A mayfly from tropical Brazil capable of tolerating short-term dehydration

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Abstract. The mayfly *Cloeodes hydation* (Baetidae, Ephemeroptera) inhabits ephemeral, rain-filled rock pools as well as pools, springs, and brooks of longer duration in savanna woodlands of the northern Pantanal region, Brazil. Larvae have the ability to tolerate repeated exposure to air for up to 9 h at a time, as confirmed by laboratory experiments. This tolerance is apparently advantageous in the highly dynamic, unpredictable habitat conditions, especially when pools are successively wet and dry almost daily during the end of the rainy season. Survival of larvae depended on the presence of sediment, and was always followed by moulting. Inducible and repeated moulting showed that numbers of moults and instars were not always indicative of development. We present data on *C. hydation* abundance in 2 rock pools throughout 1 y. From these data, larval development was estimated to require between 19 and 25 d (24–29°C).

Key words: dehydration tolerance, mayflies, Baetidae, *Cloeodes*, rock pools, Neotropics.

During studies of the headwaters of a tropical lowland river in west-central Brazil, we encountered rock pools adjacent to a spring that were densely colonized by insects. These pools frequently dried up and refilled, leading us to investigate the biology of the more abundant species to determine the necessary specialization for life in this highly dynamic habitat. The chironomid *Apedilum elachistus* Townes 1945 and the baetid mayfly *Cloeodes hydation* McCafferty and Lugo-Ortiz 1995 were the most abundant insects in the pools. The biology of *A. elachistus* has already been described (Nolte 1995), including the shortest generation time known so far for Chironomidae. The biology of *C. hydation* shows characteristics never before reported for mayflies, so we decided to present our observations, although modest research facilities did not allow us to make exact measurements.

At the beginning of our studies, the baetid mayfly *C. hydation* was new to science; it was described by McCafferty and Lugo-Ortiz (1995). Its presence in temporary rock pools is notable because few mayflies occur in such small and sparsely structured habitats (Williams 1987), although some have been found in road puddles,

cattle tanks, fountains, and short-lived desert pools (e.g., Soldán and Thomas 1983, Berner and Pescador 1988). The genus *Cloeodes* was described by Traver (1938) but was poorly known until the revision by Waltz and McCafferty (1987). It is essentially a pantropical taxon with most species known from the American tropics and subtropics (central Argentina to southwestern North America), but with a few species also known from southeast Asia and southern Africa. Species of *Cloeodes* have been reported from a broad range of lotic habitats, including shallow and deep slow-flowing waters, mountainous brooks, and backwaters and stony pools of large rivers (see review by Waltz and McCafferty 1994). Virtually nothing was known about the biology of any species before the discovery of *C. hydation*, which is the first species of *Cloeodes* known from Brazil.

Study Site

The study area, at approximately 330 m a.s.l., is adjacent to 1 of the main springs of the Bento Gomes River (15°45'S, 56°35'W), an intermittent stream that drains into the northern Pantanal (Mato Grosso, Brazil). The intermittent nature of this system, including the rock pools, is a result of variable local rainfall. The tropical climate in

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the northern Pantanal region is strongly seasonal with about 80% of annual precipitation falling from November to April, whereas no rain falls in June, July and August. From July 1992 to July 1993, 1600 mm of precipitation fell in the study area where the spring generally maintains some flow throughout the year but, according to local people, is apt to dry up in extremely dry years. Because our studies were the first undertaken in the headwaters of the Bento Gomes river, historical records of environmental conditions are not available.

Approximately 20 pools lay in depressions in rock that formed the bankside of the spring-brook (hypocrenal) along a distance of some 30 m. The pools had quartzite bedrock and thus their water was of low conductivity (20–100 μS/cm); pH ranged from 5.5 to 6.8. They generally held a thin (5 mm or less) layer of silt, sand, and detritus, except when scoured out by torrential downpours. Light showers always brought new sediment into the pools, and in the dry season wind-borne material continually accumulated there; but we did not see coarse organic matter, such as leaf litter, settling in the pools. Occasional defecation by small rodents was another source of organic matter. On the rocky ground no bushes or trees grew near the pools, which were exposed to direct sunlight. Water temperature ranged from 24 to 29°C during most of the year, but rose to 42°C in March and dropped to 8°C in July. Visible algal growth (*Spirogyra* sp.) occurred only towards the end of the rainy season, although diatoms were present throughout the year, but remained scarce.

Some of the rock pools were temporarily linked with the adjacent spring-brook, but most of them were completely isolated and dependent entirely on rain and dew for water. Particularly during the end of the rainy season, the isolated pools dried up and refilled within a few hours, and thus were of special interest to us with respect to their fauna. In this study, we present data obtained from the smallest and the largest of the rock pools, i.e., 2 sites only, to show both ends of the scale of abiotic conditions which *C. hydation* experienced at our study site. The smallest pool was a simple, unstructured, shallow hollow with a maximum surface area of only 980 cm² and a holding capacity of 2.8 L. The amount of pool water depended upon temperature and rainfall. Although only 60 cm from the spring-brook, this pool provided a com-

pletely isolated and ephemeral aquatic habitat because it was on top of the raised part of the rock. Hereafter, we refer to this pool as pool "S" (Simple). The largest pool was a relatively complex system consisting of 3 basins that were connected most of the time and always contained some loose sand and stones. Its capacity was 14.7 L, with a maximum surface area of 3350 cm²; it held water longer than pool "S" because it was linked to the spring-brook from the rainy season until the early dry season. Hereafter, we refer to this pool as pool "C" (Complex).

Methods

From April 1992 to May 1993, samples of the fauna were taken from the 2 rock pools, and abiotic factors (conductivity, temperature, pH, surface area) were measured at intervals of 5 to 21 d, for a total of 35 sampling occasions. Because the pools were small, it was impossible to take quantitative samples that would represent the real abundance of fast moving insects such as *Cloeodes hydation*. Therefore we used a small dip net (9 × 14 cm; mesh size 125 μm) and took samples as gently as possible to minimize the sampling impact on community succession. Samples were taken by moving the net at first continuously, and not too fast, across the surface. This movement caused whirls in the shallow water, bringing some sediment and the associated fauna into suspension. The net was then swept a 2nd and 3rd time through the water to catch some of the suspended material. In this fashion one sample was taken from each pool on each collecting date, and was immediately preserved in 70% alcohol. All samples were taken in the same way to keep the series of samples comparable. Additional material was taken alive to the laboratory for experiments on dehydration tolerance.

To test the dehydration tolerance of *C. hydation*, larvae of various sizes, excluding 1st-instar larvae lacking gills, were placed in petri dishes that were filled with pool water and had a 2–5 mm thin substrate of silt and clay obtained from the rock pools. These dishes were then allowed to dry up at 28–31°C (room temperature) for periods between 10 min and 14 h. The time span that larvae were exposed to dry conditions was calculated from the moment when all visible free water had evaporated and the surface of the sediment layer started to look dry. It was clear

that the sediment still contained moisture at this moment, but because we had no equipment for measuring moisture content in sediments or larvae, we standardized our 'point zero' in this way. To obtain, in spite of modest working conditions, as much information as possible about the dehydration tolerance of *C. hydation*, we conducted 16 qualitative drying experiments designed in various ways as explained below. To allow larvae to rehydrate afterwards, the dry dishes were refilled with pool water, and the process of rehydration was then observed with a dissecting microscope.

(1) In the 1st series of experiments, 86 larvae were distributed among 9 petri dishes that were allowed to remain dry for periods between 10 min and 14 h. Then the dishes were refilled with pool water, and 12 h later the number of live larvae was recorded.

(2) In a 2nd series of experiments, we tested the capacity of *C. hydation* to recover from repeated dehydration, which they probably experience in the natural pool habitat, particularly at the end of the rainy season. In these experiments, each of 3 water-and-sediment-containing petri dishes were provided with 10 larvae. The dishes were then allowed to dry up 5 times, for periods from 30 min to 9 h. Between 2 successive desiccation periods, all reactivated larvae were allowed to rest submersed for a period of 12 to 24 hours, and then the number of live larvae was recorded.

(3) In a 3rd experiment, larvae were exposed to drying in the absence of sediment. Four larvae were tested for a period of 10 min, and 3 larvae for a period of 20 min.

Results

Field data during 1 year

Cloeodes hydation was found in the temporary rock pools as well as in the adjacent spring (eucrenal) and spring-brook (hypocrenal) which had permanent flow during the study period. Larvae of all sizes were always present, indicating a continuous, non-seasonal life history. The total number of *C. hydation* larvae collected on each sampling date from both study pools is given in Fig. 1, along with some information on the physical conditions of the pools.

Cloeodes hydation was generally more abundant in pool C than in pool S, and more abun-

dant during the transition period from the rainy to the dry season (in both pools) and during the dry season (only pool C with water) than during the rest of the year (Fig. 1). In the transition period (late March to early June), a mean of 14.1 larvae per sample was caught from pool S (N = 10; Table 1). At this time the habitat often dried up in the course of the day, stayed dry for a few hours, and then refilled with rain and nightly dew. In the dry season (early June to early September), pool S stayed completely dry (Table 1, Fig. 1). Pool C, on the other hand, remained linked to the spring brook by a slight film of water running over the rock surface until the end of July. Here, a mean of 59.8 *C. hydation* larvae per sample (N = 10) was caught during the transition period, and 148.7 larvae per sample (N = 8) during the dry season until pool C dried up (Table 1, Fig. 1).

The transition period from the dry to the rainy season (early September to early December) was characterized by isolated, but often very heavy, rainfall that scoured out the pools. At this time a mean of 2.2 larvae per sample was caught from pool S (N = 6), and 11.8 larvae per sample from pool C (N = 6; Table 1). During the following rainy season (December to March), characterized by almost daily rainfall and frequent thunderstorms, *C. hydation* remained rare in pool S, with mean densities of 1.6 larvae per sample (N = 6), whereas 25.7 larvae per sample were caught in pool C (N = 6; Table 1, Fig. 1).

Time of larval development

The length of the life cycle of *C. hydation* has not been determined precisely because of our inability to track oviposited eggs and rear individuals from 1st-instar larvae. Eggs were never observed, although many 1st-instar larvae of *C. hydation* were found. Judging from the field data, however, this species took approximately 19–25 d from egg to emergence, because on 4 September 1992 (the first rain after a 14-wk dry period, Fig. 1), the small, isolated pool S had filled but no mayfly eggs or young larvae were found; on 10 September, the 1st young larvae were recorded—all of them without gills; and on 29 September, exuviae of mature larvae and emerged subimagos were present. At this time, the temperature range was 24–29°C in the rock pools, resulting in 456–725 degree-days for *C. hydation* to complete development.
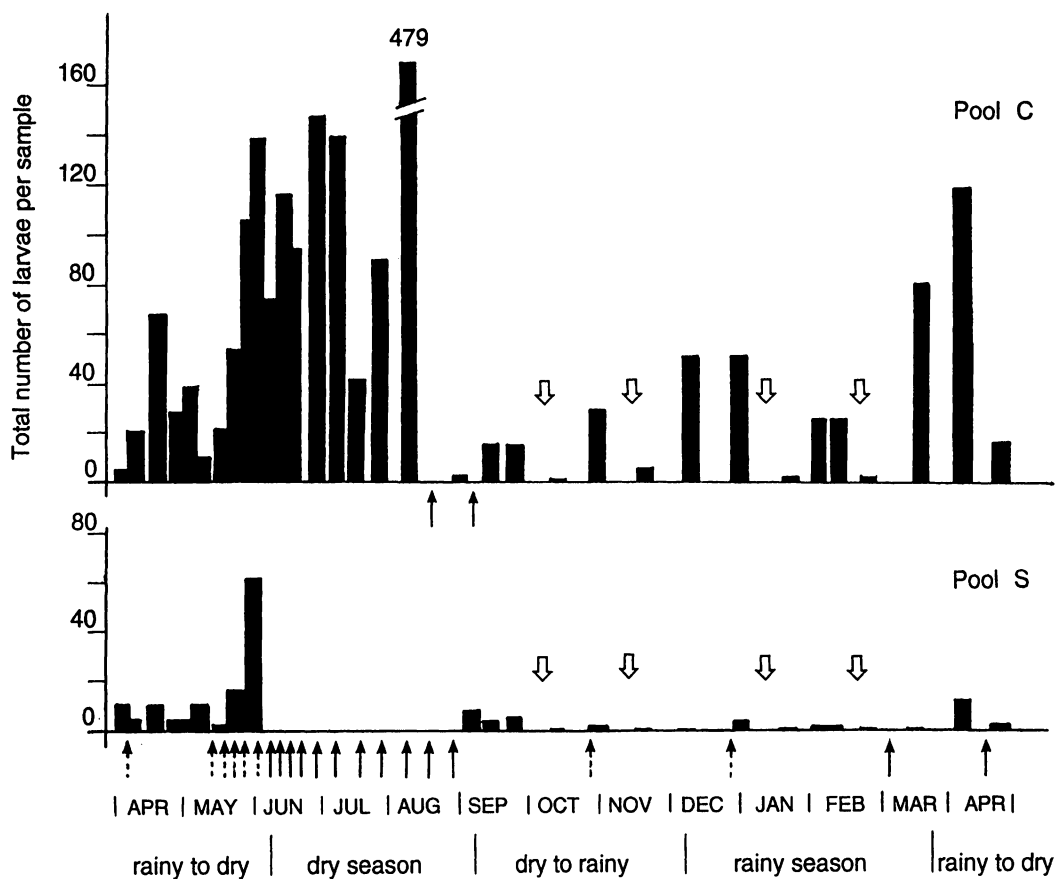
FIG. 1. Total number of *Cloeodes hydation* larvae collected during each sampling date in 2 rock-pool habitats from April 1992 through April 1993. Directly observed habitat disturbances are shown by open arrows (torrential downpours), solid arrows (dry periods > 24 h), and dotted arrows (short-term dry periods < 12 h).

Dehydration-tolerance experiments

The field data suggested that *C. hydation* was capable of tolerating dehydration. Experiments confirmed this. When allowed to dry in laboratory environments, larvae became completely immobile and hard, and looked somewhat withered. Those in environments with sediment appeared to be caked with dry sediment while the environment was dry. When the dishes were refilled with water, rehydration of the larvae occurred within 5–25 min, and larvae became active again. The process of water absorption was difficult to observe directly because of the caked cuticle of the larvae. Although sometimes individuals visibly regained a certain turgor after some minutes, rehydration only became certain when the animals started to move again.

The results from the drying-tolerance exper-

iments were as follows. No larvae survived drying in the absence of sediment. No larvae survived drying for 12 h or longer (Table 2). Larvae tolerated absence of water for up to 9 h; most individuals (70%) became active again, and 50% recovered completely, i.e., survived for at least 12 h, continued to develop, and emerged (Table 2). Mature larvae with dark wing pads were less tolerant of desiccation, withstanding only 3–6 h. When limited to this or shorter dry intervals, they were able to develop normally to emergence (Table 2).

Larvae recovered from repeated drying when individual dry periods lasted between 30 min and 9 h, with resting periods of 12–24 h between 2 such dry periods (Table 3). The survival rate of larvae decreased after 2, 3, 4, and 5 successive drying periods from 97%, 76%, 48% to 10% (Table 3).

TABLE 1.   Numbers of *Cloeodes hydation* in the simple pool S and complex pool C during 1992–1993. Seasons are given, along with the respective months, the number of sampling dates (*n*), mean number of larvae per sample, standard deviation (SD), and the number of samples taken (*N*). The difference between *n* and *N* shows when a pool was dry thus no sample was taken.

Season	Months	*n*	Pool S			Pool C		
			Mean	SD	*N*	Mean	SD	*N*
Rainy to dry	Mar–Jun	11	14.1	16.5	10	59.8	35.9	10
Dry	Jun–Sep	10			0	148.7	121.4	8
Dry to rainy	Sep–Dec	8	2.2	2.3	6	11.8	9.9	6
Rainy	Dec–Mar	6	1.6	1.5	6	25.7	20.3	6

All rehydrated larvae that became active again shed their exuviae within 12 h (*n* = 103). Only once did a larva not succeed in moulting; evidently it was unable to shed gill exuviae heavily caked with sediment, and died.

Discussion

Tropical mayflies have been little studied, and knowledge of their taxonomy and biology is limited (e.g., Brittain 1982, Dudgeon 1992, Ward 1992, Jackson and Sweeney 1995, Sweeney et al. 1995). Nonetheless, we assume that short life cycles throughout the year reflect a "normal" situation in tropical regimes (e.g., Thienemann 1934, Jackson and Sweeney 1995, Nolte 1995)

TABLE 2.   Results of the drying-tolerance experiments on *Cloeodes hydation* larvae. N total = total number of larvae exposed to experimental conditions; N wp = among these the number of larvae with dark wingpads; rehydration = number of larvae that became active again (total number of larvae, and larvae with wingpads); complete recovery = number of larvae that survived rehydration for 12 h or more, continued to develop, and emerged (total number of larvae, and larvae with wing pads); for details see text.

Drying period	N total	N wp	Rehydration Total	Rehydration wp	Complete recovery Total	Complete recovery wp	% Recovery
10 min	8	2	8	2	8	2	100%
20 min	5	1	5	1	5	1	100%
40 min	5	2	5	2	5	2	100%
3 h	10	2	9	2	8	1	80%
6 h	15	3	12	3	10	1	67%
7 h	13	2	12	1	11	0	85%
9 h	14	2	10	0	7	0	50%
12 h	12	2	1	0	0	0	0%
14 h	4	0	0	0	0	0	0%

rather than an adaptation to dynamic, unpredictable habitat conditions (e.g., McLachlan and Cantrell 1980, Gray 1981), and can be illustrated by comparing accumulations of degree-days. The range of 456–725 degree-days *C. hydation* took for completing development, agrees well with the number of developmental degree-days associated with several other mayflies, especially Baetidae, from temperate regimes (Landa 1968, Macan 1979, Hutchinson 1993). The lower limit of the degree-day range (456) indicates "fast" development, but *C. hydation* is a small mayfly (mature larvae are only 3.4–4.7 mm long, McCafferty and Lugo-Ortiz 1995) and may need relatively little time to grow. We do not know if development time differs markedly in those individuals living in the spring-brook. Gray (1981) reported life cycles as short as 7–13 d for *Fallceon quilleri* (Dodds) and 9–14 d for *Callibaetis montanus* Eaton, 2 baetid mayflies from a desert stream in Arizona that is subject to periodic flooding. Although *F. quilleri* is only slightly larger than *C. hydation*, *C. montanus* is considerably larger, with mature larvae ranging from 9 to 12 mm in length (C. T. Lugo-Ortiz, Purdue University, personal communication; McCafferty, unpublished). Thus, factors other than size almost certainly influence development time.

Throughout the 13-mo study period, abundance of *C. hydation* in the rock pools was extremely variable. It was evident that downpours during the rainy season resulted in substantial disturbances that reduced the abundance of larvae, whereas frequent short-term dryings during the transitional seasons had no such effect (Fig. 1). The same was true for *Apedilum elachistus*, the dominant chironomid in the pools (Nolte 1995). These results support the assess-

TABLE 3. Results of experiments with recurring drying of *Cloeodes hydration* larvae. N_0 = number of larvae exposed to experimental conditions; N_1–N_5 = number of larvae that recovered and were re-exposed to drying; Total gives the sum of all larvae tested.

	Dish 1	Dish 2	Dish 3	Total (% Surviving)
N_0	10	10	9	29 (100%)
1st dry period	30 min	1 h 30 min	4 h	
Following time with water	16 h 40 min	15 h 40 min	13 h 10 min	
N_1	10	10	9	29 (100%)
2nd dry period	4 h	4 h	4 h	
Following time with water	20 h 20 min	20 h 30 min	20 h 20 min	
N_2	9	10	9	28 (97%)
3rd dry period	6 h	4 h	9 h	
Following time with water	22 h 10 min	23 h 10 min	19 h 20 min	
N_3	8	9	5	22 (76%)
4th dry period	3 h	3 h	3 h	
Following time with water	17 h 30 min	17 h 40 min	17 h 20 min	
N_4	4	5	5	14 (48%)
5th dry period	6 h	6 h	7 h	
Following time with water	19 h 20 min	19 h 20 min	18 h 20 min	
N_5	2	1	0	3 (10%)
Comments	1 of these larvae emerged within the following 48 h.	The larva emerged within the following 48 h.		

ment of Dudgeon (1992, p. 37) for running water species from seasonal tropical climates that, "it may be appropriate to view the wet season as an inimical time" for the aquatic fauna. The high water to which the rock pool fauna was subjected during the rainy season was derived directly from rain, not from spates in the adjacent spring-brook.

The capacity to withstand short-term drying is an extraordinary feature in the biology of *C. hydation*, and is the 1st such observation reported for a mayfly. In our laboratory studies, *C. hydation* could survive 9 h in the absence of water, whereas 12 h were lethal (Table 2). Although we had no equipment available to measure moisture content of larvae and sediment, we assume that larvae died because of the degree of dehydration between 9 and 12 hours. This assumption is supported by our observation that all larvae exposed to dry environments lacking sediment did not survive. These experiments were clearly unrealistic but they showed that the problem was not only an absence of water but an absence of the protective covering that larvae would normally be able to use in their natural habitat. The sediment when present presumably provides protection from dehydration, either by retaining some moisture or by slowing down the dehydration process, or both. Hinton (1960) reported similar findings for the chironomid *Polypedilum vanderplanki* Hinton, a typical rock-pool dweller from Africa. Our data show that the capacity of *C. hydation* to dehydrate without injury may be thought of as drying **tolerance** as opposed to the drying **resistance** associated with cryptobiosis in certain aquatic Diptera (e.g., Hinton 1960, Adams 1984, Dodson 1987). Nonetheless, the capacity of *C. hydation* to withstand dehydration, in combination with its capacity to recuperate from recurring daily dry periods, is clearly of selective value for this rock-pool colonizer. Next to the midge *A. elachistus*, *C. hydation* was the most abundant insect in the very small and simple pool S.

The fact that *C. hydation* always shed its exuviae when rehydrated following a dry period constitutes the 1st evidence that a mayfly can be directly induced to moult by an environmental factor. The old cuticle, hardened by drying, apparently did not return to its original condition

after rehydration, and evidently a new cuticle was required to regain normal cuticular functions. Thus, the drought-induced moulting in *C. hydation* appears to illustrate the idea expressed by J. Edwards (in Hutchinson 1993, p. 325) that, "... the purpose of repetitive moulting is to restore the cuticular surface". We do not know whether the drying or the rehydration actually triggers moulting. It is certainly possible that apolysis is initiated by drying, and during this period the outer cuticle is retained as an insulating cover—somewhat like a temporary puparium—only to be shed (ecdysis) once rehydration has occurred.

The repetitive moulting of *C. hydation* coupled with cyclic wet/dry periods also shows that the number of moults during larval life, and thus the number of larval instars, is not always correlated with growth and maturation. Such a possibility has been repeatedly discussed in the context of instar definition (e.g., Fink 1980, 1984, Hutchinson 1993) but without firm evidence for it, which is now provided by our observations.

Acknowledgements

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