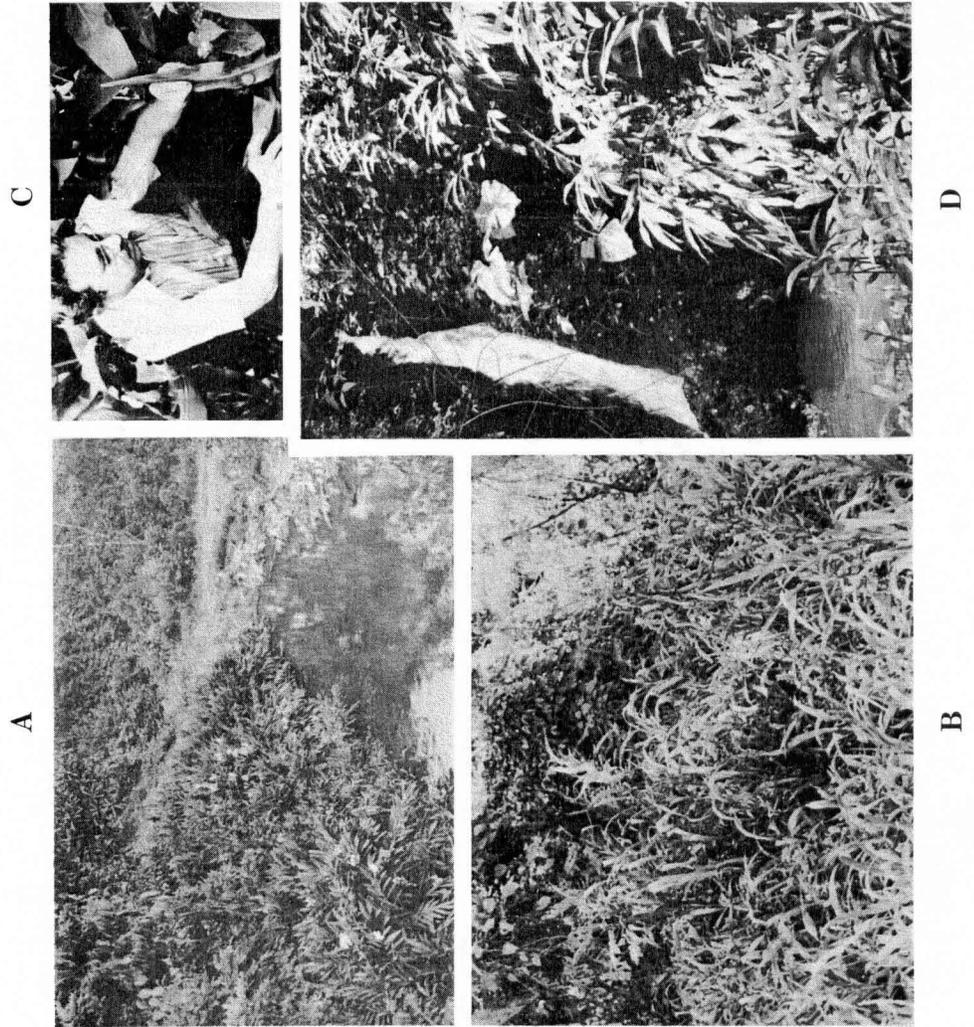


FIGURE 1. Breeding grounds of *Centrolenella fleischmanni* in Central America. —(A), portion of a stream in Finca Santa Elena (800 m) near the Matagalpa study site. A dense cover of *Hedychium* can be seen on the left margin. *C. fleischmanni*, *C. granulosa* and *C. pulverata* were breeding here. —(B), a section of the stream in the San José (Costa Rica) study site, overgrown by the grassy *Coix Lachryma-Jobi*, where *C. fleischmanni* also oviposits. —(C), a typical oviposition site of *C. fleischmanni*, the underside of a *Hedychium* leaf, that overhangs a torrential mountain stream. —(D), a waterfall at the Matagalpa study site: a dark, permanently moist environment that is favored by breeding centrolenids. Their clutches are laid mostly in the *Hedychium* leaves (foreground and right) but not in the broad-leaved “elephant ears” (*Xanthosoma*, center).

Data Analysis

In those instances where experiments did not produce clear-cut results, statistical analyses were necessary. Regression and correlation (done by the least-squares method) were used most frequently. Significant differences or similarities (usually at the 0.01 level) in the mean values of two populations were determined by the Student-*t* test.

When more than two populations were involved, the data were pooled and the mean, variance and number of observations were obtained for all the data, and then each population was compared against the entire sample.

Most invertebrates were identified by the staff of the USNM-USDA, and other laboratories, specified in the "Acknowledgments" section.

DESCRIPTION OF STUDY SITES

1. Nicaragua

The Nicaragua study site was located in Departamento de Matagalpa, near Matagalpa, on Finca San José de la Montaña, property of General J. Rigoberto Reyes. The finca is chiefly a coffee plantation and it is crossed by many small streams, around which centrolenids are most common during their breeding season. Most streams are moderate- or swift-running with clear water and rocky bottom, and typically have an abundant growth of the ginger-lily *Hedychium coronarium* on their margins (Fig. 1A). *Centrolenella fleischmanni* lay their eggs on the underside of the *Hedychium*, leaves (Fig. 1C). The topography of the region affords small waterfalls — some of them more than 10 m high — where the spray produced by the splashing water keeps the surrounding air with a high (to 100%) moisture content throughout the year, and where practically any leaf is a potential substrate for centrolenid oviposition (Fig. 1D). A 3 km section of the stream closest to the Casa Hacienda was chosen for density estimations, but virtually all others within a 5 km radius, and many more within 10 km around it, were examined at one time or another between 1974-77.

The *Hedychium* are cut one to three times a year in some streams, but in other areas (where no coffee is planted locally) they are usually left undisturbed. Since *Hedychium* grow rapidly, this cutting probably has little effect upon the centrolenid population, although many clutches are lost when the cutting takes place in the rainy season.

In the area neighboring the streams, the vegetation is more drastically altered. The understory shrubbery and small trees have been cleared away many years ago and replaced by coffee plants (*Coffea arabica*), with banana (*Musa paradisiaca*) plants regularly scattered throughout. The weedy herbs that grow under the coffee plants are cleared periodically and kept in check with herbicides. The ecology of this area is, to say the least, highly unstable.

The dominant shade trees are *Croton* (possibly *C. draco*). Their predominance and their rather small stature suggest that they may have been planted, although edaphic factors (such as the thin soil of the area) may have limited the growth

of other species. Other trees in the area — either left standing or planted to shade the coffee plantation — include *Inga vera*, *Gliricidia sepium* and *Albizia cf. carbonaria*. *Cecropia peltata* and *Guazuma ulmifolia*, indicators of second-growth conditions, are found on the margins of the plantings and along the roads. The roads are lined throughout parts of their length with a variety of cultivated plants such as *Hibiscus* and *Tithonia diversifolia*. Roadside herbs include *Lobelia laxiflora*, *Hyptis suaveolens*, *Argeratum* sp. and *Salvai* sp. Other abundant shrubs noted were *Lantana camara*, *Phytolaca* sp., *Ricinus communis* and, around the pools, *Centropogon* sp.

The cool moist climate of the area supports a variety of epiphytic bromeliads, orchids and ferns, including *Tillandsia usneoides*, *T. ionatha*, *Aechmea* sp., *Polypodium* spp. and *Epidendrum lacustre*. In the relatively-undisturbed steep banks of some parts of the stream grow, in addition, the *Hedychium*, at least two species of *Heliconia* and *Calathea*, and various creeping and climbing Araceae (of the genera *Anthurium*, *Philodendron* and *Syngonium*) form a dense tangle of vines in the floor or cling to the tree trunks, providing abundant oviposition sites for centrolenid frogs.

The stream section chosen for density and frogfly incidence determinations offered the greatest variety of habitats possible in a relatively short distance and was relatively close to the field laboratory. The width of the stream ranged from about 4m in the widest, slowest portions, to less than 1m in the fastest flowing areas, and in the waterfalls. There were at least three major waterfalls (which provided their immediate neighborhoods with abundant water spray), the largest one being about 60m high. In most of the course, the stream sides and the bottom were rocky, with little sediment, while in the backwaters and slower portions there were abundant mud and sediments; in about 75% of the course, at least on one margin had abundant *Hedychium*, and at least 60% of the streams was shaded by overhead vegetation; the water was never more than 0.7m deep, except during overflows, although greater depths (never known to exceed 1.5 m) were found in neighboring regions of the same stream; about 30% of the stream ran through active coffee groves, and much of the stream ran tortuously in less accessible terrain, which assured a minimal disturbance from peasants and domestic animals.

No weather information is available for this study area, and efforts to set up a meteorological station were unsuccessful. Although some data are available for the Matagalpa area, they were found to be poor indicators of the conditions prevailing in the study site, which is higher, cooler, and more moist than the city of Matagalpa. In 1975, for example, the rainy season started 7 weeks earlier in the study site than in Matagalpa, located only about 10 airline km away. At the study site, air temperature readings were irregularly taken and were 21-23° C during the day, and 2-3 degrees lower at night, during the rainy season. In winter, the daytime air temperature was about 19-20° C. During the rainy season, the relative humidity exceeded 85% and, thus, was beyond the measuring range of the portable hygrometer then available to me. During the dry season, it was never recorded below 75%. Moisture conditions, of course, vary enormously

in the different microhabitats; during the rainy season, it is very moist. Rains typically occur in the morning and the afternoon, and often there is a continuous sprinkling throughout the day.

A field laboratory was established in an old house in the finca, near one of the streams. The equipment available included a compound and a binocular microscope and limited laboratory wares. There were two skylights to insure that the inside of the laboratory had the same photoperiod as the outside. The temperature and humidity inside were similar to those in the nearby stream.

The Managua laboratory was temperature controlled by means of 24-hour a day airconditioning set at 20-23° C during the day, while observations were taking place. To insure maximum humidity, living materials were kept in sealed plastic cups or terraria of various sizes. Sealed containers were also used in the field stations to insure uniformity in the observations, except on those experiments which required varying relative humidity.

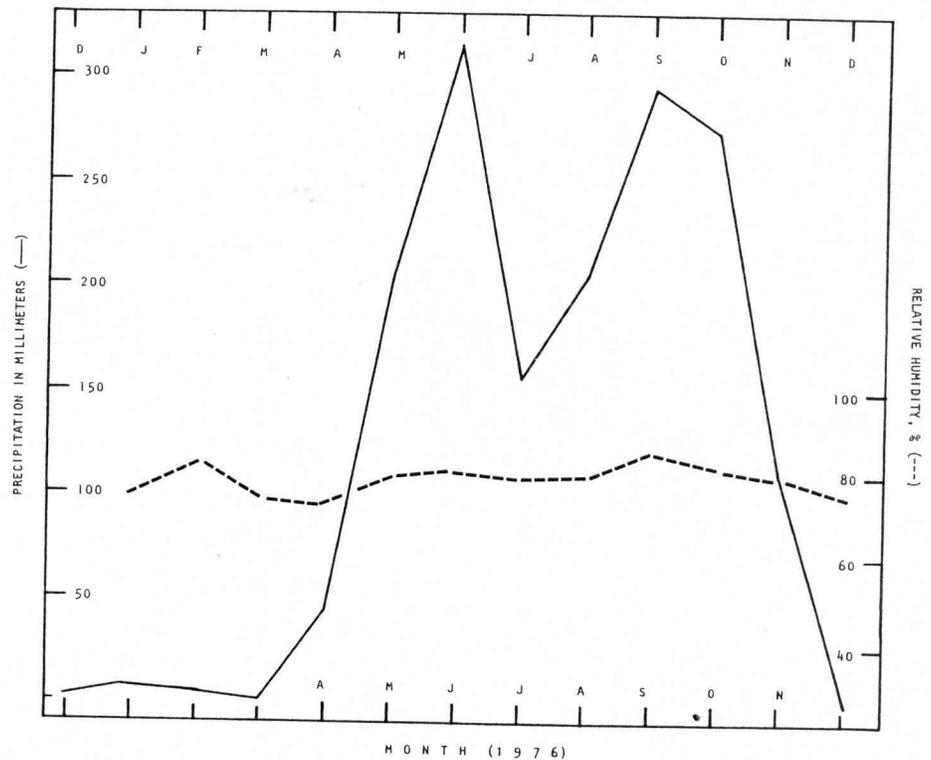


FIGURE 2. Monthly precipitation (solid line) and relative humidity (broken line) in San José, Costa Rica, during 1976. Based on unpublished information from the Servicio Meteorológico Nacional de Costa Rica. Temperatures were not available.

2. Costa Rica

The Costa Rica study site was used from June to November (1976); it was in San José province, Granadilla de Curridabat, in the property of Dr. Carlos E. Valerio. A 100m section of the stream was chosen; on either side of it were a coffee plantation and an ample backyard with tall grass and a few scattered small bushes. The stream itself is slow-flowing and swampy in spots. Although there is some *Hedychium*, on parts of the bank, most of the stream is ponded and overgrown with *Coix Lachryma-Jobi*, an abundant, maize-like plant which may be 3 meters tall, and where *C. fleischmanni* (the only centrolenid in that locality) regularly oviposits (Fig. 1B). The ponded or sluggish stream is certainly an unlikely place for breeding *C. fleischmanni* (a species most commonly found in cool, torrential streams), but they are nevertheless very common there. The situation is similar to that described in Nueva Segovia, near "Las Manos" in Northern Nicaragua (Villa, 1972). In addition to *Hedychium* and *Coix*—the dominant vegetation in the stream—there are some *Heliconia* and *Ocotea*, where *C. fleischmanni* also oviposits. Several large trees provide additional shade, and part of the stream is densely covered with the elephant-ear *Colocassia antiquorum*, where *C. fleishmanni* never oviposits. Several other plants such as the composite *Eupatorium* sp., the morning-glory-like *Ipomoea* (Convolvulaceae) and an uticant species, are abundant but *C. fleischmanni* clutches were never found on their leaves. In general, the locality is shady, moist and cool, probably more so than in the Matagalpa site. The rainy season starts earlier (in March or April) and lasts until about November. Meteorological data obtained from a nearby weather station are presented in Fig. 2.

The Costa Rica laboratory was set up in the eastern section of the Museo Nacional de Costa Rica, about 3 airline km from the above-described site. It had essentially the same equipment as the Matagalpa laboratory, and was intensively used only in June-July, 1976.

RESULTS

Distribution

Centrolenella fleischmanni "has a broad geographic and altitudinal range: lowlands and premontane slopes in humid areas from Guerrero and Veracruz, México, through Central America to western Ecuador, and through northern Colombia and Venezuela to Surinam" (Starrett & Savage, 1973:64). Henderson & Hoervers (1975) later recorded it from Cayo, Belize, in "rainforest and jungle." The species is most frequently encountered in rain- and cloud-forest habitats. In Costa Rica, Starrett & Savage (1973) found it at elevations from near sea level to over 1500m. In Nicaragua, it is found at elevations from 600 to 1200m. Although scarce in many localities, *C. fleischmanni* may be so common in some places that it composes over 90% of the individuals of arboreal amphibians.

Habitat.—In more humid localities where the dry season is not clearly defined, *C. fleischmanni* (and other centrolenids) can often be found, during the day, inactive in the vegetation throughout the forest. In drier terrain, they can be

TABLE 1. Density estimates for *Centrolenella fleischmanni* at the Matagalpa, Nicaragua, study site (1974-76)

Date	Number of individuals per km of stream length
Beginning of rainy season:	
5 June 1974	35.33
3 July 1974	42.33
3 July 1975	38.00
4 July 1976	29.00
Middle of rainy season:	
8 August 1974	29.33
9 August 1975	31.00
10 August 1976	22.00
End of rainy season:	
23 November 1976	1.00
22 December 1973	00.33
19 December 1975	00.00
22 December 1974	00.00

found less frequently, and are more concentrated in riparian vegetation. Villa (1972) described an easy method for collecting them in such habitats; by violently beating the gingerlillies, and other broadleaf vegetation, along the streams. This method obviously disturbs the preferred habitat, hence it was not used at the study sites.

In most localities, *C. fleischmanni*, are associated with fast and even torrential streams. They can be found also in the slower parts of some streams, but only rarely in ponded water. In a locality near Las Manos, near the Honduras-Nicaragua border (1000-1200m elevation) Villa (1972) found the frogs breeding in a swamp that had formed after a road dammed a fast-moving stream several years before. Apparently the population preferred to remain in that locality rather than seeking faster parts of that stream.

Density.—No serious attempt was made to census the *C. fleischmanni* at the study site because this would have involved a major disturbance to allow year-round observations. However, on several occasions, data were obtained to estimate the size of the local population. For this purpose, a 3 km section of stream near Matagalpa was chosen (described under "Study Sites"), and all vegetation likely to shelter centrolenids was thoroughly examined. The frogs were obtained, counted and released (see Table 1).

Several trends can be inferred from Table 1. During the first months of the

rainy season, the concentration of individuals is highest, presumably because that is when most breeding occurs. During the height of the rainy season, much reproduction was occurring, although breeding activity had declined somewhat. At the end of the rainy season centrolenids were rarely found, although occasionally both adults and recently-laid egg masses were discovered. It would be interesting to determine what happens to the local populations during dry periods. Possibly frogs move to moister microhabitats, such as higher vegetation, inaccessible bromeliads, or leaf litter. Efforts to find them there were few and unsuccessful due to the height and luxuriance of epiphytic vegetation providing effective shelter and concealment. It is also possible that the population declines because of predation, age, and other demographic factors.

Activity.—*Centrolenella fleischmanni* is a nocturnal frog. Its activity (primarily determined by calls heard) begins at dusk (and sometimes earlier) and continues through the night during the rainy season. During the dry season there is considerably less activity. Individuals are often found inactive even at night although climatic conditions appeared suitable for their activity.

Sympatry.—At the Matagalpa study site *C. fleischmanni* was associated with two congeners (*C. granulosa* and *C. pulverata*) with no obvious habitat separation, although the breeding sites may be somewhat different (see "Selection of oviposition sites"). In various Costa Rican localities, Starrett & Savage (1973) found *C. fleischmanni* sympatric with *C. albomaculata*, *C. euknemos*, *C. granulosa*, *C. illex*, *C. prosoblepon*, *C. pulverata*, *C. spinosa*, *C. talamancae*, and *C. valerioi*.

Food

Food items of *Centrolenella fleischmanni* were determined by examination of stomachs and feces. Stomachs were removed from freshly-caught frogs, cut open, and emptied on Petri dishes containing 70% alcohol. Living frogs were transferred soon after capture to transparent plastic cups, and allowed to defecate there for 7-10 days. The feces were soaked in alcohol before teasing the components with a dissecting needle. The combined food sample consisted of 26 stomachs containing food items, and 76 fecal dropping samples.

The main purpose of stomach and fecal analysis was to determine if drosophilid flies were being eaten, but none was found. This suggests either that (i) they were being eaten but were completely digested, or (ii) that centrolenids do not normally eat drosophilids in the field. In the laboratory several individuals took local *Drosophila* readily, and subsequently wings and other fragments appeared in the droppings examined. This supports alternative (ii).

No attempt was made to identify taxonomically the insect remains except to search for drosophilids; 58 different types of insects were recognized, but none obviously predominated over others. It seems that insects are opportunistically taken depending upon their availability.

An unexpected finding was the size of the insects eaten by these frogs. Considering the small size of centrolenids (18-25 mm SV), fly-sized insects (e.g., 3-5 mm

in body length) were expected. However, much larger prey were found; the mean of estimated total lengths for all items found (excluding filaments, antennae, etc.) was 7.5 mm, with a record size of 11 mm.

In the laboratory, *C. fleischmanni* fed readily on houseflies and nymphal crickets. Plant or other foods were not found in stomach or fecal samples nor taken in the laboratory.

REPRODUCTION

Time.—Duellman & Tulecke (1960) found eggs of *Centrolenella viridissima* (*C. fleischmanni*, *vide* Starrett & Savage, 1973) at Agua del Obispo, Guerrero, México, on June 28-29 (1958). Starrett (1960) found them at San José de la Montaña, Heredia, Costa Rica, on July 27-28. Lynch & Duellman (1973) reported numerous calling males near Quevedo, Los Ríos, Ecuador, in April. Adler (*pers. comm.*) found them in Veracruz in July (1964.) These dates correspond with the first half or third of the rainy season. In northern Nicaragua Villa (1972) noted breeding activity in September (1971) and considerably less in October (end of rainy season).

Further study in northern Nicaragua and at the Matagalpa field site provides a more complete picture of the breeding activity of *C. fleischmanni*. Males begin calling in April or May, a month or so before the onset of the rainy season, and their calling activity increased thereafter. During brief, isolated showers amplexus and oviposition occurred, but dry spells usually followed these early rains, so egg masses dehydrated and the embryos died. A considerable amount of breeding takes place during June-August, declining in subsequent months (density estimates in Table 1 may be a good reflection of breeding). By November some breeding may still occur, but in mid-December and January, only isolated calls were heard after occasional showers, but little breeding took place although some egg masses were found after a considerable search.

Amplexus.—Amplexing pairs of *C. fleischmanni* were found frequently during the rainy season. Amplexus is axillary and usually occurs on vegetation surrounding fast streams at heights varying from 0.5 to 10 m above water; amplexing pairs may be found higher up, but were not seen. Although many pairs were observed for a considerable time (up to 2 hours in some cases), oviposition was not observed. It probably takes place at night.

Selection of oviposition sites.—Compared with other frogs which lay their clutches on emergent vegetation (e.g., *Hyla ebraccata* and *Agalychnis callidryas* at the study site), *C. fleischmanni* is usually quite selective in its oviposition sites. Ideally clutches are deposited on the underside of leaves (Fig. 1C) directly overhanging rapidly flowing streams, although the back-waters, pools, or slower portions of the streams, where available, are used. If no leaves are available immediately above water those near the sides of the stream are used even if the tadpoles are not able to fall directly into the water.

There are also very clear preferences as to the type of vegetation used as

oviposition site. Where available, the ginger-lily *Hedychium coronarium* is more frequently used than any other plant. Clutches were laid usually on fresh, clean, turgid leaves at least 50 cm above the water surface. Lower leaves are usually avoided as they are the first to be damaged by moderate stream overflows (Fig. 3A). Older or non-turgid leaves or those which have been stained by mud, are never used if a choice is available, probably because the jelly adheres better to clean leaves.

There are obvious selective advantages for choosing oviposition sites so criti-

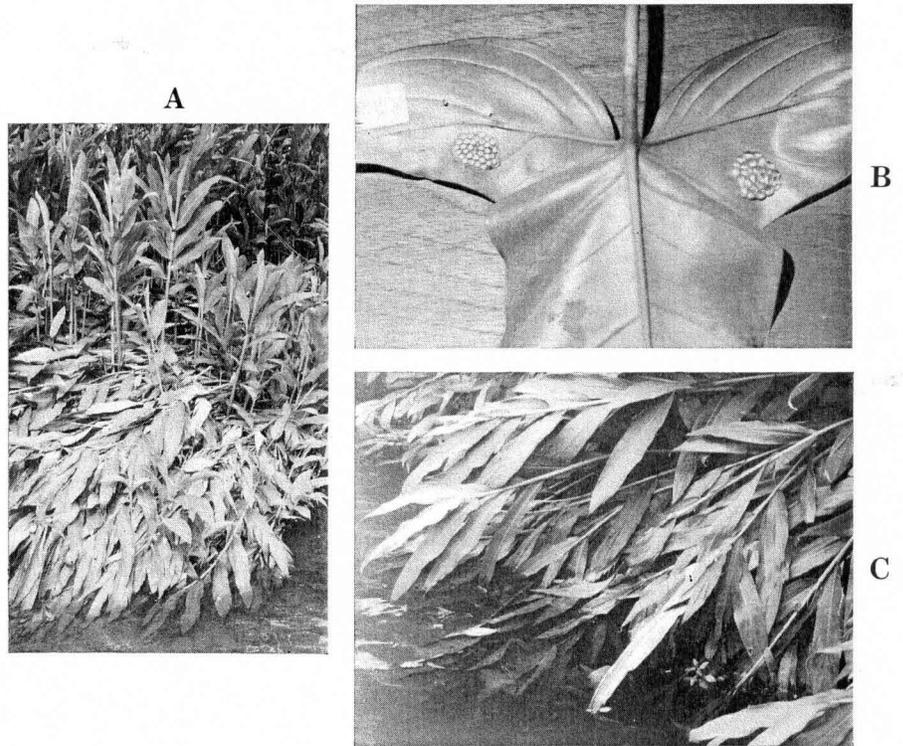


FIGURE 3. Oviposition sites of *Centrolenella fleischmanni*. – (A), following a moderate overflow at the beginning of the rainy season, those plants nearest the edge of the stream (where *C. fleischmanni* oviposits) are bent down and remain partially submerged (foreground). Taking their place, younger plants have already sprouted (background). – (B), an instance of 2 *C. fleischmanni* clutches on the same leaf. The clutch at left was in stage 11; that at right in stage 23. Note that although both are well separated, a greater separation would have been possible. The mean distance between clutches thus laid is 137.6 mm. Note tag affixed to leaf (left), used for field identification. – (C), in the process of aging, *Hedychium* plants at the edge of a stream bend down until they become submerged. Any clutch laid on old leaves runs the risk of being washed away by the current, or drowned.

cally. *Hedychium* plants are locally abundant. The young plants initially grow vertically on the stream margins, and after a few weeks, they begin to tilt toward the stream. Unless the stream overflows excessively, young leaves may overhang the stream for the duration of the rainy season (or at least the two weeks it takes for tadpoles to hatch). Older plants tilt excessively, and eventually bend enough to fall into the stream (Fig. 3C). If this occurs, any *C. fleischmanni* clutches would be washed away in a matter of hours. *Hedychium* plants sprout asexually throughout the year, and those plants nearest the stream bank eventually fall into the stream, hence there is always an abundant supply of young plants with fresh, clean leaves (Fig. 1A).

Although locally abundant, *Hedychium coronarium* is an introduced Asian plant (Standley & Steyermark, 1952; Kuck & Tongg, 1964) and thus is not the "natural" habitat of *C. fleischmanni*. Examination of second choice plants may give an idea of the original oviposition sites. At the Matagalpa localities there are a number of streamside native plants where *C. fleischmanni* oviposits preferentially. Most commonly, banana-like plants of the genera *Heliconia* (Marantaceae) and *Calathea* are used, as well as several species of creeping Araceae, especially of the genera *Anthurium*, *Philodendron* and *Syngonium*, which frequently cling to the rocky walls of torrential mountain streams. Other plants growing similarly, but less frequently used, are broad-leaved ferns (such as *Pesopteris crassifolia*) and non-creeping Araceae, such as *Anthurium crassifolium*. I have never found *C. fleischmanni* eggs on narrow-leaf grasses, deciduous and small-leaved plants (leaves measuring less than 8 x 4 cm) or urticant ones; two abundant plants that provide a broad undersurface, but where *C. fleischmanni* eggs have never been found, are begonias (Begoniaceae) and elephant ears (*Xanthosoma macrorrhiza* and *X. violacea*) (Fig. 1B).

In extensively cultivated hills, *C. fleischmanni* oviposits on coffee plants (*Coffea arabica*) and citrus trees (*Citrus* spp.) as well as in the espadillo (*Yucca elephantipes*). In a disturbed habitat in northern Nicaragua, Villa (1972) observed many *C. fleischmanni* egg masses on the grass *Coix Lachryma-Jobi* which grew abundantly in a swamp. A similar situation was also found in a San José (Costa Rica) field site (Fig. 1B).

By comparison, *Agalychnis callidryas* oviposits almost indiscriminately—although rarely on begonias and elephant-ears—and may even lay on inanimate surfaces such as metal pipes, pool walls, etc., usually but not always in a favorable position above water. The size of their clutches is considerably larger than *C. fleischmanni*'s (Table 2) and some times more than one individual oviposits on a single leaf, thus many leaves are unable to hold the weight of the egg mass and collapse into the pond.

Usually *C. fleischmanni* eggs are laid on the underside of *Hedychium* leaves, preferably the inner five-eighths of the leaf. Leaf stems and tips are avoided. *C. granulosa* frequently oviposits near the distal end of the leaf. When eggs are found attached to the upper side of leaves, they usually have been disturbed by wind, rain or animals, in such a way that the upper side faces the water.

Although most eggs are laid on leaves overhanging the stream, they are often (to 30% in some localities) laid in such a way that the tadpole's fall is obstructed by the leaves, branches, vines, etc.; it may also fall on the ground a few cm to 0.5 m from the water's edge. Field experiments showed that tadpoles are quite hardy, and usually can tolerate the obstruction even if they should hit a rock after a 0.5 m fall.

Since most hatching occurs during the showers or rains, the water film cushions the fall, prevents the desiccation of the tadpoles and allows them to respire while on the ground. Hence, after falling, they can flip themselves, like a fish out of water, for at least an hour (observed), before finally reaching the stream. Such

TABLE 2. Variation in the number of eggs per clutch in 4 species of frogs with arboreal eggs at the Matagalpa study site.

Species & Date observed (side of leaf where deposited)		Number		mean	± SD
		of clutches	of eggs/ clutch		
<i>Centrolenella fleischmanni</i> (underside)	(1971)	11	15-22	20.9	2.06
	(1974)	93	15-48	30.15	5.24
	(1975)	204	10-50	29.66	6.03
	(1976)	51	17-43	28.92	6.19
<i>Centrolenella granulosa</i> (upper side)	(1974)	2	49-60	54.50	7.77
<i>Centrolenella pulverata</i> (upper side)	(1974)	5	44-88	64.00	14.90
<i>Agalychnis callidryas</i> * (upper side)	(1974)	9	46-103	62.44	18.49
	(1975)	42	15-97	46.02	21.08
<i>Hyla ebraccata</i> ** (upper/under)	-----	17	24-76	44.00	-----

*Up to 108 eggs may be laid by individuals of this species. For a summary of records, see Duellman (1970: 109).

**Data from Duellman (1970: 233) for a population from Toocog, El Peten, Guatemala, included for comparison. Only a few clutches were counted for this species from Matagalpa, and the number of ova was within the range cited above.

oviposition sites, however, are probably suboptimal (i.e., increase mortality) since tadpoles (i) may be unable to locate the water, (ii) may be eaten by insects or other terrestrial predators, or (iii) may be too exhausted, when they finally fall into the stream, to swim with sufficient strength to overcome the swift current.

Oviposition on the upper vs. lower side of leaves. The small size of the *C. fleischmanni* clutch allows it to be deposited on the underside of leaves rather than on the upperside, as typical of other frogs with aboreal clutches at the Matagalpa locality (Table 2).

Large clutches, such as those of *Agalychnis callidryas*, *Centrolenella granulosa* and *C. pulverata*, are laid on the upperside of leaves which, unless they are strong enough, will bend under the weight sometimes reaching an almost vertical position. The clutch will still be supported by a broad surface of the leaf (Fig. 4, B-E), but if the leaf is moved to a position parallel to the ground (as if the clutch was laid on the underside; Fig 4I) the clutch will usually break off and fall in the water after some time (which depends upon the mass and the degree of hydration of the clutch). Unless embryos are ready to hatch, falling into the water kills them.

Clutches laid on the upperside of leaves, or on their tips, are probably more visible to certain predators (such as ants, wasps, birds and snakes) which may eat clutches laid outside water (Scott & Starrett, 1974; McDiarmid, 1975, 1978; Crump, 1977, Villa, *pers. obs.*; Villa, McDiarmid & Gallardo, 1982) than are those concealed under leaves.

Clutches laid on the upperside of leaves are more vulnerable to environmental factors such as sunlight, settling dust or debris, and rain; the latter may wash off—or dilute—any protective chemical the jelly may have against fungi or other organisms, (Villa, 1979a) or produce excessive hydration of the clutch, which could kill the embryos. During the rainy season, the relative environmental humidity is usually high enough to keep the clutches adequately hydrated without the need of rain falling directly upon them. During the dry spells, however, they seem more vulnerable to dehydration than the larger, more watery clutches which, laid on leaf-tips, receive what little rain may fall. Some moisture is provided by the accompanying parent who sits on them and may occasionally urinate on the egg mass.

During the dry spells, however, clutches laid on the underside of *Hedychium* leaves are protected from dehydration by the leaves themselves, which curl downward when their turgidity decreases, and thus shield the clutch from the drying air (Fig. 4, F-G). If the eggs were laid on the upperside of the leaves, the same curling would not only not protect the clutches, but would also stretch the masses' surface (Fig. 4H, I) providing a greater area for evaporation and thus increasing the rate of dehydration.

Number of eggs per clutch.—Little information has been published on the number of eggs comprising a *C. fleischmanni* clutch. In Costa Rica, Starrett (1960) found 15-22 ($\bar{x} = 18$) eggs per clutch, and Villa, (1972) recorded 17-22 (\bar{x}

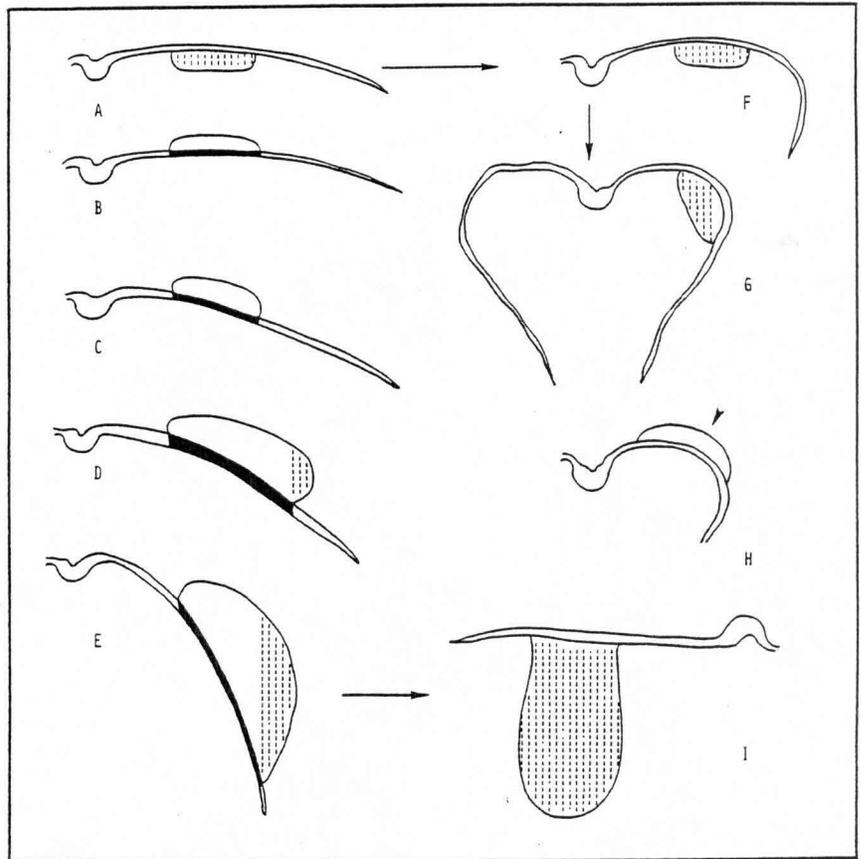


FIGURE 4. Effect of the size and oviposition site on an egg clutch. —(A), normal size and localization of a *Centrolenella fleischmanni* clutch. —(B), the same clutch placed on the upper-side of a leaf, showing a negligible effect on the leaf (black area represents portion of leaf that supports the weight of the clutch). —(C), a larger mass causes the leaf to bend down, and even further in (D) and (E), where part of the mass shifts outside the region of attachment (shaded areas). The latter two cases are exemplified by the clutches of *C. granulosa*, *C. pulverata*, *Agalychnis* and *Phyllomedusa*, in Nicaragua and Costa Rica. —(F), effect of decreased relative humidity on the typical clutch of *C. fleischmanni*: when humidity is lower, the leaf loses turgidity and its edges bend down somewhat. —(G), with an even lower relative humidity, *Hedychium* leaves curl downward and inward, and the clutch is protected against dehydration. —(H), if the clutch was laid as in (B) not only would it not be protected against dehydration inside of the leaf, but would also tend to stretch out during the curling of the leaf, and the larger surface area would increase the dehydration rate of the clutch. —(I), if the leaf in (E) is purposely turned upside down, without additional support, the mass will elongate and quickly become detached from the leaf.

= 20.9) in northern Nicaragua. More extensive quantitative data are now available. At the Matagalpa study site 358 clutches were counted in 1974-76, and 105 were counted from the San José site in 1976 (see Figs. 5-6 and 17).

The mean number of embryos counted at the Matagalpa site was significantly

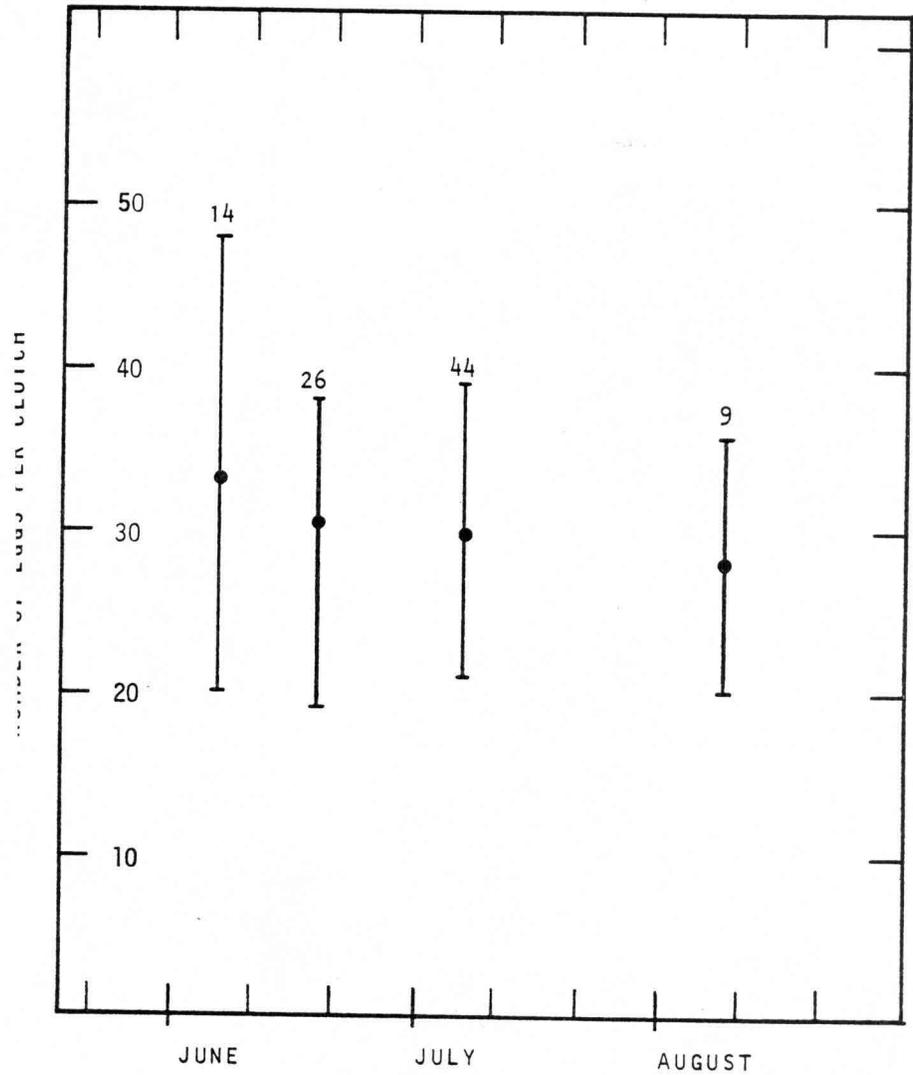


FIGURE 5. Size of *Centrolenella fleischmanni* clutches in four samples taken at the Matagalpa site during the early part of the 1974 rainy season. Vertical bar represents the range, the circle represents the mean, and the number above the line indicates the number of clutches counted for this purpose.

different ($P < 0.01$) than those reported for northern Nicaragua (Villa, 1972); the number varied from year to year (means for Matagalpa: 1974, 30.15; 1975, 29.66; 1976, 28.92), but the differences in mean values were not significantly different ($P > 0.05$). There was also some variation in the size of clutches laid at the Matagalpa site during the beginning, middle, and end of the rainy season (Fig. 5-6). In Nicaragua the range of variation was greater in the beginning of the rainy season (June-July) than during the rest of the year. In the middle of the season (August-September) the means were lower and decreased further by the end of the rainy season. The differences, however, are not significant ($P > 0.05$) in 1974 or 1976, and in 1975 only two samples (one at the beginning and the other one at the end of the rainy season) were significantly different when compared with the samples taken that year (Fig. 6).

The clutch size at the San José site was significantly ($P < 0.01$) smaller than at the Matagalpa site (Fig. 6, lower), with a mean of 25.75 for 1976. Although the smaller clutch size refers only to the San José site, it may be a reflection of a smaller clutch size for Costa Rica in general, as suggested by the low number of eggs reported by Starrett (1960). However, Taylor (1952) obtained a single clutch (not certainly laid by a *C. fleischmanni*) from near Cartago (Cartago Province), Costa Rica, and it contained 53 eggs, a number greater than any *C. fleischmanni* clutch I examined. If this number was not erroneously reported, it would be considerably higher than the number of embryos found elsewhere in Costa Rica, and perhaps evidence of local variation similar to that between the northern Nicaragua and the Matagalpa sites.

The pattern of variation among samples taken at different times at the San José site (Fig. 6, lower) was different from that at Matagalpa. At that site the values were bimodal with the highest values, and greatest variation, occurring at the beginning and the end of the rainy season and — apparently because of the reduced rainfall (see Fig. 2) — the lowest values found in the middle part of the season; a statistical analysis of the samples revealed significant differences ($P < 0.01$) between the midseason clutches and the rest of the samples (Fig. 6). The reason for the variation in clutch size in both countries (if other than rainfall), and its biological significance, can only be guessed at this time, but should be investigated further.

No information is available on the number of clutches laid by individual frogs. A few females were periodically dissected to check their reproductive condition and all had ova of assorted sizes, ranging from large oviducal to nearly-microscopic ones. This suggests that eggs are being continuously produced and that two or more clutches may be laid per season.

The clutch size of other frogs with arboreal eggs at the Matagalpa site is usually higher than for *Centrolenella fleischmanni* (Table 2). Compared with other amphibians such as *Bufo marinus* (which lays 8000 to 35,000 eggs during a breeding period, according to Oliver, 1949, and to Straughan, 1966), *C. fleischmanni* has a *K* type of reproductive pattern in the sense of MacArthur & Wilson (1967). The number of eggs is small, the size of the ovum is moderately large, embryonic (prehatching) developmental time is long, and hatchability

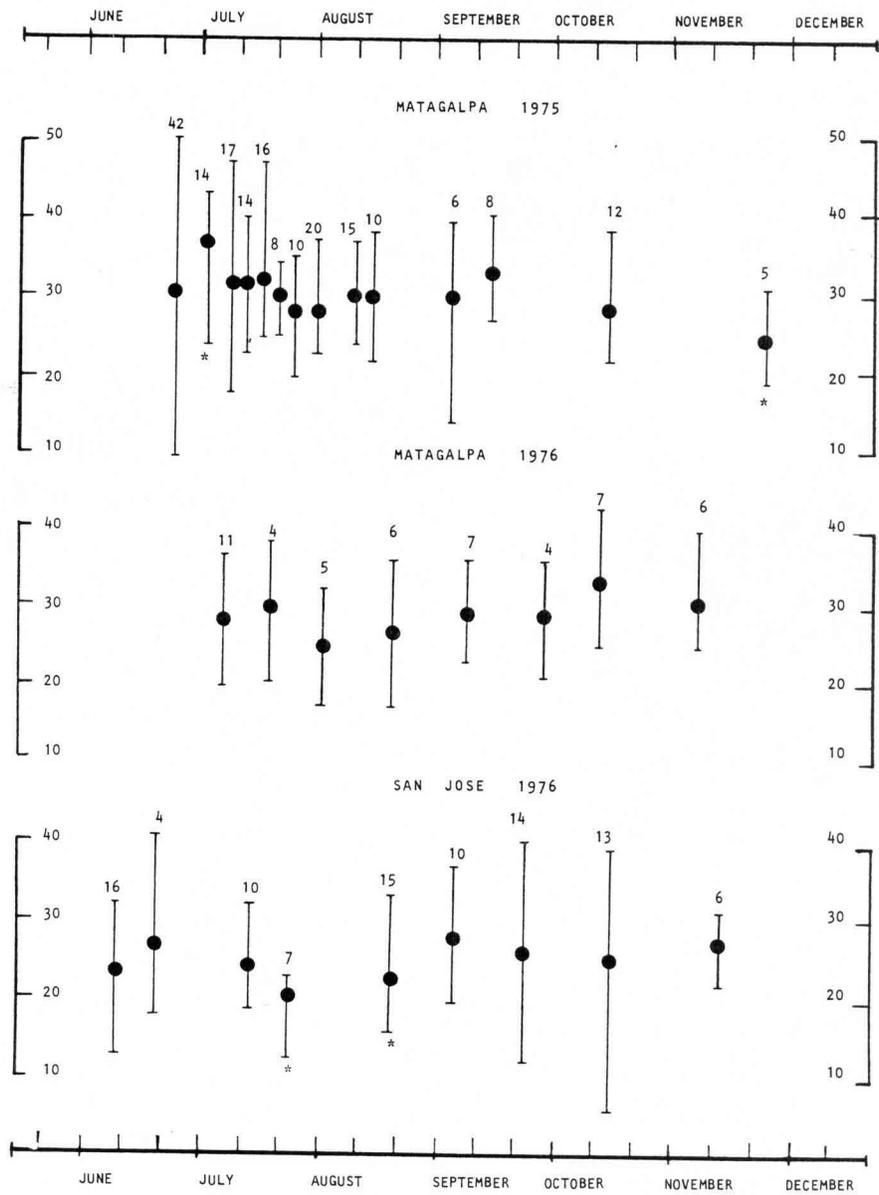


FIGURE 6. Size of *Centrolonella fleischmanni* egg clutches in samples taken at the Matagalpa and San José sites during the 1975-76 rainy seasons. Symbols as in Fig. 5. Asterisks indicate values significantly ($P < 0.01$) different from the total population mean.

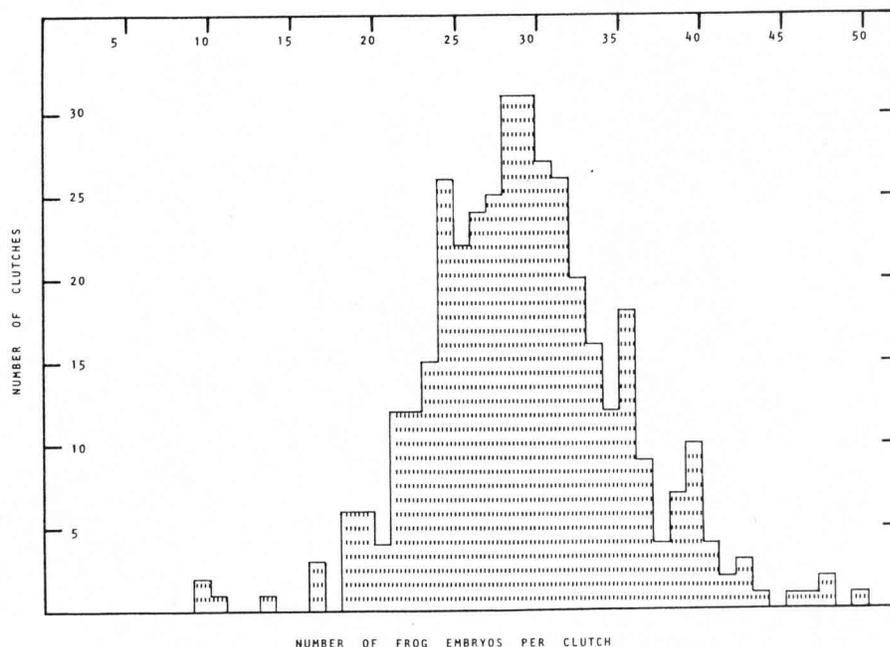


FIGURE 7. Histogram representing the size frequency of all *Centrolenella fleischmanni* clutches taken at Matagalpa in 1974-76 ($n = 385$).

is high, all indicating a higher reproductive efficiency. In Crump's (1974) list of reproductive strategies, *C. fleischmanni* fits well in "Type 4," and in Mode 5 of Duellman (1978).

Number of clutches per leaf.—Normally *C. fleischmanni* lays one clutch per leaf. This may be due to (i) the abundance of oviposition sites in relation to the number of amplexing pairs, (ii) established territories, or (iii) both. Some centrolenids are known to accompany (or "guard" clutches that are presumably theirs); approximately 30% of all clutches observed at night had an accompanying parent, almost always a male. None accompanied them during the day. Although in *C. fleischmanni*, the reason for this accompaniment does not seem to be to protect (or "guard") the clutch, the presence of a male squatting on top of a clutch, or near it, may discourage amplexing pairs from ovipositing in the immediate vicinity. McDiarmid & Adler (1974) noted territoriality and "encounter calls" emitted by *C. viridissima* (= *C. fleischmanni*) in México and *C. valerioi* in Costa Rica. I found the same to be true in Matagalpan *C. fleischmanni*, and noted that fights between males were more easily elicited during the beginning and middle of the rainy season than toward the end, when breeding was only occasional. More detailed studies of the calls and territorial behavior of *C. fleischmanni* were recently published by Greer & Wells (1980) and Wells & Schwartz (1982).

There were several instances when two clutches were found on a single leaf: (i) in two cases one of the clutches was considerably smaller than the other one (3 and 8 embryos respectively), and were both in the same developmental stage. Probably in both cases the clutch-pairs were produced by the same female whose oviposition was interrupted for some reason; in the clutch-size analysis, the number of embryos in each clutch pair was added and counted as one clutch. (ii) in nine cases clutches were found with embryos of very different ages (e.g., stages 13 vs 25; see Fig. 3B), or one of them had recently hatched, while the other was still developing. Apparently, the older clutch was no longer being accompanied by a parent, or the initial resident male had moved to another territory, before the second clutch was laid. (iii) in 6 cases (1.22% of 489 observations) 2 clutches were found with a normal embryo complement, identical developmental stages, and on the same leaf. The clutches were usually laid at opposite ends of the leaf, at least 13 cm apart. Most of these clutch-pairs were found in the first third of the rainy season, when the breeding population seemed at its highest.

More often, however, clutches were laid on leaves where previous clutches (if there had ever been any) had already disappeared from "optimally placed" plants; it was possible to determine where these clutches had been laid if they had been previously tagged.

Duration of embryonic stages.—The duration of embryonic (or pre-hatching) stages (0-25) of *C. fleischmanni* was established by Villa (1972, Fig. 95) in northern Nicaragua. The estimate was somewhat erroneous because of (i) the small (25) sample size, (ii) no clutch was continuously observed from stage 0 to stage 25, and (iii) the assumption was made that embryos in stage 10-13 were about 24

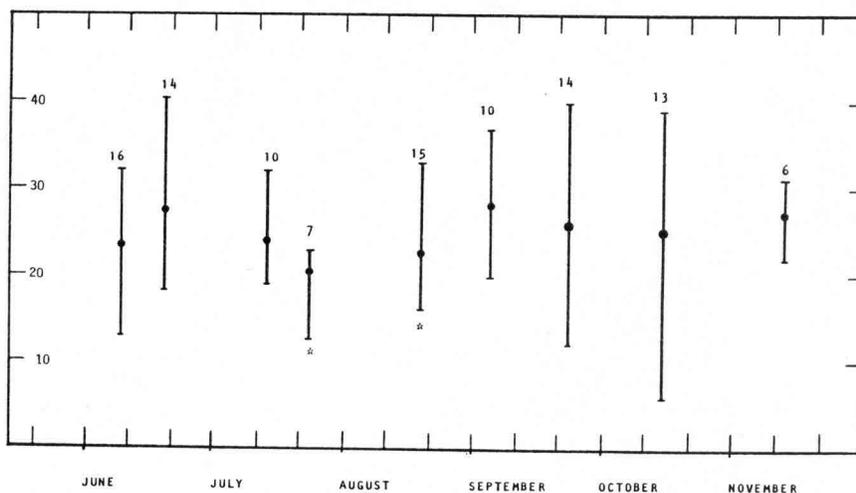


FIGURE 8. Size of *Centrolenella fleischmanni* clutches in samples taken at the San José site during the 1976 rainy season. Asterisks indicate values significantly ($P < 0.01$) different from the total population mean.

hours old. Although no clutches have yet been observed from the moment of oviposition to hatching, some of those found in the first hours of the morning were in stages 3-6 (as compared to 10-13 in the previous study), and were probably laid some time during the previous night; 12 hours was the assumed age of these clutches, and several of these were observed throughout stage 25. The longest time necessary to reach this stage was 370 hours, but shorter times were estimated for several others (Fig. 8). Although development varied even among the embryos of the same clutch, approximately 250 hours were necessary, on the average, to reach stage 25 (50 hr. more than estimated by Villa, 1972). Stage 25 was considered critical because (as will be discussed later), maggots hatching when the embryos are in this stage have no chance of survival. It is also the last of the pre-hatching stages; any time after reaching stage 25, successful hatching is probable.

The embryonic stages are not noticeably different from those normally found in other tree-frogs, thus Gosner's (1960) nomenclature was used. However, stages 8-10 were difficult to separate because of the very slight differences in the pigment of the animal and vegetal poles; stage 23 was also difficult to distinguish because of the pale pigment. To observe blood circulation in the tail fins (which also characterizes stage 22) was difficult without removing the embryo from the egg capsule. With some practice, however, these stages could be interpolated with minimal error.

Hatchability.—The percentage of tadpoles hatching from a known number of eggs (termed "hatchability" by Bragg & Bresler, 1951) is dependent of several variables. At the Matagalpa study site the following were noted as most important: (i) the number of fertilized ova (viability); embryonic mortality due to (ii) developmental abnormalities, (iii) environmental humidity, (iv) stream overflows, and (v) parasites or predators. In a paper just published, Hayes (1983) reported predation on *C. fleischmanni* egg masses by phalangids (*Prionostemma frontale*) and gryllids (*Paroecanthus tibialis*). He also observed grapsid crabs (*Sesarma roberti*) consuming *C. granulosa* embryos.

Of 141 *C. fleischmanni* clutches marked *in situ* during the 1975 rainy season, only 28 (19.86%) were not parasitized, preyed upon, washed by stream overflows, nor dehydrated during dry spells. The proportion of tadpoles hatching from the clutches not destroyed by these factors probably depends largely on the number of fertilized ova. Most of the embryos failing to hatch in this sample did not develop at all, and others (usually located at or near the margin of the clutches) developed abnormally. Thus "hatchability" approaches the "potential natality" concept normally used by ecologists (e.g., Odum, 1959) if used for the tadpole stages. Because other variables (above) were difficult to separate, and because complete information was not available for all clutches, other demographic parameters (such as "realized natality" and "realized mortality"), during the embryonic-tadpole stage, were not determined.

Hatchability for the 1975 rainy season was 71-100% ($\bar{x} = 97.22 \pm 5.99$), in the clutches not destroyed by the events mentioned above. This figure is comparable to those found in other species of frogs (see review by Woodruff, 1976).

Tadpole survival.—After the egg capsules rupture, tadpoles fall and eventually find their way into the water. What happens to them until they become sexually mature is largely unknown. Free-living *C. fleischmanni* tadpoles (and of other centrolenid species as well) are almost never seen swimming in streams. Judging from the few known finds, they are apparently fossorial; digging in the soft mud of the sides (not bottom) of streams, sometimes buried 10-20 cm deep. When found, they are invariably bright red. This unusual color, found in tadpoles of no other anuran family, is not due to chromatophores but to their blood showing through almost unpigmented skin. Villa & Valerio (1982) believe this to be a respiratory adaptation to fossorial life. They reported red tadpoles in other centrolenids, and white one in an unidentified Venezuelan *Centrolenella*. In the laboratory, tadpoles are extremely difficult to raise. Lutz (1947) noted that her centrolenid tadpoles were not "raised right through as their requirements are difficult." Starrett (1960) kept tadpoles of 5 centrolenid species for periods up to 5 months, and none reached a size greater than 20 mm. I have had the same experience, even after providing them with a variety of artificial habitats and foods. Usually the tadpoles would cease growing, become emaciated, and die one by one. Furthermore, they were always brown, never attaining the bright red color found in nature. Possibly patient rearing and experimentation will enable us to learn more about larval life of *C. fleischmanni*, but this remains to be done (Villa & Valerio, 1982).

Emergence of Froglets — No data are available on the duration of larval life of *C. fleischmanni*. In northern Nicaragua, I found metamorphosing tadpoles and froglets shortly after the breeding season had begun (Villa, 1972). The same pattern was also found in Matagalpa in 1974- 76. It seems improbable that those froglets had hatched only 1-2 months before, so they may be about 12 (or more?) months old. Although metamorphosing tadpoles can occasionally be found throughout the year, they are more common (as are adults) during the wet season. Because of the small size, (11-12 mm S V) froglets are prone to rapid dehydration if they emerge during the dry season.

Possibly in areas with a reasonably defined dry season, tadpoles "wait" until the next rainy season (1, perhaps 2 years) to complete metamorphosis, thus emerging in a moist environment, whereas, in areas with a less defined dry season (such as in the Meseta Central of Costa Rica), there is less pressure to emerge during the rainy season, and metamorphosis may be completed in less than 12 months.

Frog-Fly Interactions

The known interactions between flies (Diptera) and amphibians fall into three distinct categories: (i) flies ovipositing on dead or decomposing bodies, and the resulting maggots (fly larvae) consuming the amphibian's flesh; (ii) flies ovipositing on living amphibians, and the maggots eating away the living tissues, and (iii) flies ovipositing on healthy amphibian egg clutches and their maggots eating the developing frog embryos.

The first of these interactions is of little biological interest since the fly species involved are opportunistic scavengers, able to oviposit on virtually any type of vertebrate or invertebrate carcass; the second type (myiasis) is of more interest, since of all possible species of scavenger flies only a few seem capable of causing myiasis in amphibians (possibly the amphibian's skin secretions have some influence on this). There are only a few literature records of this type of association, which merits further study (see Villa, 1977 and MS). The third type of association is similarly interesting, since it involves peculiar adaptations or preadaptations on the part of the maggots, and since the fly's early development appears to be synchronized with, and dependent on, the frog's larval development. Such is the interaction studied in *Centrolenella fleischmanni*.

There are only a few literature references reporting this third type of interaction in frogs, and most of them record only extremely fragmentary information (often even leaving out the fly's or the frog's scientific name). Liu (1943) noted that some foam egg masses of the rhacophorid frog *Rhacophorus* (= *Polypedates*) *leucomystax*, in Western China, were laid in manure pools, and these were "mostly infected and spoiled by maggots which eat up all the eggs in the foam". These observations were recently repeated in Malaysia (near Kuala Lumpur) by Yorke (1983), who also found infested egg masses in flooded fields. In both cases it is not clear if they fly (now identified as *Lucilia* sp., a calliphorid) oviposits on healthy or diseased egg clutches.

Costa-Lima (1946) described a new species of fly, *Rhysops berthae* (Diptera: Syrphidae; Melanostomini) raised from the egg clutches of the Brazilian *Centrolenella eurygnatha* collected by the late Bertha Lutz; no other information was recorded. Bokermann (1957) reported that the larva of *Gastrops niger* Williston (Diptera: Ephydriidae) oviposit on the eggs of the leptodactylid *Physalaemus cuvieri* in Brasil, and the maggots consume the developing eggs. Wirth (1958) revised the genus *Gastrops* and described *G. willistoni*, reared from the eggs of the same leptodactylid, *Eupemphix* (= *Physalaemus*) *cuvieri*, also collected by Lutz. The same fly was recently found consuming the developing eggs of *Leptodactylus pentadactylus*, by Lacey (1980) in Brasil, and by R.W. McDiarmaid in Costa Rica (Villa *et al.*, 1982). The eggs of other leptodactylids have been found containing fly larvae, but the species involved are not known (Villa *et al.*, 1982).

Tyler (1962) found maggots of an unidentified fly species destroying the egg masses of the hylid frogs genera *Hyla* (= *Litoria*) and *Nyctimystes* in New Guinea (near Nondugl), but little other information was given about the frog-fly interaction. Silverstone (1973) found maggots of an unidentified fly "attached to a mass of yolk" in a clutch probably belonging to the dendrobatid frog *Dendrobates histrionicus* in Colombia (Department de Choco). The maggots were later identified as probably belonging to the cyclorrhaphous family Phoridae (Villa, 1980). Scott and Starrett (1974) noted unidentified maggots on egg clutches of Costa Rican *Agalychnis spurrelli* but did not determine if viable egg masses were being consumed. A third species of *Gastrops* (probably *G. flavipes*) was found in the egg clutches of two peruvian hylids (tentatively

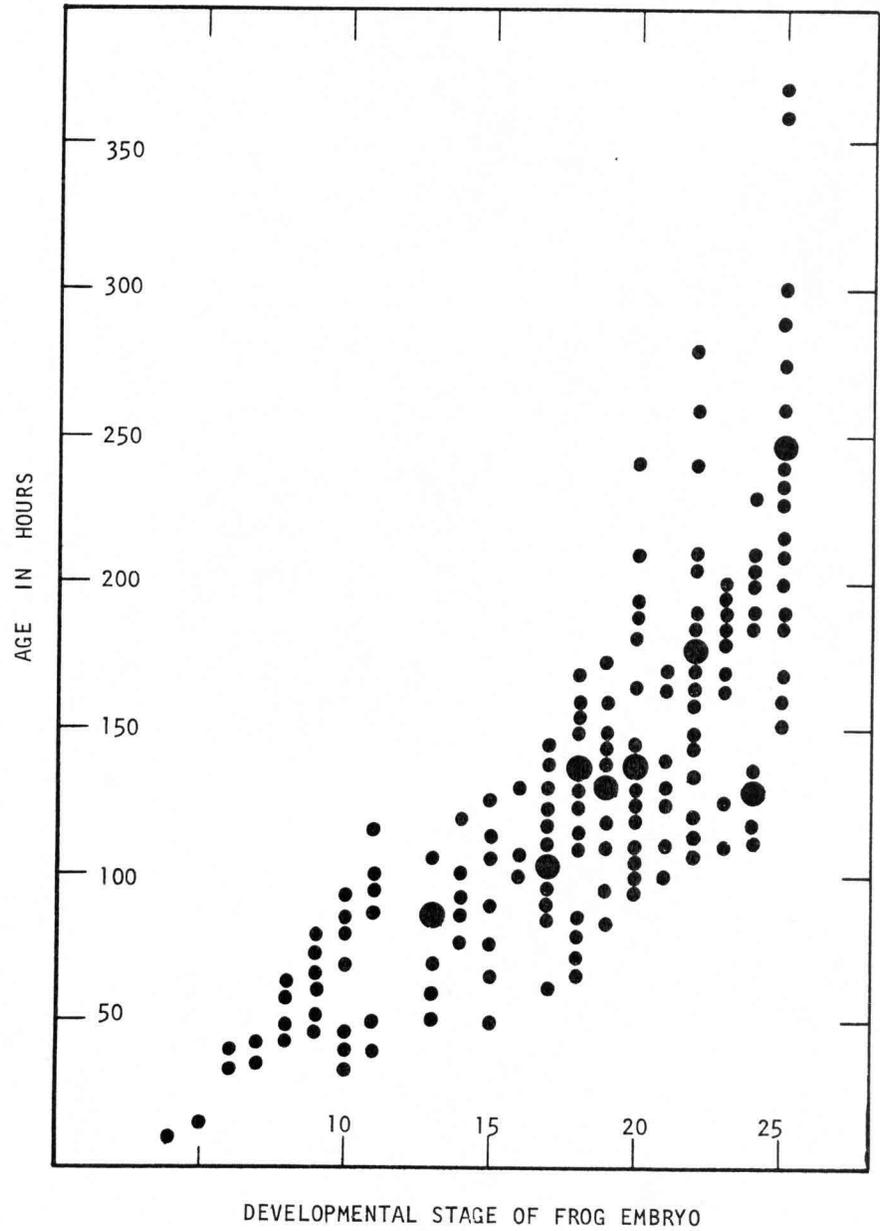


FIGURE 9. Duration of embryonic stages of *Centrolenella fleischmanni* clutches successfully reaching stage 25 under the environmental conditions of the Matagalpa site. Each symbol represents the estimated age of one or more clutches. Larger symbols represent coincident data points.

identified as *Hyla saracuyensis* and *H. punctata*) in the Bosque Nacional de Iparia (Departamento de Guanuco) by J. P. Bogart. The maggots apparently killed and consumed the developing frog embryos (Villa, 1980).

During my study of the biology of *Centrolenella fleischmanni* I noted the presence of maggots in many of the frog's egg clutches. The flies, originally identified as *Drosophila* sp. (Diptera:Drosophilidae) (Villa, 1977) were later assigned to the drosophilid genus *Zygothricha* (Villa, 1978, 1980). The preliminary study of the interaction (Villa, 1977), was followed by a dissertation (Villa, 1978) that showed that the relationship between the fly and the frog was not an opportunistic one, but instead a case of symbiosis, and that the fly's life cycle depends on that of the frog's. The maggots can only grow on the egg masses of *C. fleischmanni* and a few other tree-frogs; to this date, no alternative developing medium had been found, indicating that the relationship is obligatory for the fly. This is the first case known of such an obligatory association involving frogs and flies; others are suspected. The term "frogfly" (for lack of a better one) was coined for the unnamed fly, and details of the interaction between the drosophilid frogfly and other frog species were given (Villa, 1978). Further details are reported herein.

After Villa's (1977, 1978) observations were recorded, others have appeared in print detailing cases of interactions involving several groups of insects. These include chironomid, phorid, ephydrid and drosophilid flies in Central and South America (Villa, 1980), phorid flies and lepto-dactylid frogs (*Eleutherodactylus coqui*) in Puerto Rico (Villa & Townsend, 1983), chironomid flies and a salamander (*Ambystoma maculatum*) in Canada (Leclair & Bourassa, 1981), planarians in the eggs of a ranid frog (*Rana maulata*) in Nicaragua (Villa, 1979a), of the hylid frog *Hyla rosenbergi* in Panamá (Kluge, 1981) and even fungi on the eggs of the hylid tree frogs *Agalychnis annae* and *A. callidryas* in Costa Rica and Nicaragua (Villa, 1979b). All these cases support the contention that amphibian eggs are a microhabitat that may be utilized in several ways by many organisms to complete one or more stages of their life cycles (Villa, 1978). The details of the symbiotic association between the drosophilid frogfly *Zygothricha* sp. and *C. fleischmanni* are given below.

The Drosophilid Frogfly

Localization & Recognition.—In a given locality a variable percentage of the clutches of *Centrolenella fleischmanni* and other tree-frogs may contain frogfly eggs, larvae or pupae. Their presence is easiest to determine in the form of full-grown maggots. At this time anywhere from a few to all the frog embryos may be destroyed or deteriorating, and the mass will have a sick, milky appearance (Fig. 10A); closer inspection will reveal translucent or nearly-transparent maggots of various sizes, feeding on the frog embryos.

Pupae are very distinctive (Fig. 10B and E) but not as easy to locate as are maggots in the field. Pupae are usually covered by the remaining jelly-mass with only the respiratory-horn filaments emerging from the surface of the jelly

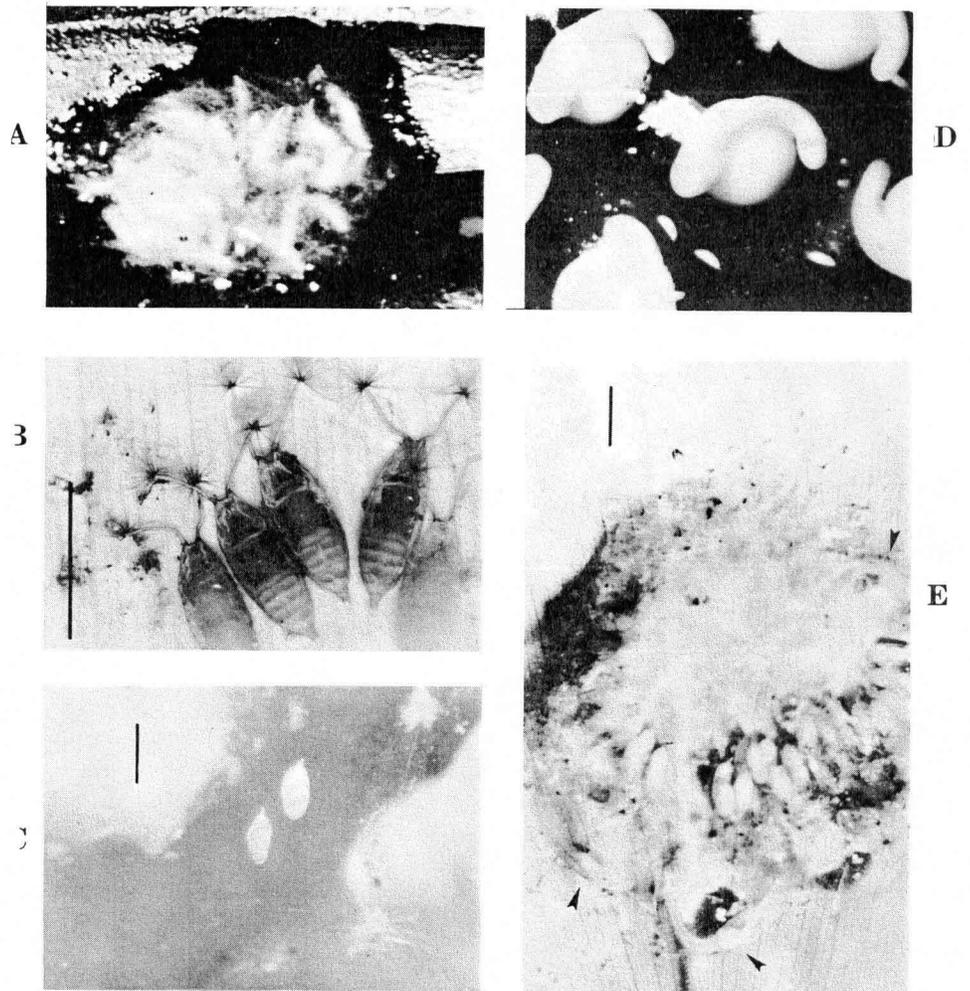


FIGURE 10. Diagnostic features of a frogfly infestation. —(A), a *Centrolenella fleischmanni* clutch totally destroyed by the feeding activity of the maggots; the latter can be seen faintly outlined in the remaining greenish-white jelly. —(B), pupae at various stages of development (scale = 0.5 mm). —(C), an empty (left) and a developing frogfly egg (scale = 0.5 mm). —(D), unhatched frogfly eggs on a clutch with embryos in stage 18. —(E), a clutch containing some surviving tadpoles (arrows) and some frogfly pupae (center); scale = 5 mm.

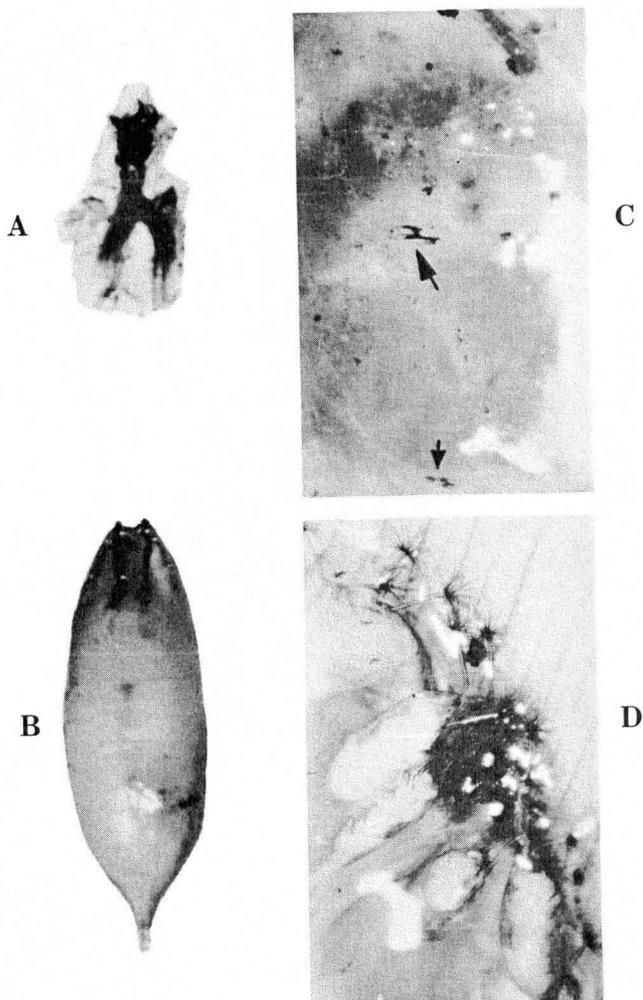


FIGURE 11. Additional characteristics of frogfly infestations. — (A), cephalopharyngeal skeleton of second-instar larva, with a total length of approximately 0.35 mm; gray portion corresponds to the remainder of the slough. — (B), abnormal (stunted) puparium. Such puparia may occur especially when maggots are not allowed to pupariate inside the jelly mass and the environmental humidity is low. In the laboratory only 21% will produce flies; in the field, without the protection of the jelly mass, they are probably subjected to predation by ants and other insects. — (C), close-up of a section of a jelly mass showing the cephalopharyngeal skeleton shed by the maggots during their second molt (arrows: lateral view above, dorsal view below). — (D), normal pupation occurs inside the jelly mass, with the production of long spiracular stalks tipped by slender filaments which protrude from the jelly becoming in contact with the air. The head of a surviving tadpole can be seen in the upper-left margin of the photograph.

(Fig. 11E and 12D) usually at the lateral edges of the mass. After the frog larvae have hatched, or the jelly has cleared or dehydrated, pupae are more easily detected (Fig. 11B). Their presence can be ascertained a long time after both tadpoles and flies have left and after the jelly has disappeared. Empty puparia remain attached to the leaf for a few weeks or even months, depending upon the amount of rainfall.

Not as easily observed in the field is the presence of frogfly eggs or mouthparts. Eggs (Fig. 10C and D) are usually found just below the surface of healthy-looking egg masses in stages 9-18 (They may be found, but are more difficult to detect, after stage 20); they are white, oval-shaped and are about 0.5 mm long excluding filaments. They can be observed in the field with the aid of a 2X hand lens, and the experienced eye can usually detect them unaided. It is easy to confuse them with small seeds, spores or pollen grains, but their characteristic shape is easily recognized in the laboratory with a 10X stereomicroscope.

It is possible that in a formerly-infected clutch, maggots or pupae are no longer present. They sometimes leave the mass spontaneously, for no obvious reason, or they may be picked off by birds or other predators although no actual observations are available. Their former presence, however, may be determined by the mouthparts they shed some 40-60 hours after hatching. Mouthparts are black with a characteristic shape (Fig. 11A and D) and measure 0.25-0.32 mm; they are easily observed with 20X magnification.

Incidence.—The percentage of clutches upon which frogflies have oviposited was determined periodically in the Matagalpa and San José sites.

In Matagalpa, data are available for the first half of the 1974 rainy season, and show (Fig. 12) that fly oviposition occurs less frequently (35% or less of the clutches affected) during the beginning of the rainy season (May-July), increasing steeply during the middle of the season. The same pattern occurred in 1975 and 1976 (Fig. 13) No data are available for the rest of 1974, but in 1975-76 extremely high peaks of incidence were found in August to October (with 100% infestation in some cases) and a decline—although still with high values—after October and November (end of rainy season).

Although the sample size was small in some cases (because of fewer clutches or reduced fieldwork) basically the same trends are evident in all the rainy season samples studied; this provides a strong indication that the differences in incidence of infestation are not artifact of collecting but instead represent trends that are repeated annually.

Data are not complete for the 1976 rainy season in Costa Rica, but those available for the San José site show basically the same trends, except that the peaks occurred earlier (July-August) than in Matagalpa, perhaps because of an earlier onset of the rains; in addition, the incidence was usually lower in San José (never exceeding 80%), and the decline started earlier—also being less steep—than in Matagalpa (Fig. 16).

These data indicate the importance of frogflies upon the tadpole population of *C. fleischmanni*. At any time during the middle of the rainy season at least 50% of the clutches are being partially (and about 20% totally) eaten by frogfly maggots. Although the impact seems less in San José, the percentage of clutches affected is still high. It would seem adaptive, therefore, for the clutches to be laid at the onset of the rainy season (May-June in Matagalpa, probably earlier in San José) when fewer (less than 35%) clutches are being destroyed. Frogs could also oviposit at the end of the season when incidence is similarly low, but the resulting tadpoles would find the habitat already occupied by the survivors of ear-

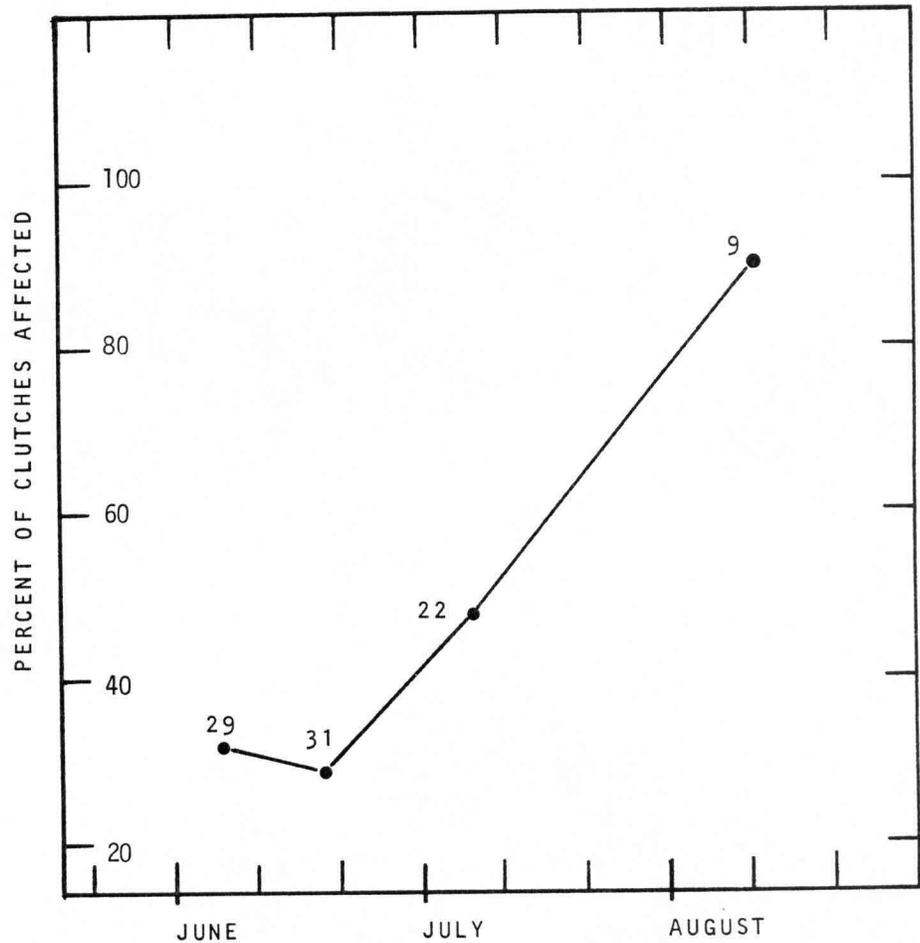


FIGURE 12. Incidence of frogfly infestation in four samples of *Centrolenella fleischmanni* clutches taken at the Matagalpa site in the early part of the 1974 rainy season. Numbers above the line indicate the sample size.

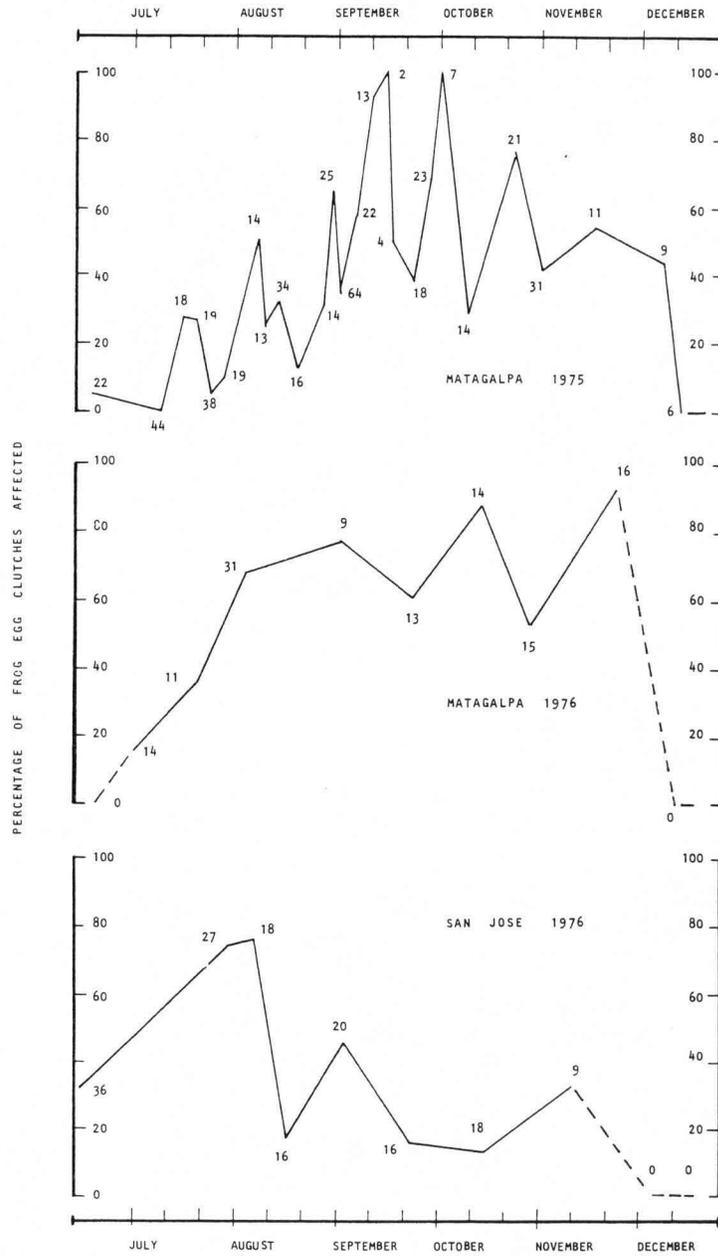


FIGURE 13. Incidence of frogfly infestation in *Centrolenella fleischmanni* clutches taken at the Matagalpa and San José sites during the 1975-76 rainy seasons. Broken lines indicate assumed declines (no egg clutches found).

lier clutches; in addition, they would have a shorter time to develop before the start of the following rainy season.

The incidence estimates may also be a good indicator of frogfly breeding, which seems timed to occur most frequently in the middle of the *C. fleischmanni* breeding season. After this, it becomes less frequent and virtually stops. Frogflies apparently do not breed during the dry season even if clutches are available: after many hours of searching, 3 clutches were found in Matagalpa in December 1974, and 6 more in December 1976, none of which had frogflies.

The contrary seems to occur in *Gastrops niger*. Bokermann (1957) found a greater percentage of infested *Physalaemus cuvieri* clutches by the end of the rainy season in Brazil, which he believed was due to fewer clutches available. However, in Matagalpa and San José, few clutches also had a lower infestation rate at the end of the rainy season.

All the available evidence indicates that frogflies reproduce only once a year, coincidentally with *C. fleischmanni* breeding. This would be an unusual reproductive pattern for a neotropical dipteran as compared to those which, under favorable conditions, breed throughout the year (C.O. Berg, *pers. comm.*, 1976). It would imply that at least some frogflies would have to survive a minimum of 6 months (through the dry season) before reproducing. This longevity would be an unlikely although not impossible feat for a drosophilid, and there are some indications that it may occur.

Laboratory-raised frogflies dissected up to a week after emergence had immature gonads (M.R. Wheeler, *pers. comm.*; and Villa, *pers. observ.*) at a time when laboratory *Drosophila* are developing eggs. This suggests a long pre-reproductive period. An alternative breeding medium was sought (both in the field and the laboratory) during the dry seasons of 1974-77, but none was found. Other *Zygotricha* species are known fungus-breeders, and fungi were especially searched for, and were inspected for fly eggs, larvae and pupae, in 1977. However, fungi are less common, and are usually dehydrated, during the dry season.

Thus, until an alternate breeding medium can be located for the frogfly, the possibility of a restricted reproductive period must be entertained. Possibly only a few of those frogflies emerging during a rainy season survive until the first rains of the next season and oviposit in the first frog clutches laid then. Their offspring, however, would be able to reproduce during the same season (perhaps a month after emergence), which would explain the steep increase of infestation incidence occurring afterward. Possibly two to five generations may breed during that season before reproduction ceases, and the last frogflies to be born would not breed until the following season, perhaps going through a diapause to increase longevity. These are only guesses that may be proven wrong once it is possible to raise frogflies through the duration of their life cycle.

Size of the egg complement.—The number of frogfly eggs in a clutch was determined by careful counting under 10-25 X magnification. The exact number could only be determined after several counts because eggs in different parts of

the frog egg clutch could very easily be overlooked. Data obtained in Nicaragua and Costa Rica (1974-76) are presented in Fig. 14.

It is extremely interesting to note that the mean number of frogfly eggs was not significantly different ($P > 0.05$) from the mean number of frog embryos in Matagalpa; the same was true in San José, although fewer eggs were laid there by both frogs and frogflies (Fig. 14). Differences in the mean number of frogfly eggs and frog embryos between the Matagalpa and the San José localities were

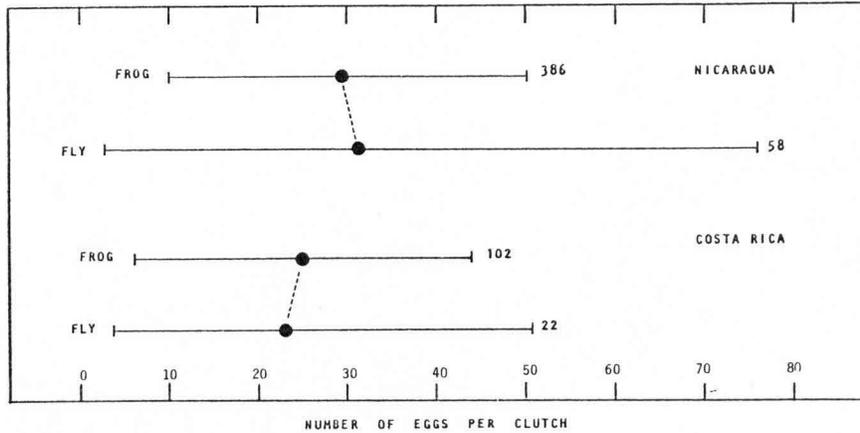


FIGURE 14. Relationship between the number of frog embryos and frogfly eggs in the Matagalpa (Nicaragua) and the San José study sites. Horizontal bar represents the range; solid circle represents the mean, and the sample size is indicated by the number beside the bar. Broken line indicates no statistical differences between mean values. Note that the number of frogfly eggs and frog embryos are not statistically different within a locality, but are significantly different ($P < 0.01$) between both localities.

statistically significant ($P < 0.01$). Thus, frogflies seemed to have adjusted to laying a number of eggs similar or approximately equal to the number of eggs in the *C. fleischmanni* clutch, even if the latter varies from locality to locality. Obviously, frogflies are taking advantage of larger clutches by laying more eggs, and limiting the number of eggs laid in localities where clutches are smaller.

This, however, has occurred at the populational—not at the individual level. Thus, a large number of frogfly eggs may be laid on a very small *C. fleischmanni* clutch or vice versa. If the number of frogfly eggs found in a clutch is plotted against the size of that clutch (Fig. 15) no correlation is found in the Matagalpa and the San José sites (correlation coefficients: $r = 0.0875$ and -0.085 respectively). This can be interpreted as suggesting that frogflies have not become so specialized as to oviposit a number of eggs dependent upon the size of the clutch where oviposition takes place, although at the population level, the mean number of frog and fly eggs are not statistically different.

It has not been possible to determine if these numbers correspond to eggs laid by one or more flies. Laboratory-raised flies offered a variety of food sources lived only a few days and have failed to mature; dissection of such flies offered no information about the number of eggs they could produce. In some cases frog egg

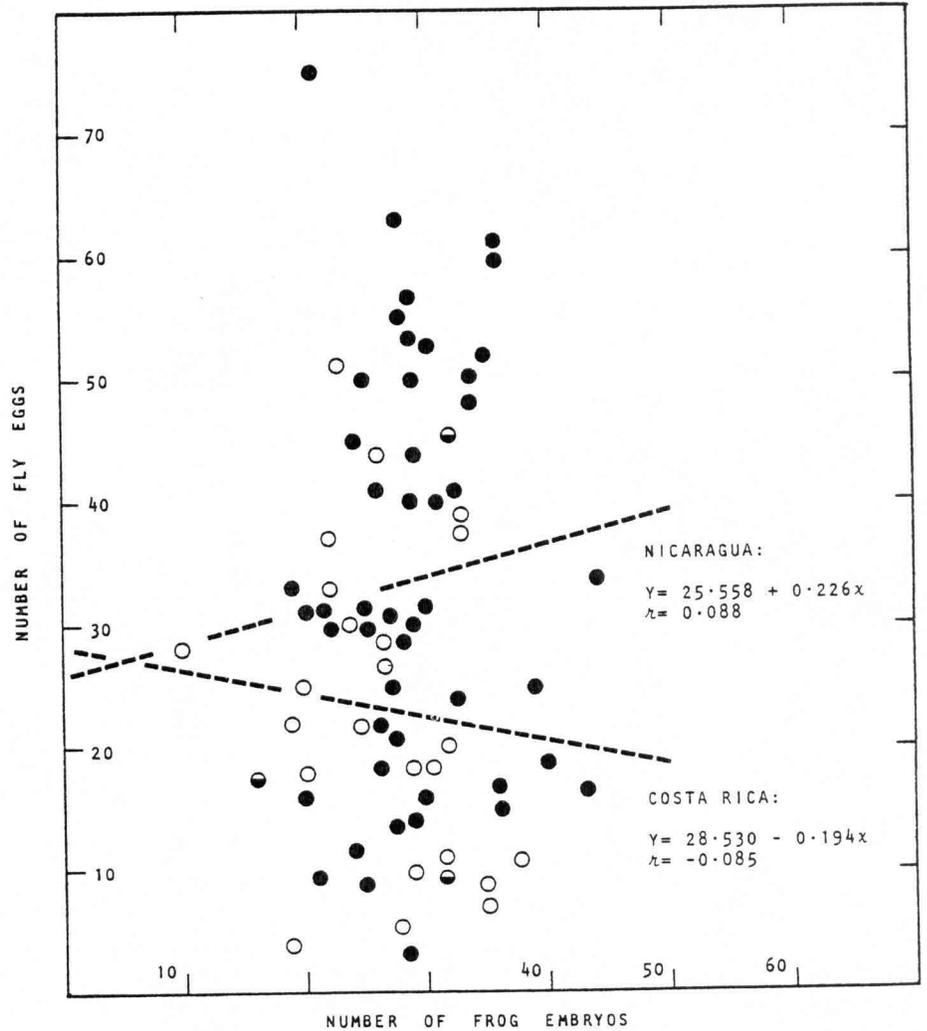


FIGURE 15. Relationship between the number of frogfly eggs and frog embryos in the Matagalpa (solid circles) and San José (open circles; coincident values are represented by half-solid circles) study sites. In both cases the correlation coefficient (r) is not significantly different ($P > 0.05$) from Zero, indicating no correlation between the number of eggs and embryos in each clutch.

clutches are found containing old pupae and young larvae, which seems to suggest that fly eggs have been laid at different times. This may possibly be the case, especially when large (50-70) egg complements are found. There are two reasons, however, which seem to contradict the hypothesis of multiple frogfly infestation: (i) the time between fly-egg laying and hatching is variable; frogfly larvae do not all hatch simultaneously, but several days may elapse between the hatching of the first and last egg (see below); (ii) when larvae and pupae of different developmental stages are found in a clutch of frog eggs, there is no clear-cut age separation, which would indicate 2 or more fly ovipositions, at different times.

Obviously more research is needed to determine the frogfly reproductive potential.

Viability and Mortality.—Not all fly eggs hatch or achieve pupation. Although definite figures are lacking for individual clutches in the laboratory, the number of fly eggs found in the field compared with the number of pupae also found in the field show a high degree of mortality at Matagalpa, although not at San José (Fig. 19).

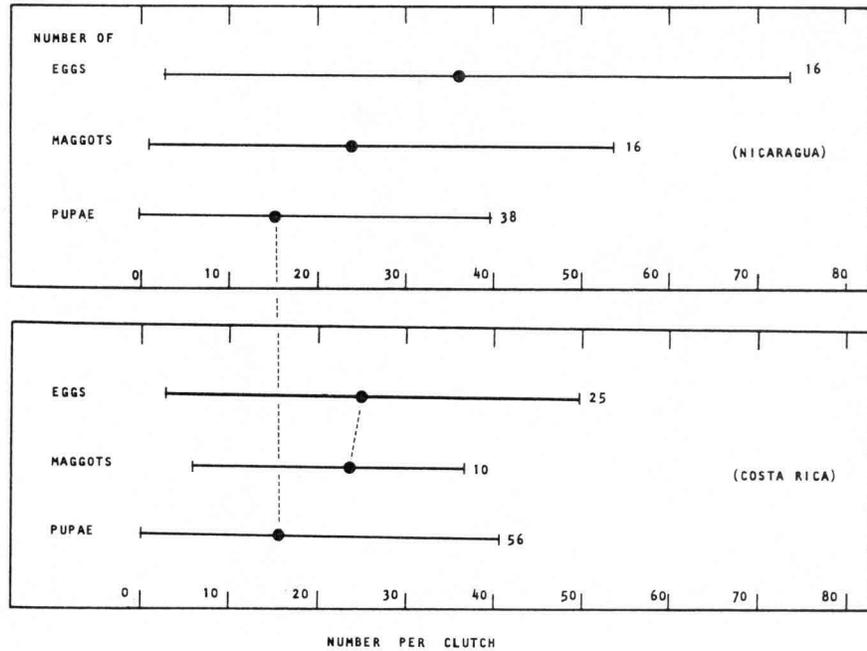


FIGURE 16. Comparison between the number of eggs, maggots and pupae in the Matagalpa and the San José study sites. Symbols are as in Figure 14. Note the graded mortality in the Nicaraguan sample, and the greater viability of the Costa Rican frogfly eggs.

Egg clutches containing frogfly eggs brought to the laboratory were followed closely. In some, all of the frogfly eggs failed to hatch indicating they were probably infertile. Although this happens infrequently, it is not rare that between 20 and 60% of the fly eggs fail to hatch. It has not been practical to obtain more accurate information on this, especially in clutches where intense maggot activity has given the mass a milky-white color, making unhatched frogfly eggs difficult to distinguish.

The mortality rates during the frogflies' life cycle were estimated by counting eggs laid, larvae and puparia present in each clutch (Fig 16). Since transportation to laboratories increased mortality, estimates were only made on recently-caught wild specimens.

The mean number of maggots found in Matagalpa was significantly ($P < 0.01$) smaller than the mean number of frogfly eggs, but the same was not true in San José. The differences are due to both nonviability of some eggs and maggot mortality. The number of pupae was significantly smaller than the number of maggots in both sites, indicating a similar mortality rate of the maggots.

It is very interesting to note that although the number of frogfly eggs laid in Matagalpa and San José were significantly different ($P < 0.01$, Figs. 15-16), the number of resulting pupae was not, mainly because of the greater viability of the eggs and the lower mortality of the maggots at San José. The San José populations, therefore, can be considered more effective than the Matagalpa one in reproductive terms, since a lower number of eggs (i.e., smaller reproductive effort) is necessary to achieve the same results as at the Matagalpa site.

The natural mortality of the pupae is negligible at both sites. Only a few puparia, of nearly 3 thousand counted, contained dead pupae. The presence of parasitoids (Gordh, 1977), or prolonged dry spells, however, drastically increased pupal mortality.

Time of Reproduction and Time of Oviposition.—The frogfly reproductive season appears to be necessarily coincident with the breeding of *C. fleischmanni* and similar frogs; it is therefore restricted to 3-5 rainy season months (perhaps longer in areas with less-defined seasons) unless there exists an alternative, as-yet-discovered, reproductive medium.

Since frogflies have not been observed ovipositing in the field or the laboratory it is not possible to determine exactly when eggs are laid. However, an analysis of the earliest occurrence of frogfly eggs in frog clutches of known age can give us some idea of the oviposition time.

Frog embryos in stages 3-6 are estimated to be approximately 12 hr. old, and were usually collected in the first part of the morning. None in these stages contained frogfly eggs, although the sample is too small (12 clutches) to be conclusive. The youngest clutches containing frogfly eggs were in stage 8 (6 cases), probable aged 20-24 hours. Since frogflies are diurnal they probably also oviposit during the day, any time after the embryos are 8-10 hours old (one night

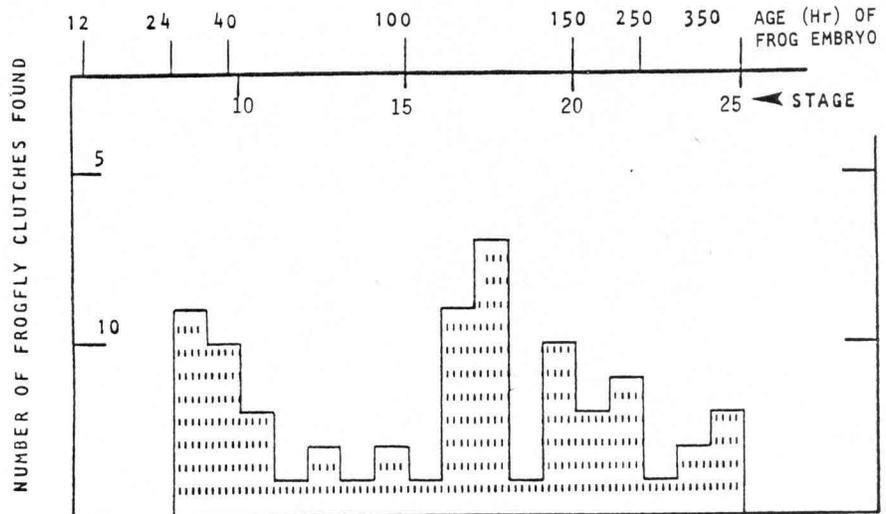


FIGURE 17. Occurrence of unhatched frogfly eggs in *Centrolenella fleischmanni* clutches of various ages. Age is measured in approximate number of hours (above upper abscissa) and in Gosner's (1960) developmental stages (below upper abscissa).

old). Frogflies may search for frog eggs during the morning, but may not find freshly-laid clutches before noon. Between noon and evening embryos are in stages 5-6 to 8 (12-20 hr. old). Oviposition may take place some time in mid-afternoon. The frequency with which unhatched frogfly eggs have been found is presented in Fig. 17.

Although it is advantageous for frogflies to oviposit as soon as possible (see section below), they cannot be thought of as dropping their eggs immediately after the frog has finished laying their own eggs. On the contrary, they will frequently lay their eggs too late (in stage 23 or later) when maggots have little or no chance to feed.

Frog clutches left *in situ* for daily observations and recorded "not infested" in stages 10-18 have later been found in stages 19-20 (4-5 days later), having freshly-laid frogfly eggs. Eggs laid on stage 20 embryos have a good chance for survival. Frogflies have, therefore, 6-7 days (150-180 hrs) after a frog-egg mass has been deposited, to achieve a successful oviposition (Fig 21).

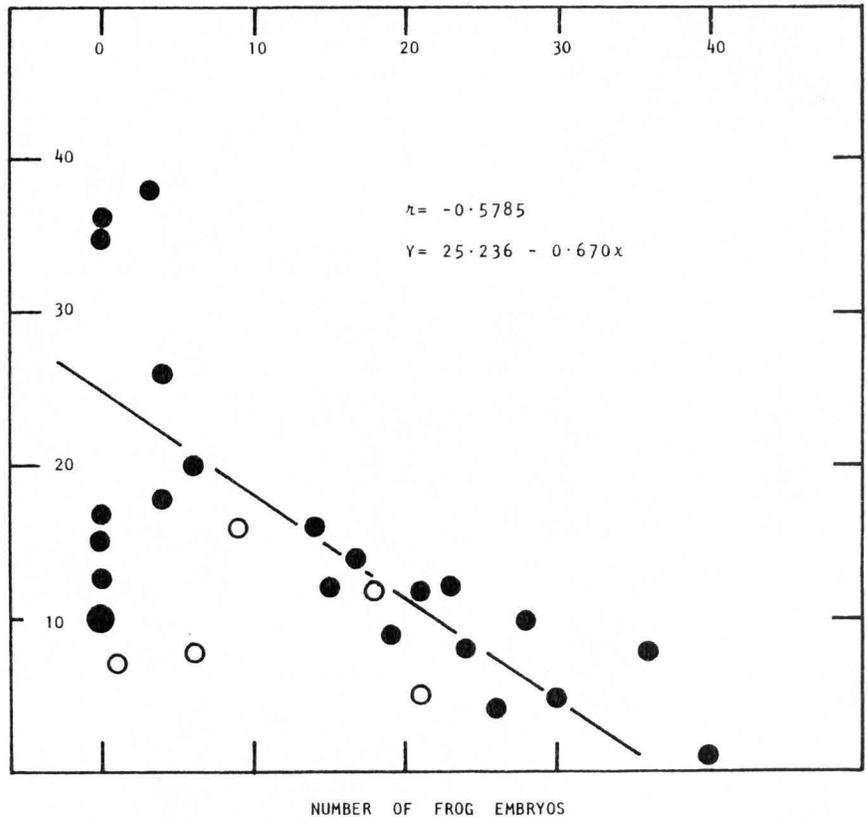
THE FROG-FLY RELATIONSHIP

Destruction of Frog Embryos

Upon hatching maggots begin moving through the jelly until an embryo is found and feeding starts. When first observed, the number of frogfly larvae or pupae was noted to be negatively correlated with the number of unharmed em-

bryos left in the clutch (Fig. 18). It was not clear (i) if flies were laying less eggs on larger clutches or (ii) if embryos were actually being destroyed (since the initial number of embryos was not known). At the time of these observations no drosophilid larvae were known to be carnivorous (see Carson, 1971), so alternative (ii) seemed unlikely.

Direct observation of the living maggots, however, very strongly indicated that embryos were actually being eaten. Since other drosophilid larvae normally feed on yeast or bacteria living in a variety of media, it was considered possible that embryos were being killed so microorganisms could grow on dead tissue and be consumed by the maggots. Would maggots subsist only on the microorganisms growing around dead embryos, or were the embryos themselves being consumed?



Two different types of experiments were designed to answer this question. Embryos were killed and frogfly eggs or first-instar maggots were introduced before the mass decayed (see Table 3 for further details). Although microorganisms grew abundantly on the dead embryos, maggots did not feed and either starved or abandoned the clutch. These results are very revealing but are not conclusive, since it can be argued that the biochemical characteristics of the mass were altered by the water, heat or cold used to kill the embryos. However, additional support was provided when maggots were observed abandoning the masses when, in nature, the embryos had died for other reasons, such as dehydration; the second type of experiment, summarized below, lends further credence to the conclusion that embryos were actually consumed.

For the second experiment advantage was taken of the facts that (i) frogfly maggots are nearly transparent and their gut content can be observed without disturbing the animals, and that (ii) frogs lay eggs of different ground-colors (Table 4). Thus, maggots could be inoculated to eggs of different colors; if the color of the gut contents was the same as that of the embryos, the latter were passing through the gut. Otherwise, some alternative food source had to be investigated. Microorganisms growing around deceased embryos were translucent or whitish, not colored like the embryos they were feeding upon. Embryo color is best defined up to stage 20, before melanophores develop and begin to mask the ground-color, thus embryos older than stage 20 were not used for these experiments. Frogfly eggs or first-instar maggots were inoculated into clutches of *Centrolenella prosoblepon* and *Rana maculata* (blackish), *Agalychnis callidryas* (sometimes polymorphic yellow, green or turquoise) and *Hyla ebraccata ebraccata* (yellow and brown). *C. fleischmanni* eggs (light green) served as control for all inoculations.

In all cases the maggots' gut became filled with material colored identical to the frog embryos they were inoculated into (see color plate for some examples). Clearly, pieces of embryo are passed through the maggots' gut. Whether they were being digested or not remains to be clarified, but it seems logical to assume that they were.

From these observations and experiments, therefore, it can be established that frogfly maggots actually feed on the frog embryos; this is the first known case of drosophilid larvae feeding on vertebrate tissues.

Rate Of Embryonic Destruction

Frogfly infestation does not necessarily mean the total destruction of a clutch. The rate of destruction varied in different samples, between 33.3 and 75.0%, with a mean of 51.14% in all samples studied at the Matagalpa site. In nature (with the initial number of embryos not known) the lowest number of maggots causing the total destruction of a clutch was 13, with a mean of 34.4 in all samples; the highest number of maggots present in a partially-destroyed clutch was 1, with a mean of 16.25 for all clutches.

The number of embryos destroyed in each clutch depends, as expected, upon

TABLE 3. Summary of experiments involving the inoculation of frogfly eggs or hatchling maggots into various media.

Where inoculated (number of replicates)	Summary of Results
Standard <i>Drosophila</i> medium; Cornell Genetics Lab (5 buffered, 5 unbuffered)	Maggots died within 20-40 hr. (apparently starved)
Standard <i>Drosophila</i> medium, U. of Costa Rica (5 buffered, 5 unbuffered)	Same as above
Instant <i>Drosophila</i> Medium [®] ; Ward's Scientific Co. (5 buffered, 5 unbuffered)	Same as above
Ripe or decaying fruit:	
Banana, <i>Musa cavendishi</i> (5 buffered, 5 unbuffered)	Same as above
Pineapple, <i>Ananas sativa</i> (5 ripe, 5 decaying; buffered)	Same as above
Watermelon, <i>Cucumis melo</i> (5 ripe, 5 decaying; buffered)	Same as above
Citrics, <i>Citrus</i> spp. (5 ripe, 5 decaying; buffered)	Same as above
Coffee molasses, <i>Coffea arabica</i> (5 fermenting, buffered)	Same as above
Unidentified local fruits, 6 spp. (5, not buffered)	Same as above
Unidentified fleshy fungi, 2 spp. (5, not buffered)	Same as above
Unidentified slime molds, 2 spp. (5, not buffered)	Same as above
Mammalian feces; dog, cat, man, racoon (5 buffered, 5 unbuffered)	Same as above
Agar-Agar, bacteriological, 2 types (5 buffered, 5 unbuffered)	Same as above
Homogenized <i>C. fleischmanni</i> clutches mixed 1:1 with Agar-Agar (5 buffered, 5 unbuffered)	Same as above
Jelly mass of unidentified spider (3 unbuffered)	Maggots unable to penetrate embryo

C. fleischmanni, dead clutches* (10 unbuffered)
C. granulosa, normal clutch (3)
C. granulosa, jelly removed (3)

Normal feeding until clutch decayed
No feeding; maggots died within 20-40 hr.
Normal survival if supply replenished
when clutch decayed

Clutches of other frogs:
Agalychnis callidryas (10)

Normal survival in Petri Dishes; reduced
survival in suspended clutches

Centrolenella prosoblepon (10)
C. pulverata (6)
Hyla ebraccata (6)
Phyllomedusa annae (10)
P. lemur (4)

Same as above
Same as above
Same as above
Same as above

Rana maculata (10)

Normal survival in Petri Dishes; reduced
survival in suspended clutches
Normal survival in clutch placed in moist
dish and replenished when decayed

Controls: *C. fleischmanni* clutch of comparable age
(2-5 replicates per experiment)

Normal feeding, survival, and pupation
(80-100%)

*Living *C. fleischmanni* clutches were killed by freezing (8 hr. at night in domestic freezer) and allowed to thaw before experiment; by heating (3 minutes, microwave oven) and allowed to cool, or by drowning (3 hr. submerged in stream water).

the number of maggots feeding on that clutch; this is implied in Fig. 18 (although in that sample the initial number of embryos was not known) and was confirmed experimentally. In addition, the number of embryos destroyed *per maggot* depends (also predictably) upon how early or how late the maggots hatch. To determine this, a known number of eggs or newly hatched maggots (between 5 and 10 in each experiment) were artificially inoculated in a clutch of known age containing at least 30 viable *C. fleischmanni* embryos, and were allowed to feed, pupate and emerge. At the conclusion of the experiment the number of surviving tadpoles was subtracted from the initial number of embryos to determine the number of embryos destroyed, and this amount was divided by the number of maggots successfully inoculated. In 1974 only frogfly eggs were inoculated; in 1975 newly-hatched maggots were used. This made inoculations more successful because (i) it was easier to transfer maggots than

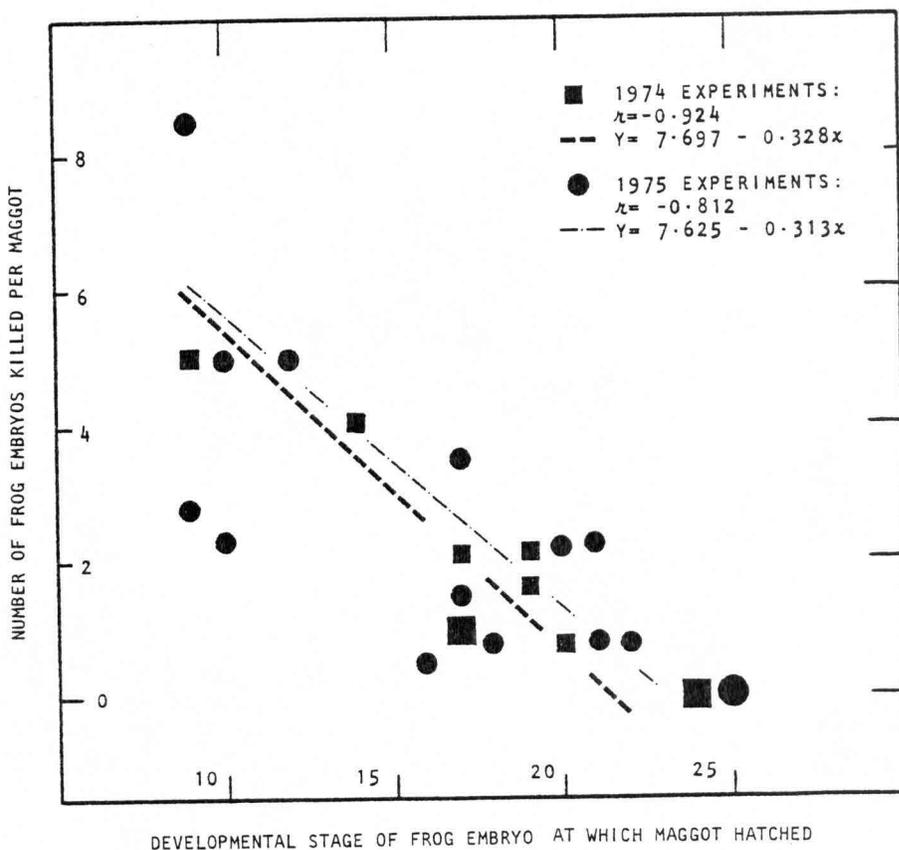


FIGURE 19. Experimental determination of the number of frog embryos consumed or killed by maggots hatching at different stages of development. Large symbols represent coincident data points.

eggs, (ii) the time of initial feeding could be observed without long delays, and (iii) it prevented the waste of time which resulted when nonviable eggs were inoculated. Because of the nature of the inoculations, the 1974 and 1975 results were analyzed separately, but were not found to be significantly different ($P > 0.05$); because of the larger sample size, the 1975 results are considered more representative.

The number of embryos destroyed per maggot ranged from about 0.3 to an excessive 8.2 (Fig. 19). A minimum of half an embryo was necessary for each maggot to develop and pupate successfully, although 2-4 per maggot were frequently eaten. That 4 or more were destroyed per maggot in some cases may represent an unnecessary waste of the resource (because presumably not all embryos destroyed were totally eaten), and this occurred more frequently in maggots hatching when embryos were in stage 14 or earlier. Although this occurred experimentally when maggots were purposely inoculated, it probably occurs only rarely in nature, since maggots tend to hatch when tadpoles are older (usually between stages 15-22; see Figs. 20- 21).

No embryos were destroyed if maggots hatched in stage 24-25 clutches. Maggots were observed attacking these old embryos but the latter would react by frequently thrashing and jerking until the maggot would become detached or the egg capsule would break.

Nature of the Frog-Fly Association

Many kinds of associations are possible between two different species; these may range from occasional or casual (faculative) to absolute dependence (obligatory), and from mutually beneficial (mutualism) to detrimental for one of the species (predation and parasitism) or both (competition).

The close and protracted interaction between individuals of different species

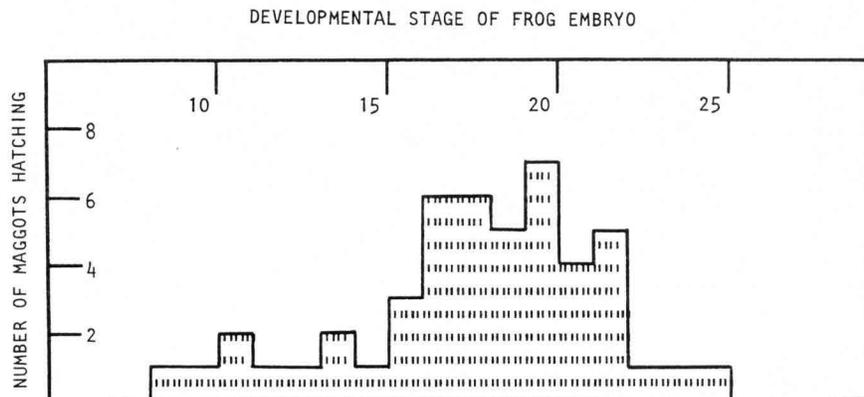


FIGURE 20. Hatching frequency of frogfly eggs in relation to the embryo's developmental stage.

TABLE 4. Some characteristics of the clutches laid by 6 species of frogs found in the Matagalpa site. Modified from Villa (1977).

Species	Oviposition Site	Developing Egg Color	
		Animal Pole	Vegetal Pole
<i>Agalychnis callidryas</i>	Over Poned Water	Green to Yellow	Light Green to Yellow
<i>Centrolenella fleischmanni</i>	Over Mountain Streams	Light Green	Cream
<i>Centrolenella granulosa</i>	Over Mountain Streams	Black	Cream
<i>Centrolenella pulverata</i>	Over Mountain Streams	Dark Green	Light Green
<i>Hyla ebraccata</i>	Over Poned Water	Brown	Yellow
<i>Rana maculata</i>	Submerged in Mountain streams	Black	Cream

is called *symbiosis*, a term simply meaning "living together" in its original (de Bary, 1879) and most modern meaning (Vernberg & Vernberg, 1970; Wilson, 1971). Symbiosis is said to consist of three types of associations: *mutualism* (both species derive benefit), *commensalism* (one species derives benefit), and *parasitism* (one species benefits, the other is harmed).

Odum (1959) divided interspecific associations into positive and negative categories, positive associations being those in which neither partner suffers any harm (commensalism, proto-cooperation, mutualism) and at least one of the pair derives benefit, and negative associations being those where one species takes advantage and damages the other one (predation and parasitism).

Odum's scheme has been widely accepted by ecologists because of its simplicity and usefulness. The frog-fly relationship can be considered to represent a negative interaction, since the fly larvae prey upon the frog embryos and destroy a significant portion of them, whereas the frog does not apparently benefit from the interaction. It must be pointed out, however, that predaceous and parasitic interactions, although detrimental for the individual prey or host, may result in long-term benefit at the populational level and in this sense may not be a "negative" interaction.

Although presently there is not evidence to conclude that frogflies somehow benefit the *C. fleischmanni* population, it should be pointed out that *C. fleischmanni*—the species most affected by frogfly infestation—seems to be the most successful of the centrolenid species. It has the broadest distribution (ecologically and geographically, Starrett & Savage, 1973) and, at least in Central America, is usually the most abundant of the centrolenids where present. There may be no correlation between the two facts, of course, but the possibility should not be dismissed *a priori*.

It is difficult to decide whether frogflies are predators or parasites. The characteristics commonly associated with both categories, taken from a survey of the major works on ecology and parasitology* are presented in Table 5.

Some authors attempt to define parasitism (or predation) using a single criterion. Other authors have attempted to define parasitism on the basis of size, reproductive potential, obligatory dependence, morphological specialization, etc. The practice of using a single criterion is useless and should be discouraged in favor of examining all possible characteristics of the association before attempting to categorize it.

The same literature survey reveals that although pathogenicity (or some kind of harm to the host) usually indicates parasitism, many organisms are known to be harmful to some species** (and even to some individuals) under certain conditions, but not to others, or under different set of conditions; thus a parasite can be a commensal first, and a parasite later, or vice versa.

Frogfly maggots exhibit characteristics of predators, such as killing their prey (host), consuming it completely, having a reduced biotic potential, but also show characteristics typically associated with parasites, such as the intimate degree of association with the host species, the unilateral and obligatory dependence upon the frog embryo, their living within the food source, and others (see Table 5).

As parasites, frogflies lack the remarkable specializations found in "true" parasites such as leeches, tapeworms, or ticks; but as predators they are more finely tuned to their prey than "true" predators such as tigers, snakes or hawks. In a sense, they can be considered transitional between both categories.

If further delimitation of the relationship is required, frogfly larvae can be considered parasitoid because, by destroying their host, they function more like predators, but nevertheless possess some parasitic traits. The adjective "parasitoid" (see Berg, 1964, for discussion) has been used for organisms that are intermediate, or transitional, between parasites and predators (Noble & Noble, 1964; Berg, 1964; Knutson & Berg, 1966).

Some authors use the term for a "parasite that slowly kills the victim, this event occurring near the end of the parasite's larval development" (Wilson, 1971) or for cases where the insect larva lies on "host tissue so that the host will live until completion of larval development" (McGraw-Hill, 1978: 1167). Among the parasitoid Hymenoptera and sciomyzid Diptera the larvae "eventually kill

*Allee et al. (1949), Askew (1971), Baer (1952), Caullery (1952), Cameron (1956), Cheng (1964), Croll (1966), Le Clercq (1969), Noble & Noble (1964), Odum (1959), Read (1970, 1972), Rogers (1962), Smyth (1962), Street (1975), Soulsby (1966), Vernberg & Vernberg (1970), Wilson (1971).

**Such seems to be the case of the phorid fly *Megaselia scalairs*, a saprovores of unviable eggs of *C. fleischmanni* but whose maggots kill and consume eggs of the frogs *Agalychnis annae* and *Eleutherodactylus coqui* (see Villa & Townsend 1983).

TABLE 5. Summary of criteria commonly used to distinguish parasites and predators, obtained from a literature survey (citations in p. 49, footnote). All criteria are generalizations and have few or many exceptions (e.g., many parasites kill their prey). When a parasitic (or predatory) trait is considered *usually* applicable to frogflies, it is designated (+); if it does not, it is designated (-). A (0) is used if the trait is intermediate.

Parasites	Predators	Frogfly traits resemble:	
		Parasites	Predators
Live on "interest"	Live on "capital"	-	+
Rarely kill their hosts (by consumption)	Usually kill their prey	-	+*
Only part of the host tissues (or nutrients) consumed	Most of prey consumed	-	+*
Host death may occur because of toxins excreted	Prey death related to consumption	-	+
All nutritional requirements are obtained from host	Other sources needed (e.g., water, vitamins)	+	-
Produce many (hundreds, thousands) offspring	Relatively few offspring	-	+
Individuals more numerous than hosts	Less numerous than prey	-	+

Much smaller than host	Much larger than prey	0	0*
Living in intimate association with host	No intimate association	+	-
Restricted to one or few host species	No such prey restriction	+	-
Living in its food supply and feeding on it	Do not live in food supply	+	-
Has mechanisms to insure transmission	Do not have transmission mechanisms	+	-
Part of life cycle is independent of (primary) host	Always dependent on prey	+	-
Cannot move to choose host	Able to pursue prey	+*	-
Has physiological and/or morphological adaptations for living in its host	No such adaptations	+	-
<hr/>		8	
Parasitic traits of frogflies			
Predatory traits of frogflies			6
<hr/>			

*If the entire clutch is considered the prey (or host), or if it is the embryo individually, the criteria may be interpreted differently.

their victims but before doing so spare them as much as possible, in order that they may continue to feed and grow and thus yield fresh nutriment just as is needed" (Wheeler, 1923); however, this is not the case with frogflies, which may kill the frog embryos (or at least stop their development) at the beginning, not the end, of their larval life. In this sense, frogfly larvae may not fit the recent definitions of parasitoid. Following Berg (1964 and *in litt.*, 1977), I prefer to use "parasitoid" in the broader sense of an intermediate between predators and parasites; this is more in line with the term as originally proposed (deBary, 1879; Askew, 1971) than with the more restricted definitions cited above.

Localities

The following list includes all localities known to me from which frogflies have been obtained, their approximate altitude (when available) and the person(s) responsible for the collection (my initials are given as JV). Probably other localities where *C. fleischmanni* occurs should be added to the list, but frogflies have been found consistently absent in some localities.

MÉXICO: *Oaxaca*: stream at 4.4 mi S Yetla; R.W. McDiarmid. — Veracruz: 2 km N of Teocello (100 m); K. Adler (fly species uncertain; eggs only). — NICARAGUA: *Matagalpa*: Finca "La Cumplida" (700 m). — Finca "Santa Emilia" (740 m); Finca "San José de la Montaña" and environs (800-1100 m); Finca "San Pablo" (1200 m), all between Matagalpa and Tuma; JV & M. Villa. — *Boaco*: Finca "Oro Verde", Cerro Masigüe (600-800 m); JV. — *Ocotral*: 2-8 km S of Las Manos, near Honduras border (1250 m) JV & M. Villa. — COSTA RICA: *San José*: no other data; N.J. Scott, Jr.; 0.5 km N Curridabat, Rio María Aguilar; R.W. McDiarmid. — Ciudad Universitaria; S. Ortega. — Granadilla de Curridabat (1320 m); C.E. Valerio, JV. — Bajo de la Hondura (1200 m) S. Ortega & JV. — Río Paracito (1450-1500m) L.D. Gómez & JV. — Various unnamed streams around San José-Moravia area; L.D. Gómez, S. Ortega, M. Villa & JV.

POSTSCRIPT

Few neotropical tree frogs have been studied extensively in their natural conditions. While the present work did not intend to be exhaustive, it has revealed many peculiarities which *Centrolenella fleischmanni* may share with other congeners, and some aspects of its natural history which merit further study. Among these are: sex ratios, and other demographic aspects, of its natural populations; whether one or more egg clutches are produced during the rainy season, and observations of actual oviposition. Its egg color is light green, whereas other congeners lay brownish and even black eggs. Sometimes eggs of different colors, belonging to different species, are found in the same area and the same vegetation. The adaptive significance of egg color remains to be studied experimentally in these and other species.

Centrolenid frogs are popularly known as "glass frogs" (after Starrett & Savage, 1973), because the venter is "so transparent that some of the internal organs are clearly visible through the skin" (Savage, 1967). This feature is unique

among adult anurans, and its adaptive significance (if any) has not been investigated. Furthermore, the pigmentation of the visceral, parietal, cardiac and hepatic peritonei is distinctive in the different species and reliably characterizes the species groups. This is another interesting aspect which remains to be studied.

The tadpole of *C. fleischmanni* has its own set of unique features. The transparent skin, red color, embryonic eyes and wormlike appearance were explained in terms of fossorial habits (Villa & Valerio, 1982), but the picture is far from complete and details of the tadpoles' ecology and behavior are presently under

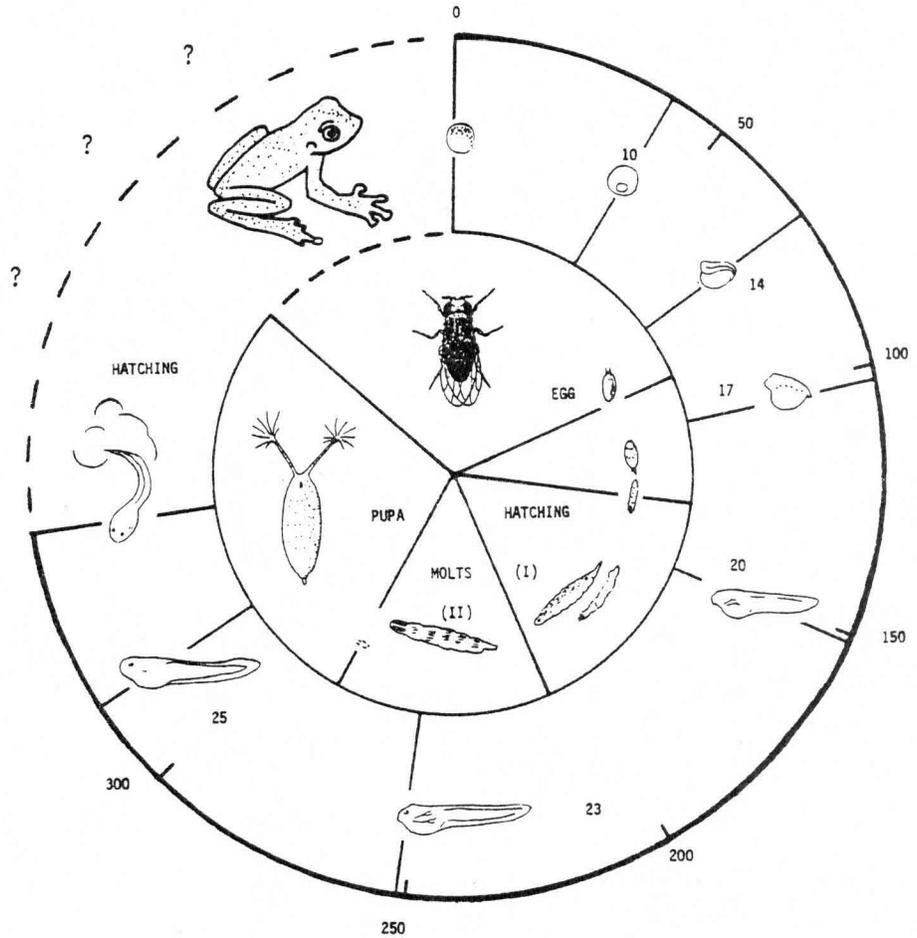


FIGURE 21. Simplified representation of the life cycles of the frog (outer circle) and the fly (inner). Numbers outside the circles represent hours after the frog's oviposition; those inside refer to the frog embryo's developmental stages (of Gosner, 1960). Where the duration of developmental stages is unknown the circles are left open.

study. Similar observations are lacking for tadpoles of almost all other centrolenid species.

Another very fertile area of research is that of interspecific relationships involving *C. fleischmanni*. Its resistance to infections by a lethal *Mycelia sterilia* fungus which affects eggs of other local species has been documented, but not fully explained (Villa, 1979b). The role of the adult accompanying eggs needs to be investigated in relation to the eggs' resistance to fungal infection. The role of the phorid fly *Megaselia scalaris* in the dispersal of the fungus to other egg masses (and any other possible means of dispersal of the fungus) also requires further study.

The *Centrolenella-Zygothricha* relationship is an especially fertile field. The high incidence of fly infestation on *C. fleischmanni* eggs in some localities and virtually no infestation of eggs of other frogs in the same locality is noteworthy and presently under investigation. The frog's success in its distribution and abundance, in spite of heavy fly infestation and low egg number, are remarkable, and perhaps even linked. Breeding of adult *Zygothricha* in the laboratory remains to be accomplished, and is the key to answering many questions about the biology of the fly itself and its relationship with *C. fleischmanni*. Basic information such as the number of eggs laid by the fly, the number of clutches produced per season, and the possible existence of alternative breeding site(s) also remain to be answered. Similarly, interactions between other organisms and the eggs of other amphibians (see Villa, 1980 for review), and the importance of these associations in the life of amphibians in general, are areas of research which are only beginning to bear fruit.

RESUMEN

Centrolenella fleischmanni (Anura: Centrolenidae) es una ranita diminuta que se encuentra en el neotrópico desde el Sur de México y en Centroamérica hasta Ecuador, en la vertiente del Pacífico, y hasta el Norte de Colombia, Venezuela y Surinam en la del Atlántico. Su distribución altitudinal va desde casi el nivel del mar hasta unos 1500 metros. Generalmente abunda donde se encuentra, siendo más común en la vegetación cercana a ríos y riachuelos, de velocidad moderada o rápida. Se reproduce principalmente durante la estación lluviosa (Mayo a Octubre) y, en menor grado, durante todo el año en localidades cuya estación seca no es muy marcada. Los machos cantan cerca de los ríos, posados en las superficies superiores o inferiores de las hojas, y muestran cierta territorialidad. Los huevos son adheridos casi siempre a las superficies inferiores de las hojas encima o muy cerca de los ríos. Uno de los padres, generalmente el macho, acompaña los huevos, posándose encima de ellos durante la noche y durmiendo cerca (pero no encima) de ellos durante el día. El número de los huevos de cada puesta varía de localidad a localidad, lo mismo que en cada localidad a través de la estación lluviosa. El número promedio de huevos de cada postura varía, en las localidades estudiadas, entre 18 y 30.15. Los renacuajos emergen entre 250 y 350 horas después de puestos los huevos, y después de que desaparecen las agallas externas (el estadio 25 de Gosner). Más

del 95% de los huevos que no son destruidos por factores bióticos o abióticos nacen, pero casi el 80% de las masas puestas perecen. Al nacer, los renacuajos caen al agua, y rápidamente desaparecen en el lodo o bajo las piedras. Poco se sabe de su desarrollo posterior en condiciones naturales, pero los renacuajos aparentemente viven en el lodo o la basura de los bancos, frecuentemente sobre el nivel del agua y fuera del río, bajo piedras o en el lodo cercano. Al nacer son de color verde claro, pero en condiciones naturales adquieren un color rojo brillante, por el que difieren de los renacuajos de todas las demás familias de anuros. El color rojo no se debe a cromatóforos sino que a la sangre que se vé a través de una piel casi sin pigmento. Esta parece ser una adaptación respiratoria para una vida subterránea. En situaciones artificiales es sumamente difícil criar los renacuajos a partir de huevos recién puestos, y nunca adquieren el color rojo. La duración del estado larval se desconoce, pero se estima en al menos uno, y posiblemente dos, años.

Moscas del género drosófilido *Zygothrica* depositan sus huevos en las masas ovíferas de *Centrolenella fleischmanni*, y sus larvas (gusanos) matan y consumen muchos o la mayoría de los huevos de la rana. El porcentaje de las masas de huevos afectadas varía de localidad a localidad y a lo largo de la estación lluviosa, alcanzando hasta 80-100% en algunos casos, y luego disminuyendo abruptamente. Se encontró una correlación positiva entre el número de huevos de la rana y de la mosca, y existe evidencia de que las moscas depositan sus huevos días después de que las ranas depositan los suyos; los huevos de mosca depositados muy tarde no alcanzan su desarrollo completo y frecuentemente mueren, según se determinó en experimentos de laboratorio. Otros experimentos indicaron que el desarrollo de las larvas de la mosca ocurre principalmente en los huevos de *C. fleischmanni* y algunos otros centrolénidos, aunque no todos. Este parece ser el primer caso documentado de una relación obligatoria de insectos en huevos de rana, y de una mosca drosófilida en embriones de vertebrados.

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