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Natural History of the Four-toed Salamander, Hemidactylium scutatum, in West Virginia

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Natural History of the four-toed salamander, *Hemidactylium scutatum*, in West Virginia

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Abstract

A 2-year study was conducted to determine the reproductive and nesting habits, embryonic and larval development, and tolerance to acid conditions of *Hemidactylium scutatum* in West Virginia. Five study sites located in or adjacent to the Otter Creek National Wilderness Area, Monongahela National Forest, Randolph County, West Virginia, were monitored to determine nesting habits and length of incubation and larval periods in *Hemidactylium*. Time of breeding was determined by spermatogenic wave analysis and time of egg deposition was determined by examination of ovarian follicles and field observations of gravid females migrating to nest sites. Breeding occurred in autumn and again in spring when climatic conditions were favorable. Migration to nest sites occurred in early April and oviposition occurred in mid-April to early May. Nests were found within 25 cm of permanent and temporary pools adjacent to wooded areas in the following 3 substrate types: *Sphagnum* sp. moss, non-*Sphagnum* sp. moss, and *Eriophorum virginicum* roots. A 7 to 8 week incubation period was followed by a 9 to 10 week larval period that ended in mid-August. Eggs were laid with a mean diameter of 3.7 mm and hatched with a mean diameter of 6.3 mm. Larvae averaged 8.9 mm snout-vent length (SVL) and 12 to 15 mm total length (TL) at time of hatching and 13.3 mm SVL and 16.6 mm TL at

transformation. Larvae had the following 4 morphologically distinct developmental stages: 1) post-embryonic; 2) growth; 3) gill resorption; and 4)transformation. Eggs and larvae developed normally in both neutral and acid environments.

Rana sylvatica embryos and *Hemidactylium* embryos and larvae were tested in the laboratory to determine their tolerance to low pH conditions. The 96-hour TI_m (median tolerance limit) test was used as the measure of acute toxicity to low pH. Regression analyses revealed that *Hemidactylium* embryos were more tolerant of acid conditions than larvae and *R. sylvatica* embryos.

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Introduction

Hemidactylium scutatum, the four-toed salamander, is a monotypic species that was first identified by Schlegel in the mid 1830's (Neill, 1963). The type locality is in Nashville, TN (Neill, 1963). It is a member of the tribe Hemidactyliini, subfamily Plethodontinae, and family Plethodontidae (Duellman and Trueb, 1986). It is morphologically similar to other plethodontids except it has only four toes on each hind foot. Hemidactyliini is separated from other tribes in plethodontinae because it has an aquatic larval stage. In cloacal anatomy (Sever, 1987) and skull morphology (Lombard and Wake, 1986; Rose, 1995), *Eurycea* is the closest related plethodontid genus to *Hemidactylium*.

Distribution

Hemidactylium occurs throughout eastern North America. It ranges from Nova Scotia west to Wisconsin and southeast Oklahoma, and south to eastern Louisiana and the panhandle of Florida (Conant and Collins, 1991; Fig. 1). It was originally believed to occur in only eight counties in the higher elevations of West Virginia (Green, unpublished data). Green and Pauley (1987) list *Hemidactylium* in 20 counties, mostly in the eastern half of the state (Fig. 2). However, it probably occurs in every county where suitable habitat is present. Three additional county records have been found since the

Figure 1. Distribution of *Hemidactylium scutatum* in North America according to Conant and Collins (1991).

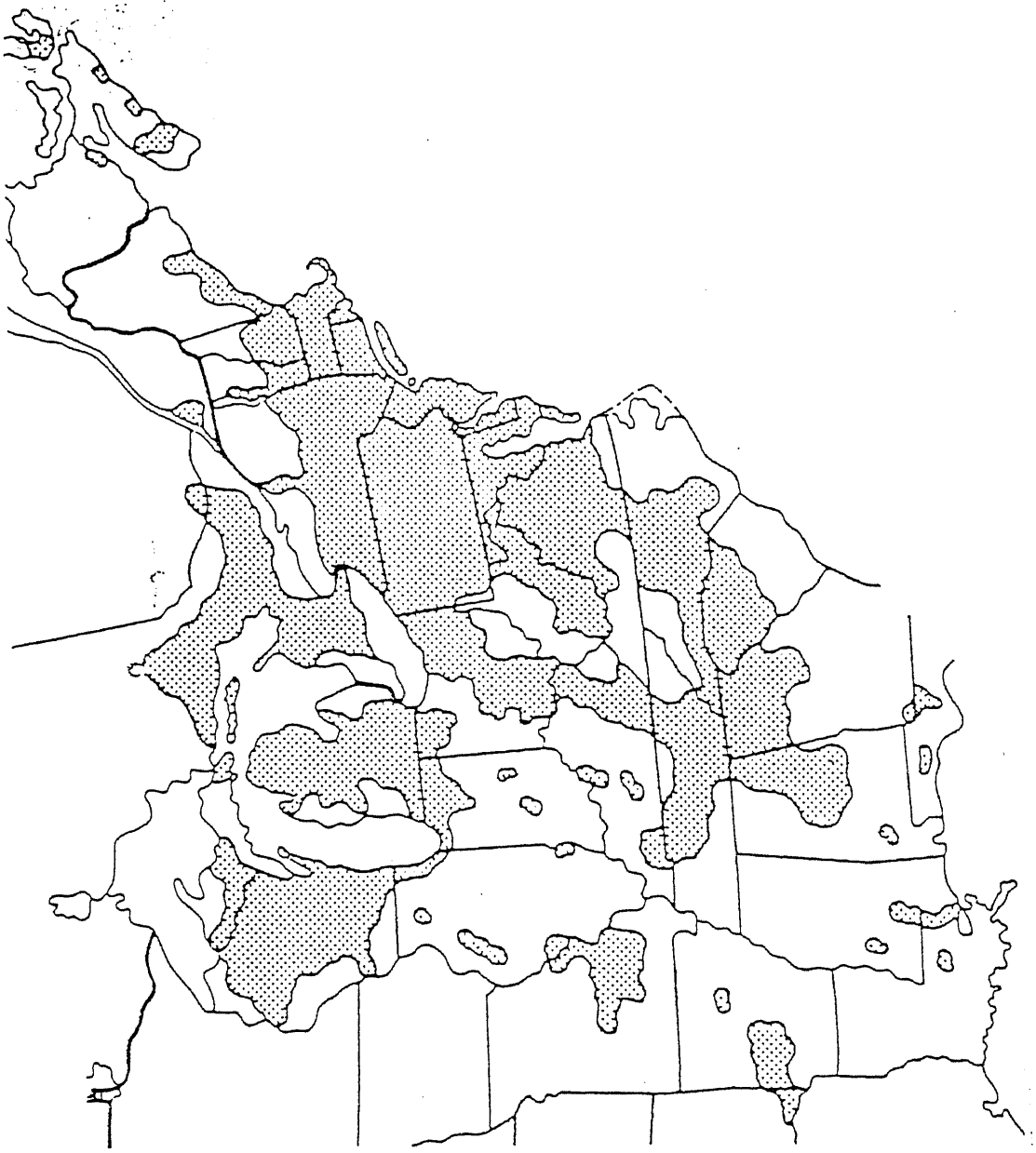
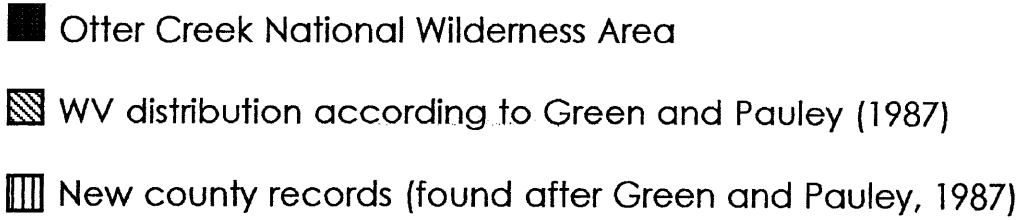
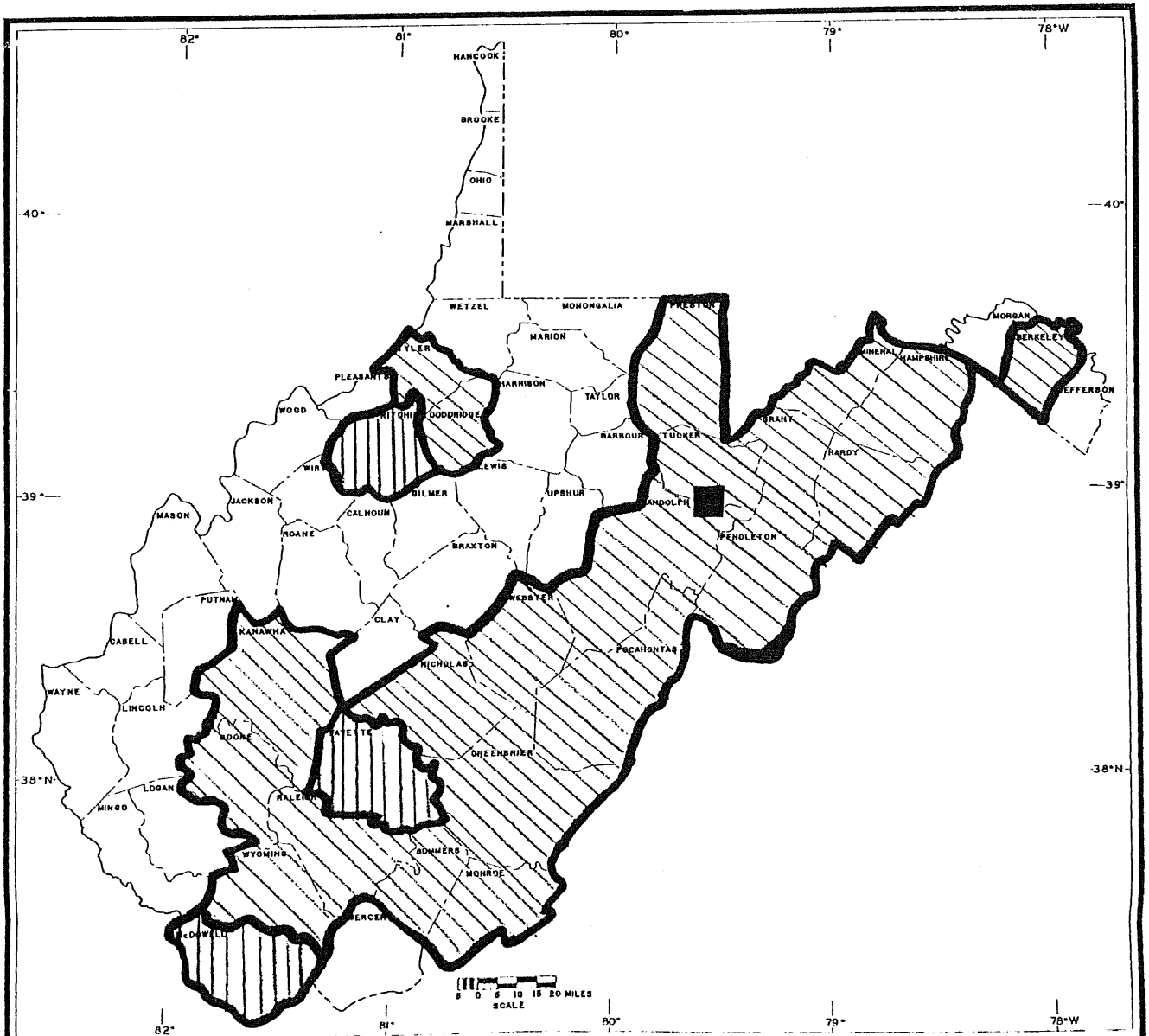


Figure 2. Distribution of *Hemidactylium scutatum* in West Virginia.




82°
81°
80°
79°
78°W
40°
39°
38°N
HANCOCK
BROOKE
OHIO
MARSHALL
WETZEL
MONONGALIA
PRESTON
MORGAN
BERKELEY
MARION
TYLER
PLEASANTS
HARRISON
TAYLOR
MINERAL
HAMPSHIRE
JEFFERSON
WOOD
RITCHIE
DODDRIDGE
BARBOUR
TUCKER
GRANT
HARDY
WIRT
LEWIS
GILMER
UPSHUR
RANDOLPH
JACKSON
MASON
CALHOUN
ROANE
BRAXTON
PENDLETON
PUTNAM
WEBSTER
CLAY
KANAWHA
CABELL
POCAHONTAS
NICHOLAS
WAYNE
LINCOLN
BOONE
FAYETTE
GREENBRIER
LOGAN
RALEIGH
MINGO
SUMMERS
WYOMING
MONROE
MERCER
McDOWELL
0 5 0 5 10 15 20 MILES
SCALE

publication of the work of Green and Pauley (1987), one in the New River Gorge in Fayette County (Pauley, 1993), one in North Bend State Park in Ritchie County, and the other in Panther State Forest in McDowell County.

Description

Hemidactylium scutatum is one of the smallest salamanders in West Virginia (Green and Pauley, 1987) and ranges in total length from about 5 to 10 cm. *Hemidactylium* has a reddish-brown dorsum that becomes gray laterally, and a bluish-white venter with irregular, black spots (Neill, 1963; Fig. 3). The ventral spot pattern is unique to each individual within a population (Bishop, 1941; Harris and Gill, 1980; Harris *et al.*, 1995). *Hemidactylium* has 13 or 14 costal grooves, a distinctly blunt snout, and a distinct constriction at the base of the tail that marks the point of detachment in autotomy (Bishop, 1941). Mature males are morphologically distinguished from females by their smaller average size, more slender form, relatively longer tail, and enlarged premaxillary teeth (Blanchard and Blanchard, 1931; Bishop, 1941; Figs. 4 and 5). Another important character used to distinguish males from females is the shape of the snout. Figures 6 and 7 show that males have a more squarely truncate snout that is swollen in the naso-labial groove region.

Natural History

Research by Bishop (1918, 1941) and Blanchard (1923) provided an excellent account of the natural history of *Hemidactylium* in New York and

Figure 3. Photograph showing dorsal view of *Hemidactylium scutatum* male.

Figure 4. Photograph showing dorsal view of *Hemidactylium scutatum* male (left) and female (right).

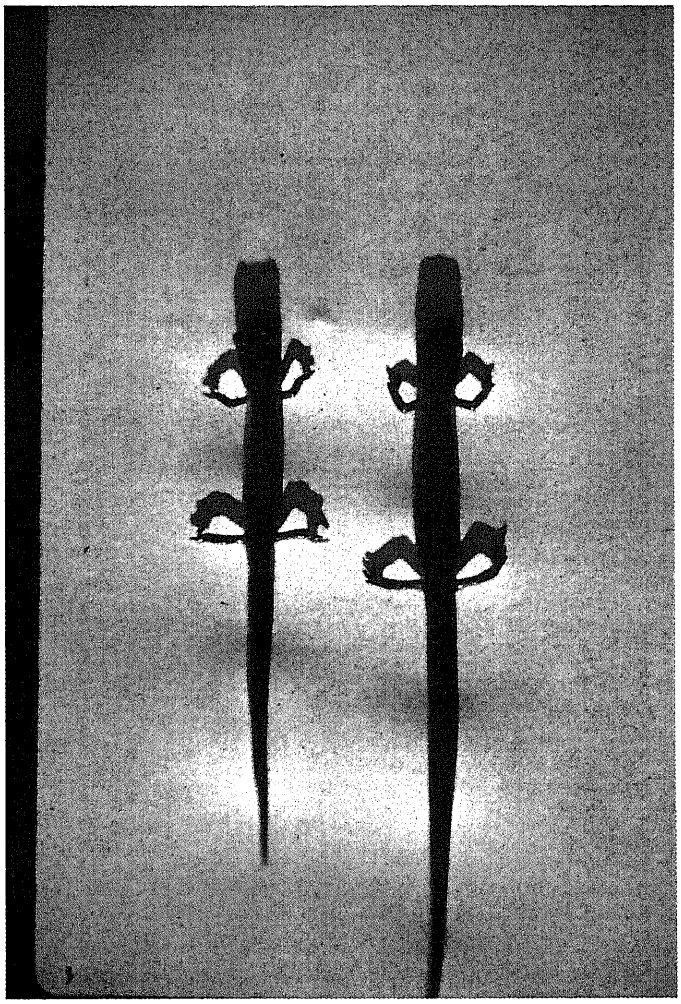


Figure 5. Photograph showing ventral view of ***Hemidactylium scutatum*** male (left) and female (right).

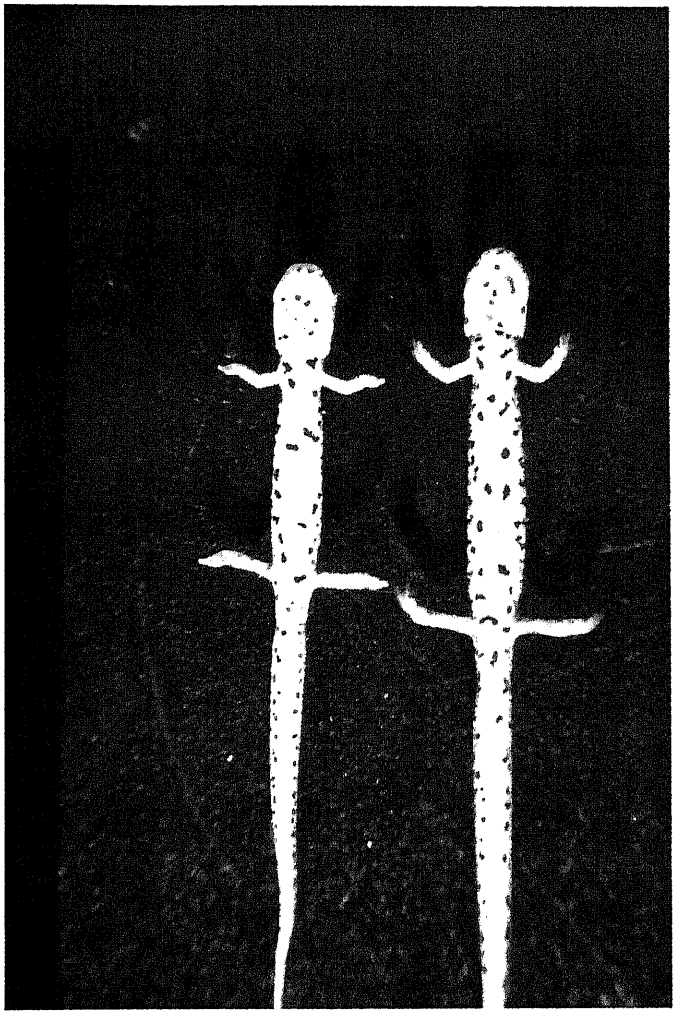
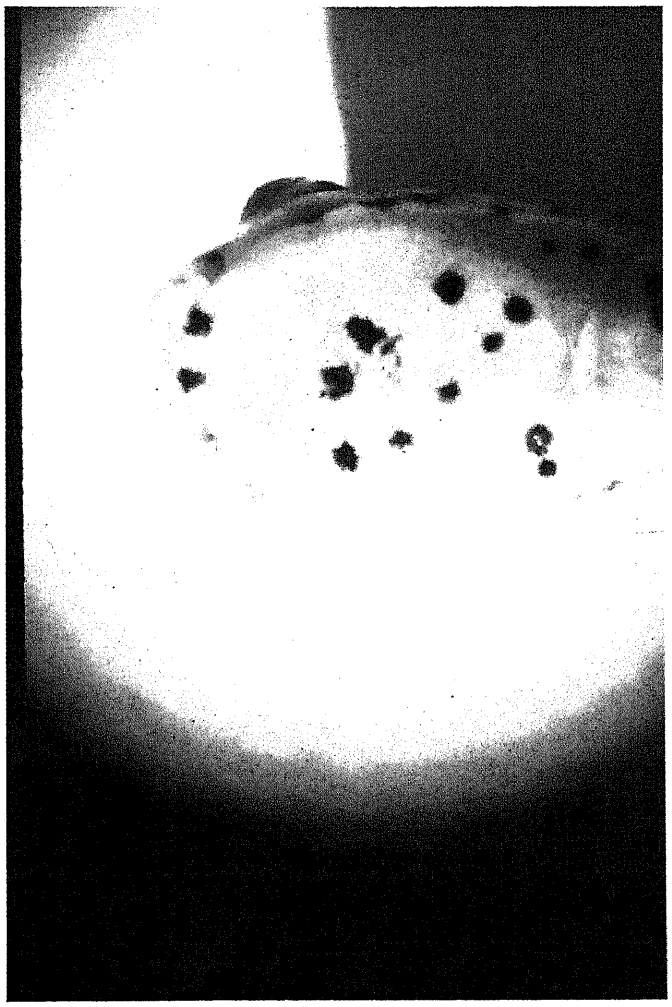


Figure 6. Photograph showing ventral view of *Hemidactylium scutatum* female head.

Figure 7. Photograph showing ventral view of *Hemidactylium scutatum* male head.



Michigan, respectively. The following information is based on these studies unless otherwise noted. *Hemidactylium* breeds in the autumn in wooded areas adjacent to nest sites. Fertilization is internal by means of spermatophores. Hibernation occurs in wooded areas in cavities of rotted wood, under leaves, or underground (Blanchard, 1933a). There is an early spring migration of adult gravid females to the nesting grounds, which are described as ponds, pools, or swamps that are directly adjacent to deciduous woods. *Hemidactylium* nests are usually associated with *Sphagnum* mosses, but they have been found in several other situations. Generally, eggs are laid singly attached to rhizoids and stems of moss or in damp cavities in rotted wood. Nests are usually laid within 8 inches (20.3 cm) of water. Several females may deposit eggs in the same nest, but only one attendant remains with the eggs. Females stay with the nests until hatching, about 38 days in Michigan and 55 days in New York. There is an aquatic larval period of about 6 weeks. Juveniles remain at or near the nest site until they reach maturity, approximately 2 ½ years after transformation (Blanchard and Blanchard, 1931). Females outnumber males in a population during the juvenile stage (Blanchard, 1935).

Habits and development of this species are well documented for the northern part of its range, but little research has been done in West Virginia

(Green, unpublished data; Green and Pauley, 1987). Most research conducted on this species is related to nesting habits. No long-term studies have investigated the range of environmental parameters associated with nests or development. *Sphagnum* moss species, which are known for their acidic properties (Conard and Redfearn, 1979; Bold *et al.*, 1987; Smith, 1990), are considered excellent habitat for *Hemidactylium* nests (Bishop, 1918; Blanchard, 1923) but no studies have investigated acid tolerance in *Hemidactylium.*

The main objective of my study was to examine natural history of *Hemidactylium* in West Virginia. This was a 2-year study focused on nesting habits, egg and larval development, and environmental factors such as pH tolerance associated with the natural history. The study is described in 3 chapters. Chapter I contains detailed information on breeding and nesting habits in WV. Chapter II concentrates on egg and larval development. Chapter III combines field and laboratory studies to determine tolerance levels of *Hemidactylium* to acid conditions.

Description of Study Sites

Five study sites were used to examine the natural history of *Hemidactylium scutatum* in West Virginia. All sites are located either in or near the Otter Creek National Wilderness Area, Monongahela National Forest, Randolph

County, West Virginia (Fig. 8). Sites 1 and 2 are located on the Bowden Quadrangle and sites 3 - 5 on the Parsons Quadrangle. Aspect is flat at all sites. Complete descriptions of each site are listed below.

Site 1: Condon Run (CR) (Fig. 9)

Elevation: 929.6 M / 3050' Mountain Range: Shavers Mountain

General description: Riparian pool / Mixed deciduous forest

This site is a 20 m x 5 m riparian pool on a tributary of Condon Run and is located 0.4 km west of the confluence of Condon Run with Otter Creek (Fig. 8, #1). Maximum water depth is 92 cm. Edges of the pool are lined with *Sphagnum quinquifarium, S. girgensohnii,* and *Polytrichum ohioensis.* Dominant vegetation surrounding the site includes thick *Rhododendron maximum* and *Tsuga canadensis.*

Site 2: Bickle Knob (BK) (Fig. 10)

Elevation: 1139.9 M / 3740' Mountain Range: Shavers Mountain

General description: Vernal pool / Deciduous forest

This site is located on FSR 91 approximately 0.024 km southwest of FSR 774 (Fig 8, # 2). This site is a series of small vernal pools surrounded by a deciduous forest. During the study period, water depth at this site varied from 0 cm (totally dry) to 16 cm (flooded with all pools connected). The main study area at this site was two vernal pools that measured about 5 m

Figure 8. Map of Otter Creek National Wilderness Area showing the 5 study sites.

1. Condon Run
2. Bickle Knob (position on map not accurate)
3. Moore Run Trail #1
4. Moore Run Trail #3
5. Moore Run Trail #5

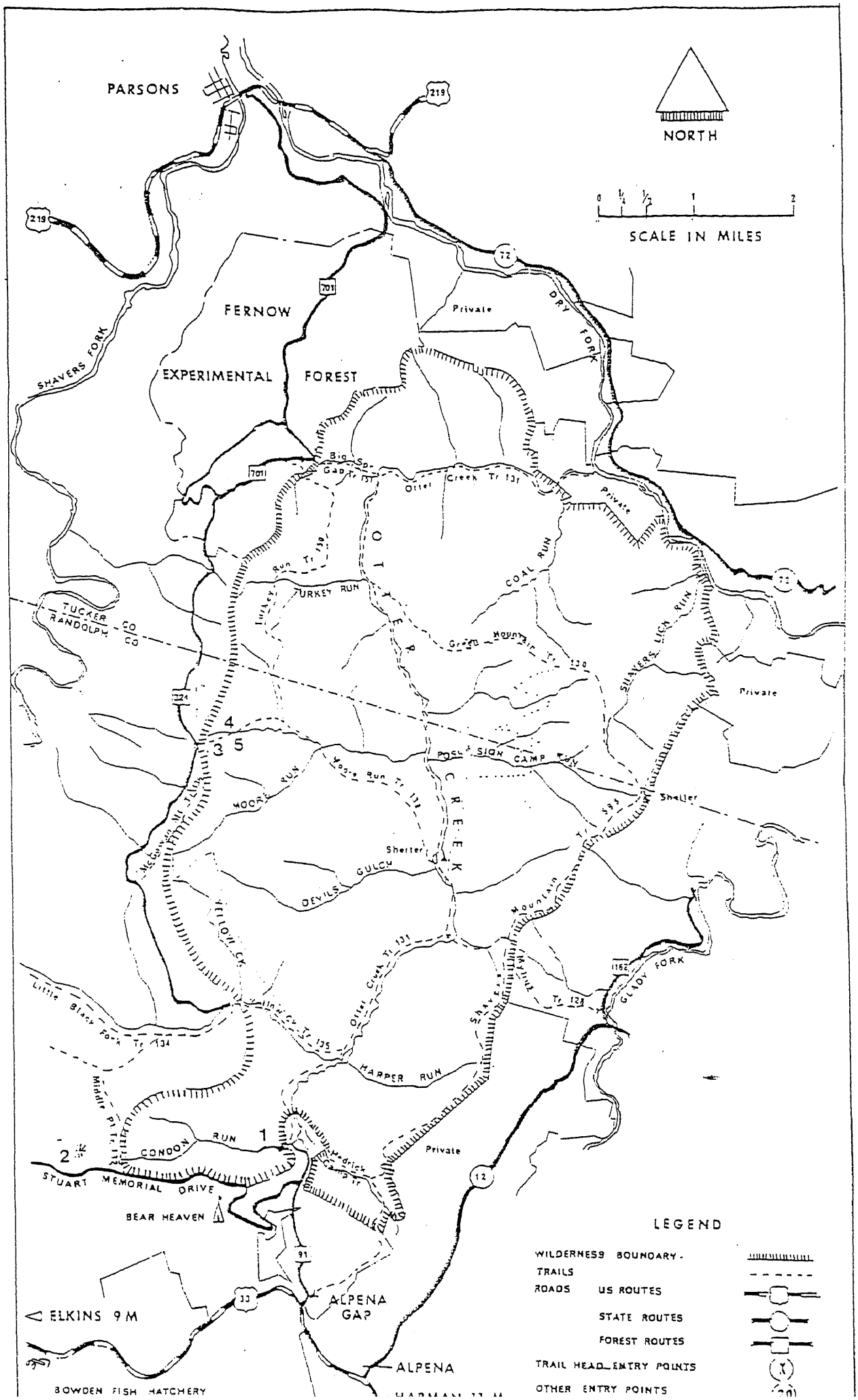

PARSONS
NORTH
SCALE IN MILES
FERNOW
EXPERIMENTAL FOREST
SHAVERS FORK
Private
DRY FORK
Big Spa Gap Tr 137
Otter Creek Tr 137
Private
OTTER CREEK
COAL RUN
Turkey Run Tr 130
TURKEY RUN
Gandy Mountain Tr 130
TUCKER CO
RANDOLPH CO
SHAVERS LICK RUN
Private
PUCKER GAP RUN
Moore Run Tr 134
MOORE RUN
Shelter
Shelter
DEVILS GULCH
Mountain Tr 133
Otter Creek Tr 131
GLADY FORK
Little Black Fork Tr 134
Tr 135
HARPER RUN
Middle Fork
CONDON RUN
STUART MEMORIAL DRIVE
BEAR HEAVEN
Private
ELKINS 9 M
ALPENA GAP
ALPENA
BOWDEN FISH HATCHERY
LEGEND
WILDERNESS BOUNDARY
TRAILS
ROADS US ROUTES
STATE ROUTES
FOREST ROUTES
TRAIL HEAD ENTRY POINTS
OTHER ENTRY POINTS

Figure 9. Photograph of Site 1: Condon Run (CR).

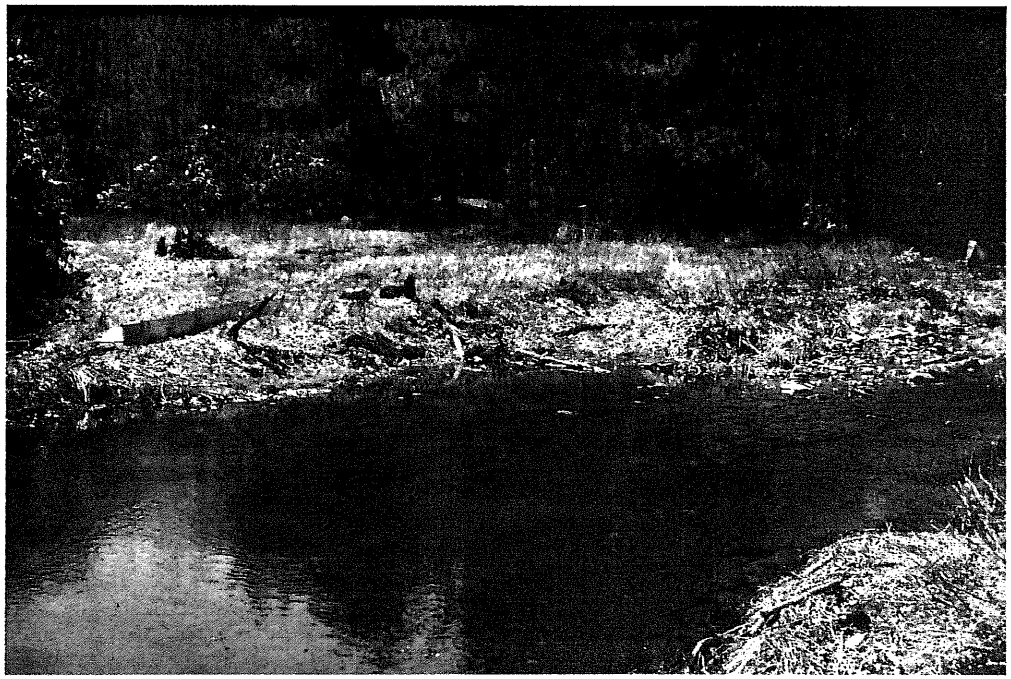


Figure 10. Photograph of Site 2: Bickle Knob (BK).

x 5 m each. *Thuidium delicatulum* and *Mnium sp.* are the dominant moss types present at this site.

Site 3: Moore Run Trail # 1 (MT-1) (Fig. 11)

Elevation: 1011.9 M / 3320' Mountain Range: McGowan Mountain

General description: Vernal pool / Bog

This site is found on Moore Run Trail (FST 138) approximately 0.4 km east of FSR 324 (Fig. 8, # 3). This site is a 2 m x 1 m vernal pool on an open *Sphagnum* bog. Water depth ranges from 0 to 11 cm. *Sphagnum quinquifarium* is the dominant moss species present at this site. Other vegetation includes *Rhododendron maximum, Vaccinium sp.,* and *Eriophorum virginicum.*

Site 4: Moore Run Trail # 3 (MT-3) (Fig. 12)

Elevation: 1005.4 M / 3300' Mountain Range: McGowan Mountain

General description: Permanent pool / Bog

This site is located on Moore Run Trail approximately 0.4 km east of FSR 324 (Fig. 8, # 4). This site is a 5 m x 1.5 m permanent pool in a *Sphagnum* bog surrounded on the south side by *Rhododendron maximum.* Maximum water depth at this site is 61 cm. Edges of the pool are dominated by *Sphagnum quinquifarium* and *Eriophorum virginicum.*

Site 5: Moore Run Trail # 5 (MT-5) (Fig. 13)

Elevation: 1011.9 M / 3320' Mountain Range: McGowan Mountain

Figure 11. Photograph of Site 3: MooreRun Trail #1 (MT-1).

Figure 12. Photograph of Site 4: Moore Run Trail #3 (MT-3)

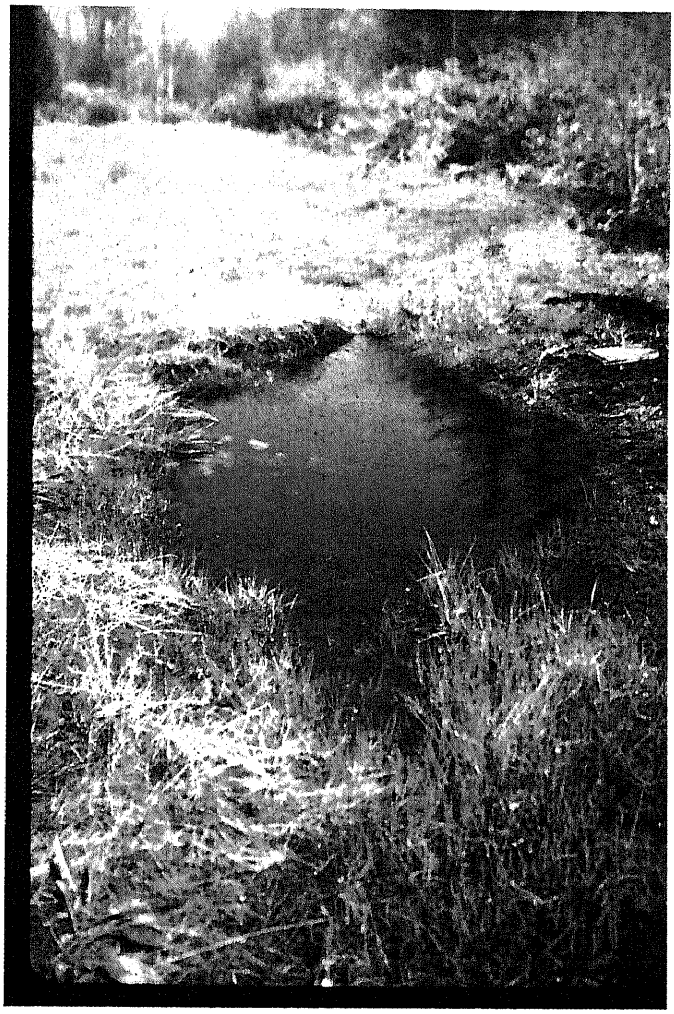
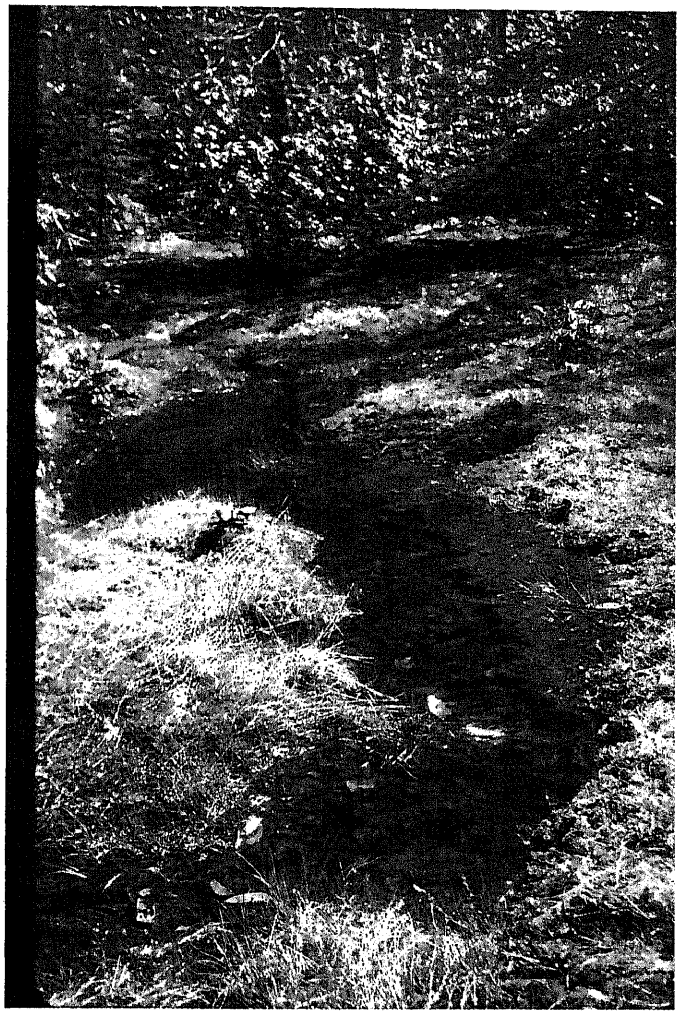


Figure 13. Photograph of Site 5: Moore Run Trail #5 (MT-5)



General description: Spring / Bog

MT-5 was not established until late in the 1995 larval period. This site is on Moore Run Trail approximately 0.4 miles east of FSR 324 (Fig 8, # 5). This site is a 20 m x 1 m spring on the edge of a *Sphagnum* bog. Maximum water depth is 26 cm. The dominant moss species is *Sphagnum megallanicum.* Dominant vegetation at this site includes *Rhododendron maximum, Eriophorum virginicum,* and *Tsuga canadensis.*

Chapter I. Reproduction and Nesting Habits

Introduction

Duellman and Trueb (1986) stated that eastern North American plethodontid salamanders generally breed in autumn, and often again in spring. Several authors have shown that *Hemidactylium* breeds in autumn (Dieckmann, 1927; Blanchard and Blanchard, 1931; Blanchard, 1933b; Branin, 1935). Branin (1935) is the only author to suggest that *Hemidactylium* courtship activities may be arrested late in autumn by climatic conditions and occur again in spring.

Courtship activities in *Hemidactylium* are similar to those of other plethodontids (Noble and Brady, 1926) and usually occur at night (Branin, 1935). Blanchard (1933b) and Branin (1935) suggested that moisture rather than temperature was the main factor that stimulated deposition of spermatophores. Blanchard (1933b) described *Hemidactylium* spermatophores as 2 mm gelatinous disks that taper into 1 mm diameter stalks and end in pale yellow tops that contain sperm. Females store sperm in spermathecal tubules until egg deposition (Dieckmann, 1927; Blanchard, 1933b; Branin, 1935). Sever (1987) noted that females can also retain sperm at least one month after oviposition.

Migration of gravid *Hemidactylium* to the nesting grounds occurs in early

spring (Bishop, 1918, 1941; Blanchard, 1934c; Breitenbach, 1982; Green, unpublished data) and is probably initiated by temperature rather than moisture (Blanchard, 1934c; Green, unpublished data). In West Virginia, Green (unpublished data) found that initiation of migration occurred only after at least 3 consecutive days of temperatures above 40° F (4.5°C). Mass migrations occur for 1 or 2 weeks before oviposition starts, but some females continue to migrate for about 2 weeks after most eggs have been laid (Blanchard, 1934c; Bishop, 1941; Wood, 1951; Breitenbach, 1982). All reports in literature suggest that males, non-gravid females, and immature individuals do not migrate, rather they attend to normal feeding activities.

Females probably select nest sites from water because sites are chosen directly above and within 8 inches (20.3 cm) of its surface (Bishop, 1918, 1941; Blanchard, 1922, 1934c; Gilbert, 1941; Wood, 1951, 1953). *Hemidactylium* nests are often found in *Sphagnum* mosses (Bishop, 1918, 1941; Blanchard, 1923; Blanchard and Blanchard, 1931), but have been found in several other substrate types. Gilbert (1941) found nests in *Thuidium, Mnium* and *Climacium* and Wood (1951, 1953) observed nests in 10 other moss genera, 4 genera of hepatics, and 1 genus of sedge. Nests have also been found under loose bark, inside rotted planks, boards and logs, in mounds of pine needles, and in hollow stumps (Blanchard, 1922; Wood, 1951; Breitenbach,

1982; Pauley, 1993). Wood (1951, 1953) found that substrate type was less important than water proximity as long as it contained or covered crevices for egg deposition. Limited environmental data have been reported in the literature for *Hemidactylium* nests. Wood (1951) recorded pH and temperature values from less than 10 nests. He reported an average water pH (n = 5) of 5.0 and average air, water, and nest temperatures (n = 9) of 28.7°C, 21.7°C, and 23.1°C, respectively.

Egg deposition occurs from mid-April to early May in Michigan (Blanchard, 1934a), New York (Bishop, 1918), and West Virginia (Green, unpublished data), and from mid-February to early March in the coastal plains of Virginia (Wood, 1951). Extra-seasonal ovulation has been documented in December in both laboratory (Branin, 1935) and field (Wood, 1955) studies. Bishop (1941) noted that females turn upside down and deposit eggs attached singly to rhizoids and stems of mosses and vegetation and that the process required several minutes for each egg laid. Studies of ovarian follicles have shown that females contained a maximum of 46 eggs in Michigan (Blanchard, 1936), 60 in New York (Gilbert, 1941), 80 in Virginia (Wood, 1951), and 68 in West Virginia (Green, unpublished data). Nests contained an average of 31 eggs in Michigan (Blanchard, 1936), 25 and 50 eggs in New York (Gilbert, 1941 and Bishop, 1918, respectively), 30 to 40 in Virginia (Wood, 1951), and 42 in West Virginia (Green, unpublished data). Blanchard (1934b) observed

nests with 22 to 1110 eggs which represented complements of 1 to 14 females. Although several females may deposit eggs together, only 1 attendant remains until eggs hatch (Blanchard, 1934b; Bishop, 1941; Harris *et al.*, 1995).

Only two species of plethodontids exhibit communal nesting behavior, *Batrachoseps attenuatus* and *Hemidactylium scutatum* (Duellman and Trueb, 1986). As a result, this aspect of *Hemidactylium* natural history has been well documented (Blanchard, 1934b; Bishop, 1941; Wood, 1953; Harris and Gill, 1980; Breitenbach, 1982). Harris *et al.* (1995) described 6 hypotheses that may explain communal nesting behavior: habitat saturation, aggressive usurpation, intraspecific brood parasitism, multiple defenders, predation dilution, and kin selection. The habitat saturation hypothesis is unlikely because communal nesting occurs from the start of oviposition (Wood, 1951; Goodwin and Wood, 1953). Preliminary research by Harris *et al.* (1995) suggested that habitat saturation, aggressive usurpation, brood parasitism, and multiple defenders hypotheses could be rejected as reasons for communal nesting behavior in *Hemidactylium*. Habitat saturation theory was rejected because communal nests occurred under conditions of low population density. Aggressive usurpation theory was rejected because usually the first female to lay eggs also remained to brood them. Brood

parasitism was rejected because no aggression was observed between females at nests. Multiple defenders theory was rejected because only one female remained with each nest. Communal nesting behavior was not directly studied in my research and is mentioned here because it is characteristic of *Hemidactylium.*

Breeding and nesting behaviors in *Hemidactylium* have been well documented for most of its range. Green (unpublished data) has documented observations such as date of oviposition, number of eggs per nest and female, and number of females per nest from a limited number of nests in West Virginia. No long-term studies have documented the ambient factors such as temperature and pH that are typical of nest sites. This chapter documents breeding and nesting behaviors in West Virginia, reports ambient environmental parameters characteristic of nesting habitats, and compares these results with those found in other localities.

Methods and Materials

Size Classes

Seventy-seven specimens from the West Virginia Biological Survey (WVBS) collection and 66 specimens from my study sites were examined to determine morphology and size classes of *Hemidactylium.* WVBS specimens represented several years of collection and many localities within the state.

A 100-meter search away from the nests at each of my sites in spring and autumn to collect adults for reproductive and morphological studies yielded no specimens. Specimens from my study sites included 2 mature males, 10 mature females, 1 juvenile, and 53 larvae (see Ch. II, Methods, for collection and processing of larval specimens). All terrestrial specimens were collected at or near nests, anesthetized in chlorotone and fixed in a 10% formalin solution. Snout-vent length (SVL), total length (TL), and cranial width (CW) to the nearest 0.1 mm were measured on all specimens using dial calipers according to procedures in Table 1. Specimens were dissected under a binocular dissecting microscope and gender was determined by examination of the gonads. Mean morphological values for each size class were subjected to a one-way ANOVA and Neuman-Keuls Multiple Comparisons Test (NKMCT) to determine variance between groups. Head length (HL) to the nearest 0.1 mm was measured on mature individuals using dial calipers as described in Table 1. Head area (HA) was calculated by multiplying CW and HL. Head length and HA data were subjected to an independent group t-test to determine significant variance between males and females.

Breeding

Time of mating was determined by examining the spermatogenic wave: i.e., movement of spermatozoa through the male reproductive system.

Table 1. Character descriptions for morphological measurements.

Character (Abbreviation)	Description
Snout-vent length (SVL)	Length (mm) from the tip of the snout to the posterior end of the cloacal opening.
Total length (TL)	Length (mm) from the tip of the snout to the tip of the tail.
Cranial width (CW)	Width (mm) of the widest part of the head behind the eyes.
Head length (HL)	Length (mm) from the tip of the snout to the gular fold.
Head area (HA)	Multiplication of characters CW and HL.

Spermatozoa are first produced in the distal region of the testes and then in the proximal region (Highton, 1956; Sayler, 1966). Spermatozoa migrate from the testes through the efferent ductules to the vasa deferentia before moving into the cloaca for deposition. To determine the presence of sperm in *Hemidactylium*, tissues from the proximal and distal regions of the testes and vasa deferentia were placed on microscope slides, smashed with the end of a probe, stained (Wright's stain), then examined under a compound microscope. Presence or absence of sperm was recorded for each region.

Time of egg deposition was determined by examination of diameter and volume of ovarian follicles. Oviducts and follicles were removed from specimens while under a binocular dissecting microscope. Mature follicles in each specimen were counted to determine size of egg complements in *Hemidactylium*. Follicle diameter was measured to the nearest 0.1 mm using an ocular micrometer and binocular dissecting microscope. Oviducts and follicles were placed in a 10 ml volumetric flask to determine volume (ul) by water displacement. Oviducts were included in water displacement measurements because follicles were often enclosed in the oviducts.

Nesting Behavior

The following methods were performed on the first visit to each nest. Moss and vegetation surrounding the water at each site were carefully searched for nests. Only eggs that had been laid within a few days were used in long-

term monitoring of *Hemidactylium* nests. Each nest was marked with a numbered metal tag for identification (Fig. 14). Number of females present and number of eggs laid were counted at each nest.

Environmental parameters including temperature and pH were recorded at every nest to determine ambient conditions during oviposition. Temperature (°C) was measured with an armored thermometer and pH was measured with an Oakton pocket pHTestr3 model 35624-30. Air temperature was measured one meter above ground. Nest temperature and pH were measured in the core of each nest. Nests were closed immediately after placing the thermometer inside to minimize heat exchange. Moss temperature and pH were recorded 2.5 cm from each nest in an area where no nests occurred to determine if any micro-habitat differences occurred in nest selection. Moss measurements were not recorded during 1996 due to time constraints. An independent group t-test was used to determine significant variance between moss with nests and moss without nests.

Physical parameters including substrate type, distance and angle of each nest from water, and distance between nests were recorded at each nest. Substrate type was the specific vegetation such as *Sphagnum* moss used as nest habitat. Mosses were identified according to Conard and Redfearn

Figure 14. Photograph showing a tagged *Hemidactylium* nest.

(1979) and the *Sphagnum* mosses were identified to the species level using the Field Guide to the Peat Mosses of Boreal North America (McQueen 1990). The single sedge species was identified according to Strausbaugh and Core (1977). Distance of each nest from the edge of the water and distance between nests were measured to the nearest 0.01 cm using a tape measure. The angle at which each nest was found from the surface of the water was measured using a protractor.

Morphological characteristics were measured for every female attending a nest. Animals were placed in a 12.5 cm x 2.5 cm transparent, plastic measuring box (Figs. 15a and 15b) and covered with a moist sponge. Measuring boxes were used to limit movement of specimens and reduce stress from handling during data collection. Measurements were taken by looking through the bottom of the box. Snout-vent length, TL, CW, and HL were measured to the nearest 0.1 mm using dial calipers. Table 1 describes where each character was measured on specimens. Weight was measured to the nearest 0.01 gram using a 5-gram Pesola spring scale. Each female was identified by the number of spots and pattern present on her chin and immediately returned to her nest after data collection.

Figure 15a. Photograph showing ventral view of a *Hemidactylium* female in the box (12.5 cm x 2.5 cm) used for field measurements of morphological characters .

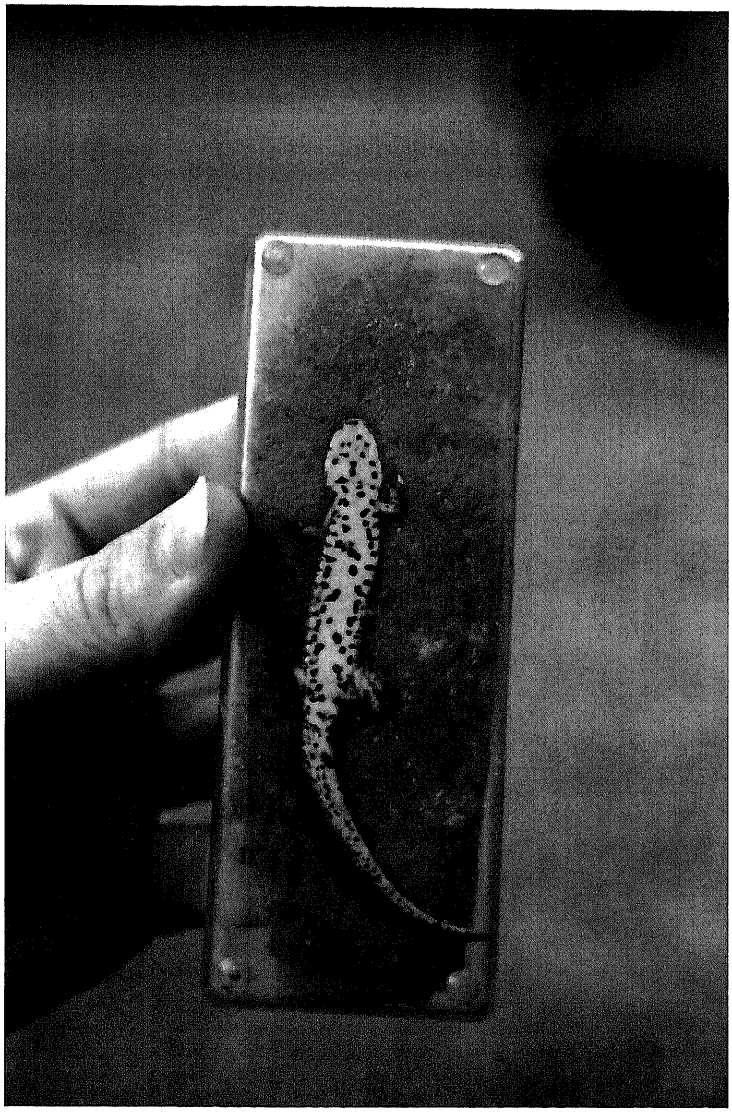
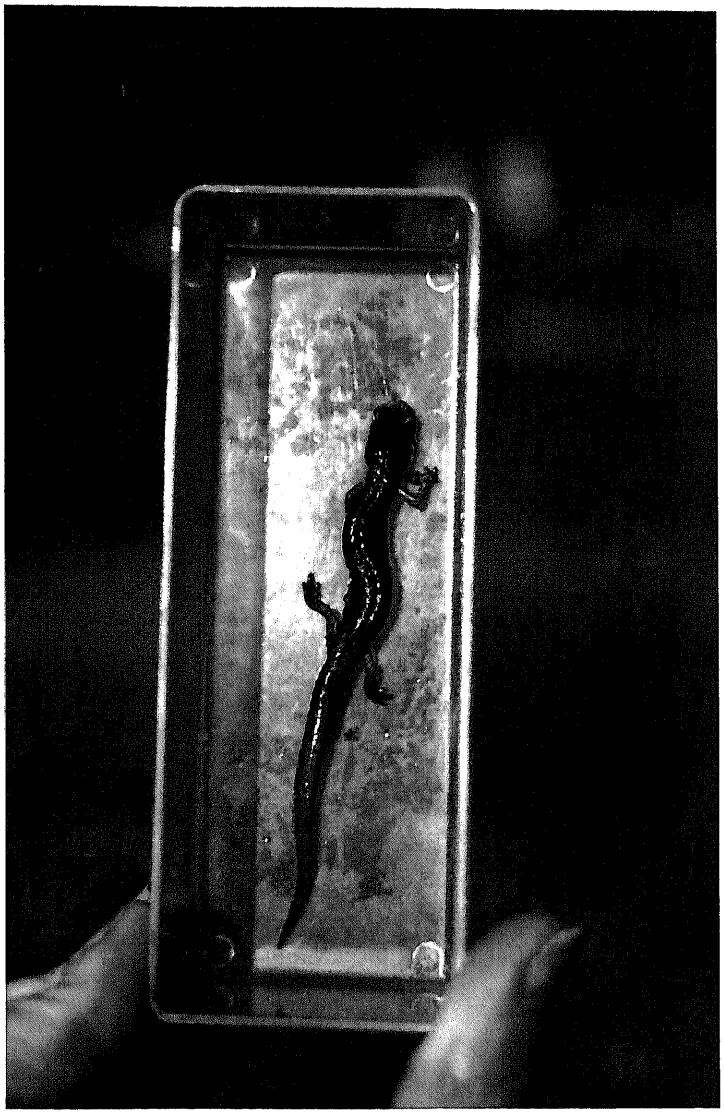


Figure 15b. Photograph showing dorsal view of a *Hemidactylium* female in the box (12.5 cm x 2.5 cm) used for field measurements of morphological characters .



Results

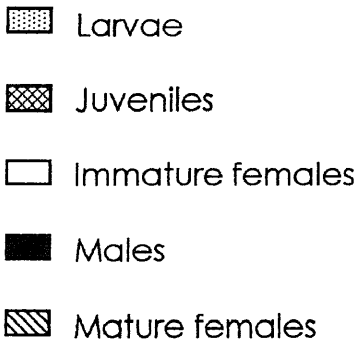
Size Classes

Results from ANOVA and NKMCT showed that larvae, juveniles, immature females and males, and mature females had significantly different mean SVL, CW, and TL. Snout-vent length was positively correlated with CW. Mature females had a greater maximum SVL, TL, and CW than mature males (Fig. 16 - 18). Mature females ranged in size from 29.0 to 46.5 mm SVL, 56.6 to 108.0 mm TL, and 4.5 to 6.8 mm CW and mature males ranged in size from 26.9 to 36.5 mm SVL, 37.7 to 83.9 mm TL, and 4.1 to 5.6 mm CW. Immature females ranged in size from 29.0 to 30.0 mm SVL, 60.8 to 63.7 mm TL, and 4.7 to 5.2 mm CW. Head length ranged from 6.5 to 9.9 mm in females and 6.3 to 8.1 mm in males. Head area ranged from 30.2 to 64.4 mm^2 in females and 25.8 to 45.4 mm^2 in males. Females had a significantly greater mean HL and HA than males ($p < 0.01$).

Breeding

Table 2 shows results from the spermatogenic wave. Mature males were collected in all 4 seasons. No spermatozoa were found in the testes or vasa deferentia of the specimen collected 22 May and all other specimens had spermatozoa present in at least one portion of the reproductive tract. Female specimens were collected in the spring, summer, and autumn. Most females were captured from nest sites in the spring. Most gravid females

Figure 16. Length-frequency histogram for the morphological character snout-vent length (SVL).



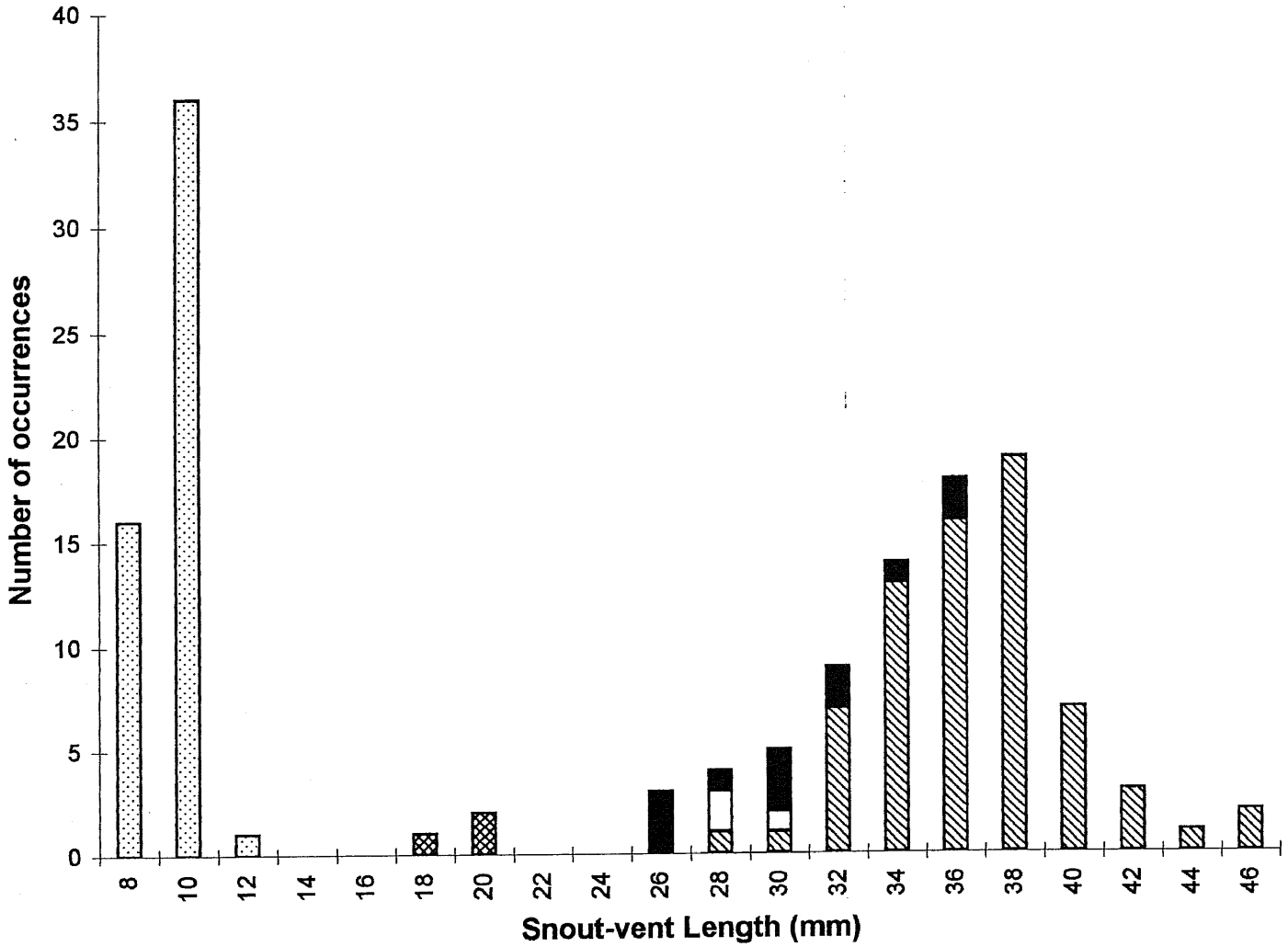
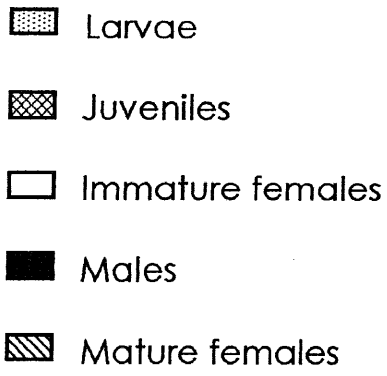

Number of occurrences
0
5
10
15
20
25
30
35
40
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
Snout-vent Length (mm)

Figure 17. Length-frequency histogram for the morphological character cranial width (CW).



Number of occurrences
0
5
10
15
20
25
30
35
Cranial width (mm)
2
2.25
2.5
2.75
3
3.25
3.5
3.75
4
4.25
4.5
4.75
5
5.25
5.5
5.75
6
6.25
6.5
6.75

Figure 18. Length-frequency histogram for the morphological character total length (TL).

Larvae

Juveniles

Immature females

Males

Mature females

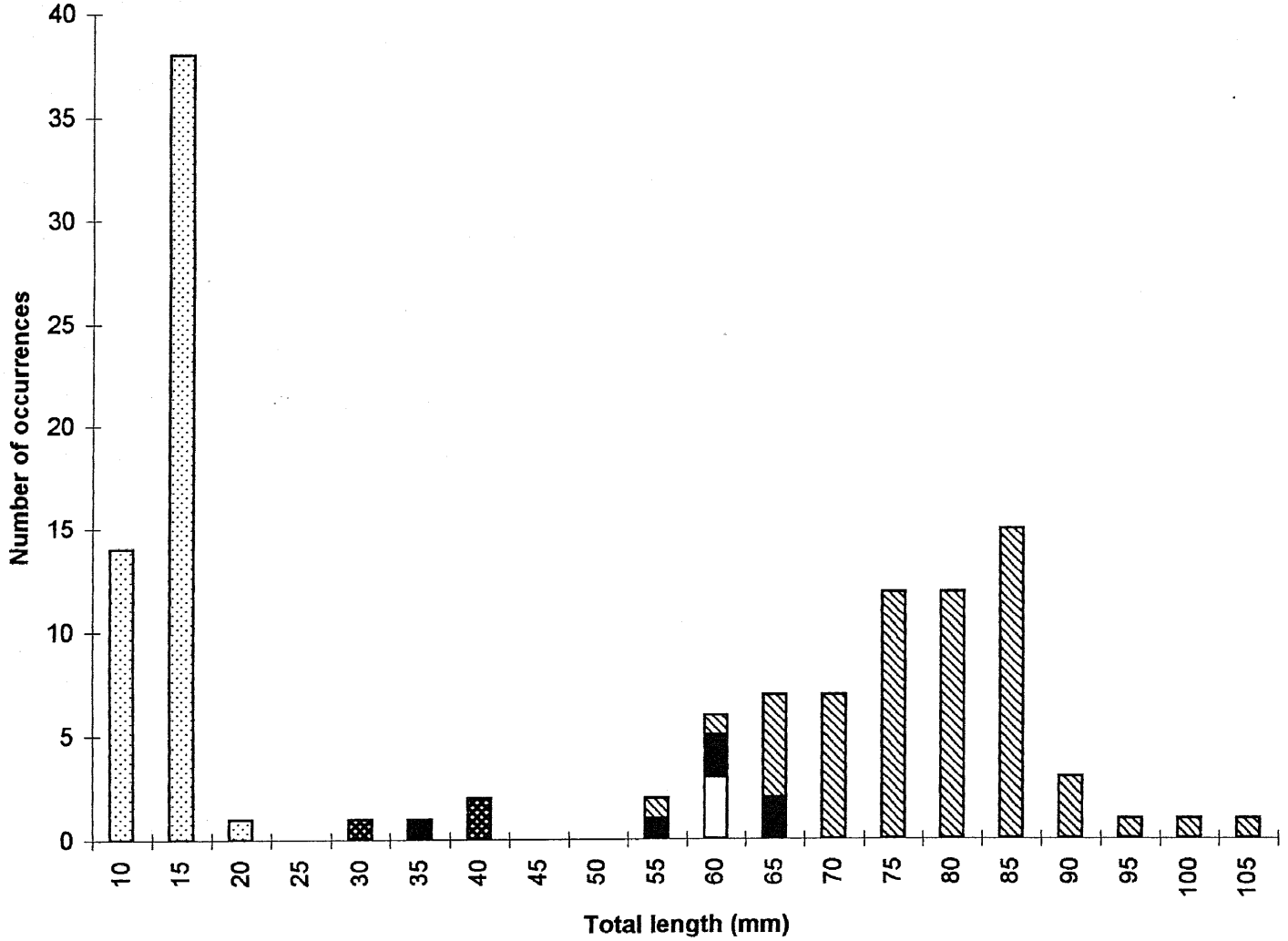

Number of occurrences
0
5
10
15
20
25
30
35
40
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
Total length (mm)

Table 2. Results of the spermatogenic wave analysis showing presence (yes) or absence (no) of spermatazoa in the reproductive tracts of male *Hemidactylium* (n=14).

Date	Posterior testes	Anterior testes	Anterior vasa deferentia	Posterior vasa deferentia
3 April	YES	YES	YES	YES
3 April	YES	YES	YES	YES
8 April	YES	YES	YES	YES
11 April	YES	YES	YES	YES
29 April	YES	YES	YES	YES
17 May	NO	NO	YES	YES
18 May	YES	YES	YES	YES
22 May	NO	NO	NO	NO
23 June	YES	YES	YES	YES
24 June	YES	YES	YES	YES
15 September	YES	YES	YES	YES
6 December	YES	YES	YES	YES
6 December	YES	YES	YES	YES
6 December	YES	YES	YES	YES

were captured from 5 April to 5 May, but one gravid specimen was collected 9 September 1995 from a nest site. Females had 17 to 56 ($\bar{x}$ = 31) mature follicles in the oviducts. No correlation was found between SVL and number of mature follicles. Follicle volume for gravid females ranged from 20 to 670 ul, with an average of 281 ul. Mature follicles ranged in size from 2.0 to 2.6 mm diameter ($\bar{x}$ = 2.4 mm).

Nesting Behavior

Table 3 shows ranges for environmental data collected during egg deposition. Air temperature ranged from 3.5°C - 29.5°C ($\bar{x}$ = 12.5°C) and nest temperature ranged from 5.5°C to 25.5°C ($\bar{x}$ = 13.0°C). Nest pH ranged from 3.6 to 6.7 ($\bar{x}$ = 4.5). An independent group t-test showed no significant difference between pH or temperature ($p < 0.01$) in moss with nests and moss without nests. Nests were found an average distance of 21.9 cm from the edge of the water and 17.4 cm from each other, with a range of 0.1 cm to 200.7 cm. The angle of the nest from the surface of the water ranged from 90° to 174° with an average of 134°.

Six moss species and one sedge species were used as *Hemidactylium* nesting substrate (Table 4). Seventy-two nests were found in *Sphagnum* sp., 19 in non-*Sphagnum* moss species, and 2 entangled in the roots of *Eriophorum virginicum*. Seven of the 9 nests observed in non-*Sphagnum*

Table 3. Ambient environmental conditions recorded on the first day of *Hemidactylium* egg deposition in WV (1995 and 1996 data combined / n = 93).

	Minimum	Mean	Maximum
Air temperature (°c)	3.5	**12.5**	29.5
Water temperature (°c)	6.5	**11.5**	23.5
Nest temperature (°c)	5.5	**12.9**	25.5
Moss temperature (°c)	6.0	**13.3**	24.5
Water pH	4.00	**4.90**	7.00
Nest pH	3.60	**4.48**	6.70
Moss pH	3.30	**4.48**	6.90
Distance of nest to edge of water (cm)	7.6	**21.9**	48.3
Distance between nests (cm)	0.1	**17.4**	200.7
Angle of nest from the edge of the water (°)	90	**134**	174

Table 4. Type of vegetation used by *Hemidactylium* as nest substrate (n = 93).			
	Species	**No. of nests**	**% of total**
Moss species	*Sphagnum quinquifarium*	59	63
	Thuidium delicatulum	10	11
	Sphagnum megallanicum	7	8
	Sphagnum girgensohnii	6	7
	Mnium sp.	5	5
	Polytrichum ohioensis	4	4
Sedge species	*Eriophorum virginicum* (roots)	2	2

moss species were beneath moss in cavities of decaying logs.

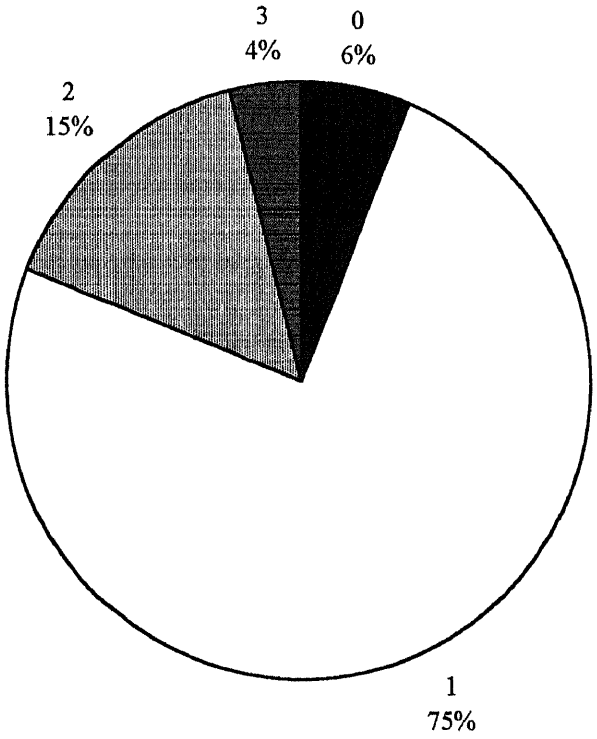
Although 75% of nests had only 1 female attendant, several had 2 or 3 (Fig. 19). Communal nests with more than 1 clutch of eggs occurred at all sites except Bickle Knob (BK). Nests contained from 6 to 145 eggs ($\bar{x}$ = 33) and number of eggs per female based on field observations ranged from 5 to 75 ($\bar{x}$ = 26). Table 5 indicates little morphological difference between non-gravid and gravid attendants. Non-gravid attendants were those that had already laid eggs. Attendant females ranged in size from 28.4 to 42.3 mm SVL, 66.6 to 98.2 mm TL, 4.5 to 7.1 mm CW, and 5.5 to 8.6 mm HL. Attendants had an average weight of 1.0 g for non-gravid females and 1.4 g for gravid females.

Discussion

Size Classes

Blanchard and Blanchard (1931) described size classes for *Hemidactylium scutatum* in Michigan and reported 2 juvenile size classes that ranged from 26 to 41 mm and 45 to 67 mm TL. They concluded that sexual maturity was not reached until after the second growing season and my study supports those conclusions. I observed two distinct sexually immature groups, juveniles (33 to 41 mm TL) and immature females (60 to 64 mm TL). However, my results were based on a very small sample size and may not accurately

Figure 19. Percent of nests with 0 to 3 female attendants (n = 93).


3
4%
0
6%
2
15%
1
75%

reflect the population as a whole. My study and that of Blanchard and Blanchard (1931) showed considerable overlap in size between second-year immature individuals and mature adults. Blanchard and Blanchard (1931) found that mature males ranged in TL from 49 to 77 mm and I found TL for males to be 37 to 84 mm. Michigan females were 62 to 89 mm TL and those in WV were 56 to 108 mm TL. Males attained sexual maturity at an average smaller CW, SVL, and TL than females and grew to a shorter maximum length. Size differences and shape of the snout have been used to determine gender in the field (Bishop, 1918, 1941; Blanchard, 1923). Statistical analyses in my study showed that mature males have significantly smaller HL and HA than mature females, but considerable overlap exists between groups and prevents a clear definition of gender based on size characteristics.

Breeding

Dieckmann (1927) reported that mating occurred prior to August and other accounts suggested that breeding occurs only in autumn (Blanchard, 1933b; Bishop, 1941). Branin (1935) suggested that mating could occur throughout the year if climatic conditions were favorable. Results from the spermatogenic wave in my study indicated a winter and spring, as well as an autumn breeding period. Several other plethodontids exhibit a semiannual reproductive pattern (Duellman and Trueb, 1986). Based on the

specimen collected 22 May (SVL = 33.3 mm) with no spermatazoa in the reproductive tract, it is possible that a pause in breeding activities occurs during late spring when females are attending nests and males are feeding.

Bishop (1941) and Blanchard (1935) noted that it was easier to collect males in autumn because they were breeding and females in spring because they were at nest sites. This skewed distribution of gender prevented even sampling of both sexes throughout the year in my study. Small sample sizes were examined in my study due to the paucity of specimens in the WVBS collection. More extensive studies are needed to determine an accurate account of breeding activity in *Hemidactylium* in West Virginia.

Nesting Behavior

It is difficult to determine what factors stimulated migration because no environmental data were collected prior to deposition. Minimum air temperature recorded on the first day of deposition was 3.5°C (38.3°F) which corresponds with observations by Green (unpublished data) that temperature must be at least 40°F for migration to occur.

Studies of ovarian follicles, gravid females, and nesting sites indicated that West Virginia *Hemidactylium* migrated in early April and oviposited in mid-April to early May. Eggs in developmental stage 8, 21 to 22 days old (Bishop, 1918), were observed in Kanawha State Forest, Kanawha Co., in mid-April

which indicates that migration and oviposition dates may be earlier in the southern part of the state. Blanchard (1934c), Bishop (1941), and Green (unpublished data) also reported migrations of females to the nest sites in early to mid-April. Results from my study support those data and also suggest an autumn migration. A gravid female and juvenile were collected 15 September 1995 at a nest site approximately 6" underground. This female contained smaller follicles (2.0 mm) than other gravid females (2.4 mm) suggesting that oviposition would not occur until spring. The location of this female also suggests that spring migration may be a series of vertical and horizontal movements to nest sites. Based on SVL and TL, the juvenile was a first year individual.

Reports in literature on migration suggest that only gravid females migrate to the nest sites (Blanchard, 1934c; Bishop, 1941; Breitenbach, 1982). Four of the 13 males examined during this study were collected from nest sites. One specimen was captured on the perimeter of the nest site on 15 September 1995 and another was collected within 2 inches of a nest on 29 April 1996. Both had sperm present in all regions of the reproductive tract. The male collected 29 April had a 30.0 mm SVL and 63.6 mm TL suggesting that it had recently attained sexual maturity. Wood (1951) observed 1 male and 4 juveniles in attendance at nests, but gave no explanation other than "accidental distribution". The male was found at a nest attended by a

female. Bishop (1918) also noted that several ½ grown specimens were observed at nest sites. Given that Bishop (1918) and Blanchard (1923) both described the females as fully twice the size of males, it is possible that small specimens observed at nests were males. Whether or not these specimens migrated to the sites cannot be answered without additional evidence, but it is likely that smaller males and juveniles found near nests had transformed and not yet migrated to adjacent woodlands. This behavior would be advantageous and would allow newly matured individuals to breed before migration to more terrestrial habitats. Other males collected from nest sites were fully grown adults that may have migrated in search of females.

All specimens collected during this 2-year study were taken from the nest sites, which suggests that entire populations may occur at or near the nest sites year round. However, *Hemidactylium* migrations from wooded areas to nest sites have been observed in other parts of West Virginia (Pauley, pers. comm.).

Another possibility for the lack of specimens found away from nest sites is that *Hemidactylium* are fossorial. The number of females that occur at nest sites suggests that there is a large population at each site. Survey methods employed in my study proved ineffective for finding male *Hemidactylium*.

Follicle size in West Virginia specimens was comparable to that reported

in literature for other regions (Bishop, 1918; Dieckmann, 1927; Wood, 1951; Sever, 1987). Average number of eggs per female was similar in my ovarian studies (31) and my nest observations (26). Maximum number of follicles per female in my ovarian study (56) was comparable to other results from WV (Green, unpublished data), Michigan (Blanchard, 1936), and New York (Gilbert, 1941), but not to results from Virginia (Wood, 1951). Blanchard (1936), Green (unpublished data), and Wood (1951) observed a positive relationship between female size and number of eggs, a correlation not observed in my study or by Gilbert (1941). This may be due to small sample sizes used in my study and by Gilbert (1941). Solitary nests were defined by Blanchard (1934b) as containing less than 40 eggs, but my ovarian studies suggest this number is probably higher. However, several papers discussed the possibility that females do not deposit their entire complement in one nest (Blanchard, 1936; Gilbert, 1941; Wood, 1951).

Nests are generally found in areas with suitable habitat adjacent to woodlands (Blanchard, 1923). While micro-habitat characters preferred by females have not been well documented, limited data from my study revealed no temperature or pH differences between substrates with nests and those without. *Hemidactylium* nests are often found in *Sphagnum* mosses (Bishop, 1918; Blanchard, 1923), but can also be found in several other types of substrate (Gilbert, 1941; Wood, 1951). I found that most nests

were in *Sphagnum* sp. and that *Sphagnum* substrates were chosen before non-*Sphagnum* moss substrates in areas with both types of habitat. This was evidenced by examination of date of oviposition in relation to substrate type. The last new nest observed at CR both years was in *Polytrichum ohioensis*. *Sphagnum* and *Eriophorum* substrates were both used from the start of oviposition in areas with both substrate types. All communal nests in these areas were laid in *Eriophorum* substrate which also contained the greatest number of eggs and females.

Wood (1951) stated that water proximity was more important than substrate type in nest selection. My study revealed nests laid farther away from water occurred in *Sphagnum* moss. *Sphagnum* mosses have a high capacity for water retention (Smith, 1990) and probably provide enough moisture for larvae to survive until they reach water. Eggs were laid an average of 21.9 cm (8.6") from water which is comparable to reports in the literature (Bishop, 1918, 1941; Blanchard, 1922, 1923; Gilbert, 1941; Wood, 1951, 1953). Blanchard (1934c) stated that nests are usually laid directly above water. This statement is quantified by angle measurements recorded during my study. I found that most nests were laid at a 90° angle from water. I observed that eggs were first laid at angles from 90° to 120°, then in areas with more obtuse angles.

As noted in the literature (Bishop, 1918; Blanchard, 1923; Wood, 1951), most nests were found in natural cavities. I observed several nests at the end of underground channels. I did not make an effort to determine the extent or origin of these channels, but their presence supports a fossorial existence for *Hemidactylium*.

Hemidactylium displayed a high tolerance to low pH conditions as denoted by egg deposition in acid environments. They can also tolerate neutral conditions. Tolerance is generally a product of acclimatization (Smith, 1990; Laws, 1993). More in depth studies are needed to define the role of pH in *Hemidactylium* natural history (see ch. IV for a detailed discussion of acid tolerance).

Summary

Hemidactylium in West Virginia breed in autumn and possibly again in spring if climatic conditions are favorable. Migration to nest sites occurred in early April and oviposition occurred in mid-April to early May. Females utilized a wide array of habitat types for nest sites. Nests were found within 25 cm of permanent and temporary pools adjacent to wooded areas in West Virginia. The 3 main substrate types used as nesting substrate were *Sphagnum* sp. moss, non-*Sphagnum* sp. moss, and *Eriophorum virginicum* roots. Eggs were laid in substrate with pH values from 3.6 to 6.7 and

temperatures from 5.5°C to 25.5°C. Several studies have examined communal nesting behavior in *Hemidactylium*, but few have researched the ecological elements of nest selection. Although my study provides a preliminary analysis, more in-depth studies are required to further discover the preferences of females during nest selection.

Chapter II. Development

Introduction

The following information is based on a description of *Hemidactylium* eggs by Bishop (1918). *Hemidactylium* embryos average 2.5 - 3.0 mm diameter and are enclosed by at least 2 gelatinous envelopes for a total diameter of 4.5 - 5.0 mm when laid. *Hemidactylium* eggs cling together and to surrounding vegetation by the adhesiveness of their gelatinous coverings. The amount of yolk in *Hemidactylium* eggs is proportionally smaller than that in *Desmognathus fuscus* and *Plethodon cinereus* and the developing embryo has more bulk than the other species. Yolk reduction in *Hemidactylium* eggs more closely resembles that of *Eurycea bislineata.*

Length of incubation depends on temperature and moisture (Blanchard, 1923; Bishop, 1941; Duellman and Trueb, 1986). Incubation of field-reared eggs required less time in Michigan (Blanchard, 1923), than in New York (Bishop, 1918, 1941), Virginia (Wood, 1951), and West Virginia (Green, unpublished data). Lab-reared eggs required less time than field-reared eggs for incubation (Bishop, 1918, 1941). Bishop (1918) and Green (1941) reported that excessive moisture caused high egg mortality, but Wood (1951) observed no mortality in eggs submerged in water throughout incubation. Harris and Gill (1980) conducted the first quantitative study of

egg survivorship. They observed no relationship between clutch size and survival. Bishop (1918) observed that female attendance had no effect on egg development as long as the moisture content approximated that of field conditions. Harris and Gill (1980) found that brooding by an attendant increased embryonic survival, regardless of the length of time spent with eggs. Wood (1951) suggested that embryonic survival was greater in communal nests, but Harris *et al.* (1995) reported it to be equivalent to that in solitary nests.

Duellman and Trueb (1986) stated that *Hemidactylium* have pond type larvae characterized by laterally compressed bodies, high caudal fins, and large gills. *Hemidactylium* larvae are unique among plethodontids in their possession of a dorsal fin on the trunk (Bishop, 1941). A review of the literature revealed that the habits and development of *Hemidactylium* larvae are not well documented. Bishop (1918, 1941) observed that larvae hatched at an average total length of 12.4 to 13.8 mm and had mostly resorbed their gills within 1 week at an average total length of 20.4 mm. Bishop (1918) estimated a 10 to 20 day transformation period for larvae that were kept in a terrestrial environment. Blanchard (1922, 1923) discovered that *Hemidactylium* larvae were aquatic and noted a 6 week larval period in southern Michigan. He observed larvae wriggling from nests into water

using the same motions typical of embryos in eggs.

Other than limited research by Wood (1951), no attempt has been made to quantify temperature and pH requirements of *Hemidactylium* habitat during development. Research has not been conducted on larval development, and work has not been documented on egg development in West Virginia. The following data define egg and larval development in West Virginia and present the range of environmental parameters that are characteristic during development.

Methods and Materials

Environmental Data

All sites were monitored biweekly throughout the nesting season in 1995 and 1996. Environmental parameters were monitored throughout the incubation period. Temperatures (°C) were measured with an armored thermometer and pH values were measured with an Oakton pocket pHTestr3 model 35624-30. Air temperature was measured one meter above ground. Nest temperature and pH were measured in the core of each nest. Nests were closed immediately after placing the thermometer inside to minimize heat loss. Sites were visited weekly throughout the larval period in 1996 only. Water depth, pH, and temperature were recorded during each visit. Depth of the water was measured where larvae were collected and

was recorded to the nearest 0.1 cm with a tape measure. Water temperature (°C) and pH were measured in the same locality where larvae were collected.

Egg Development

Nests were monitored biweekly in 1996 to determine dates of oviposition and hatching and length of the embryonic period. Two nests were monitored weekly in the laboratory. Egg diameter and stage of development were recorded on every visit. Egg diameter measured the total egg including gelatinous membranes to the nearest 0.1 mm using dial calipers. Three measurements were recorded for each field nest and 5 for each laboratory nest. Specific developmental stages were identified according to Bishop (1918, Table 6) and determined using a binocular dissecting microscope in the lab and a hand lens in the field. Lab and field data were compared with each other and with the results of Bishop (1918, 1941) to determine if *Hemidactylium* in West Virginia follows the same developmental patterns as in the northern part of its range.

Larval Sampling

Specimens were collected from Condon Run during the larval period in 1995 to determine the date of hatching and transformation and the length of the larval period. Fifty-three larvae and 3 transformed juveniles were collected, anesthetized with carbonated water, preserved in a 10% formalin

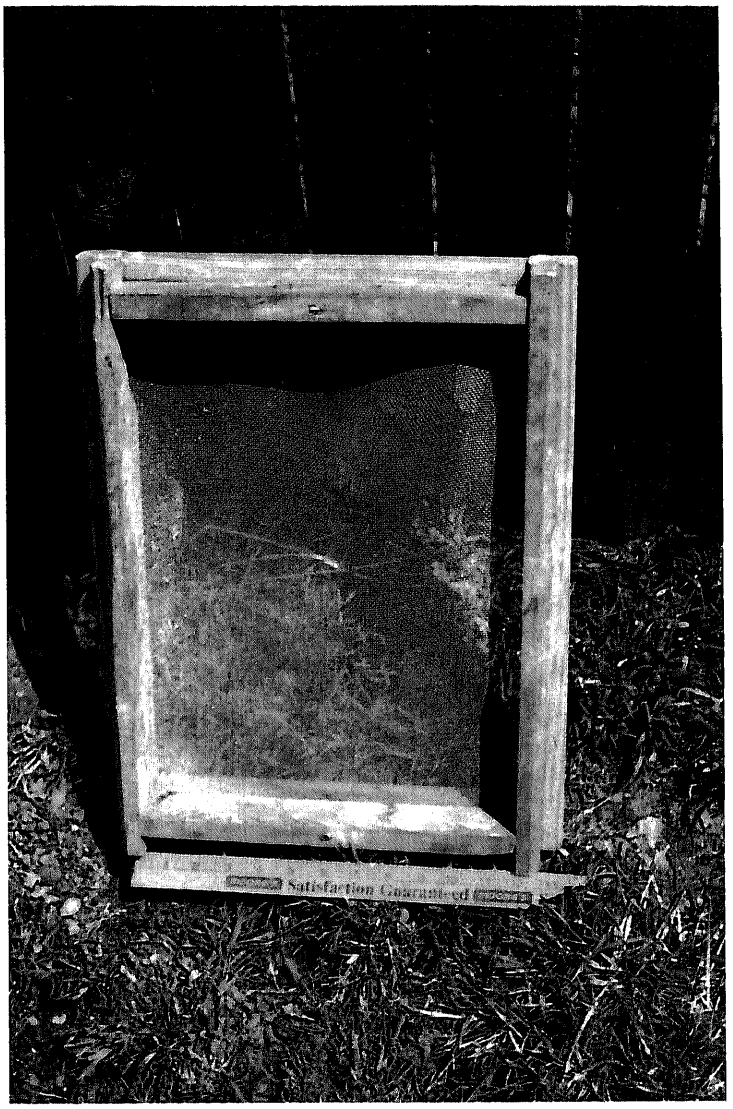
Table 6. Developmental stages of lab-reared *Hemidactylium* eggs according to Bishop (1918).

Stage	Apprximate age (days)	Description
1	2-3	Medullary folds well formed but no where approximated
2	2-3	Embryo defined in the yolk. Union of medullary folds marked by median depressed line.
3	4-5	Segregation of tissue showing indication of position, form, and extent of head, gill and body regions.
4	7-8	Limits of head well defined; gill region with large growth of tissue preliminary to actual development of gill and arm buds. Embryo folded upon itself.
5	9-10	Eyes highly pigmented; gill region much thickened and expanded laterally. Three primary divisions of body - head, trunk and tail recognizable.
6	13-14	Embryo slightly more advanced than that in stage 5. Tail distinct from trunk and constriction back of gill region more pronounced. Reduction of yolk mass evident in this and two preceding stages.
7	15-16	Gill and arm buds indicated by lateral depressions; head and tail distinct. Yolk mass confined to region of the lower abdomen.
8	21-22	Three gill buds on right side separate, two on left side and coalesced; arm buds well-formed. Pigment in irregularly sharped flecks confined to the back of the head, trunk and tail. Embryo active within the gelatinous envelopes of the egg. Eyes prominent with pupils well-developed.
9	23-24	Marked development of the gills with indications of fringes. Body compressed, back of the head and yolk confined to anal region of abdomen. Color pattern developing along sides of back and tail.
10	25-26	Gills completely developed and functional; hands with three fingers and legs with indications of developing toes. Color pattern brilliant; light and dark grays predominating with interspersed patches of yellowish green and orange.
11	31-32	Larvae just released from egg envelopes. Body and tail compressed and from above, wedge-shaped. Fingers fully developed; toes partly so. Larvae in this stage when placed in water swim freely.

solution, and returned to the lab morphological data collection. Aquatic specimens were captured with a dipnet and aquatic funnel traps. Post-embryonic larvae and transformed individuals were captured by hand under moss at the perimeter of each site. Total length, SVL, CW, and gill size were measured to the nearest 0.1 mm using a binocular dissecting microscope and an ocular micrometer. Average gill size (AG) was determined by measuring the length of all six gills and calculating the mean. A mean value was determined for each morphological character and plotted by date of collection.

One hundred and forty-one larvae were collected from all study sites during 1996. Post-embryonic larvae were collected by hand in the nests. Aquatic specimens were captured with dipnets, small aquarium nets, and aquatic funnel traps. On each visit, pools were sampled until at least 5 specimens were collected or until 20 sweeps with a dipnet and 40 sweeps with an aquarium net yielded no specimens. A screen filter (Fig. 20) was used to sift through the mud and collect specimens when the vernal sites were without standing water. All specimens were anesthetized using carbonated water and measured for TL, CW, and gill length to the nearest 0.1 mm using dial calipers. Gill length was measured as length of the longest gill on each side of the head using dial calipers and a hand lens. Larvae were revived in pond water and released at their original collection site. Due to different

Figure 20. Photograph showing screen filter used for collection of *Hemidactylium* larvae.


Satisfaction Guaranteed

collection techniques employed, 1995 and 1996 data sets were considered separate for statistical analyses. Transformed individuals were collected in 1995 only and were used with 1996 data to determine size at transformation and the length of the larval period.

Mean values for all morphological characters were evaluated using SAS (1980). All data grouped by date were subjected to a one-way ANOVA and mean values were plotted against each other to show significant differences in larval development over time. Significant morphological stages of development were extrapolated from the graph. Larval development data grouped by stage were then analyzed using a one-way ANOVA and Duncan's Multiple Range Test (DMRT) to reinforce the statistical significance of the first one-way ANOVA.

Results

Environmental Data

Nest temperature ranged from 5.5°C to 33.0°C ($\bar{x}$ = 18.1°C) and nest pH ranged from an acid 3.30 to a neutral 7.45 ($\bar{x}$ = 4.39). Larvae were collected in water ranging from 1.5 cm to 31.0 cm in depth ($\bar{x}$ = 20.4 cm). Larvae were captured either within 10 centimeters of land above decaying vegetation or underneath masses of *Sphagnum* which hung down in the water. Several larvae were collected in areas without water in 1995. These larvae were

found in the mud within 10 centimeters of the surface. After heavy rains, several larvae were obtained from a site that had been dry and searched thoroughly for specimens one week prior. These specimens were in a more advanced stage of development than larvae at other sites. Larvae survived in water pH ranging from 3.86 to 7.10 ($\bar{x}$ = 5.33). Water temperature ranged from 13.5°C to 28.0°C ($\bar{x}$ = 20.4°C). Figure 21 shows average temperature and pH over time correlated with important developmental events. Average pH remained relatively constant throughout development. Air, nest, and water temperature values were closely related and varied with each other. Hatching occurred during the first peak in temperature after egg deposition (June 7 - 20) and gill resorption occurred during the second peak (after July 21).

Egg Development

The earliest nests were observed on 23 April 1995 and 30 April 1996 and hatching occurred at all nests by 16 June 1995 and 20 June 1996 (Fig. 21). Length of the embryonic period was approximately 52 days for field-reared eggs. Figure 22 shows that development of lab-reared eggs required approximately 40 days for completion. Both lab and field reared eggs were laid at a mean diameter of 3.7 mm. Egg size increased dramatically over time in the field nests but only moderately in the lab nests (Figs 22, 23). Hatching began by 38 days in both the lab and field nests.

Figure 21. Environmental values recorded during *Hemidactylium* developmentt (1996 data only).

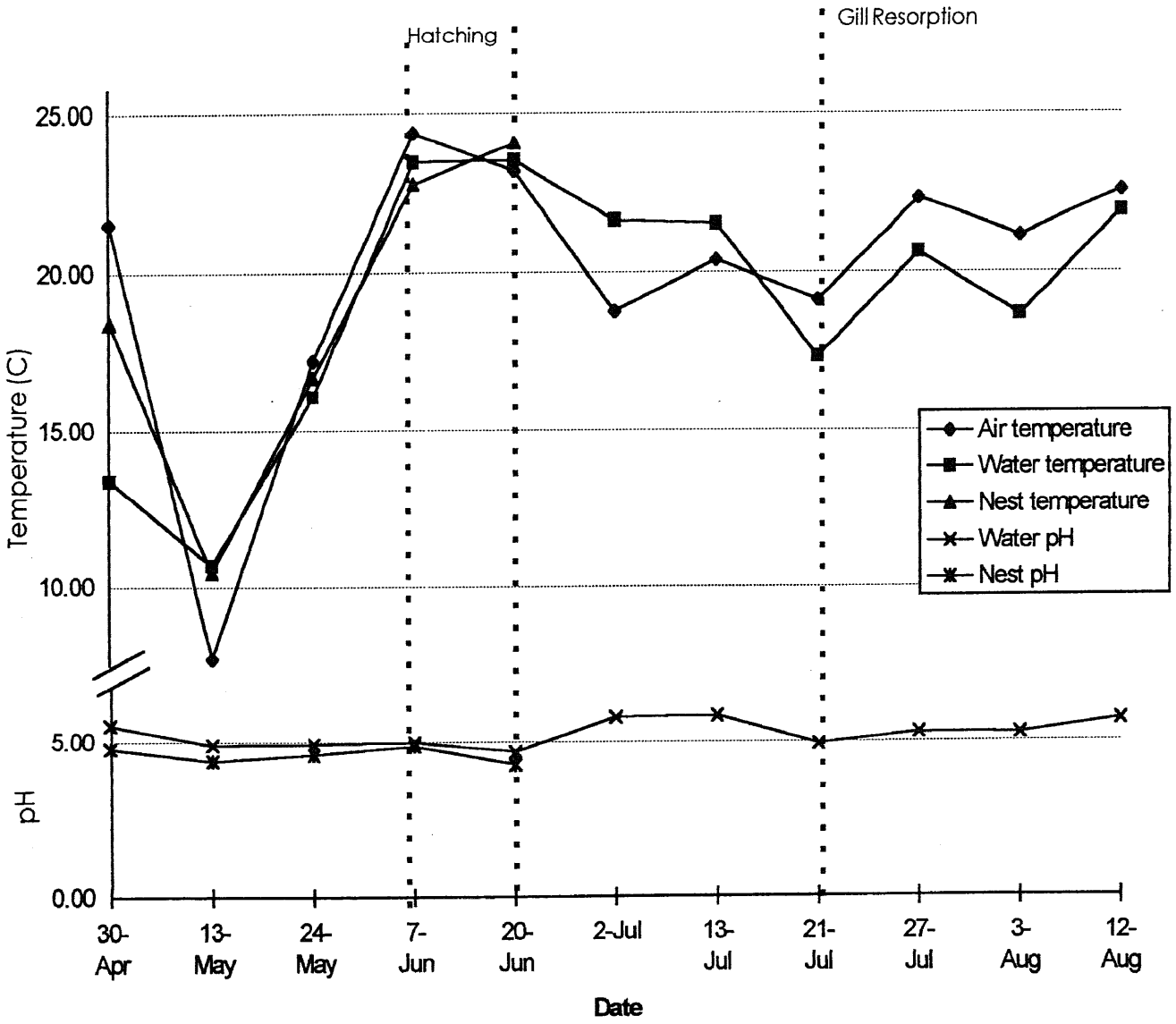

Hatching
Gill Resorption
25.00
20.00
15.00
10.00
5.00
0.00
Temperature (C)
pH
Air temperature
Water temperature
Nest temperature
Water pH
Nest pH
30-Apr
13-May
24-May
7-Jun
20-Jun
2-Jul
13-Jul
21-Jul
27-Jul
3-Aug
12-Aug
Date

Figure 22. Development of lab-reared *Hemidactylium* eggs.

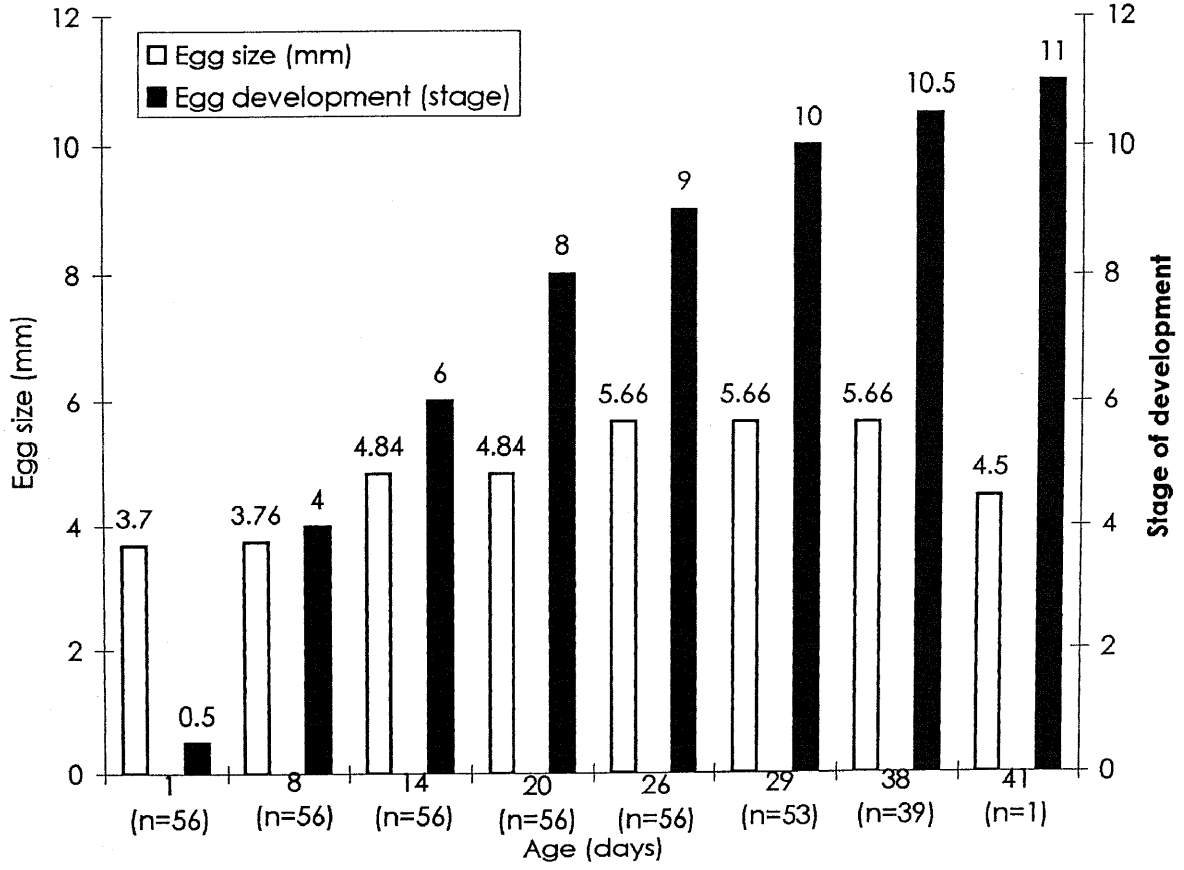
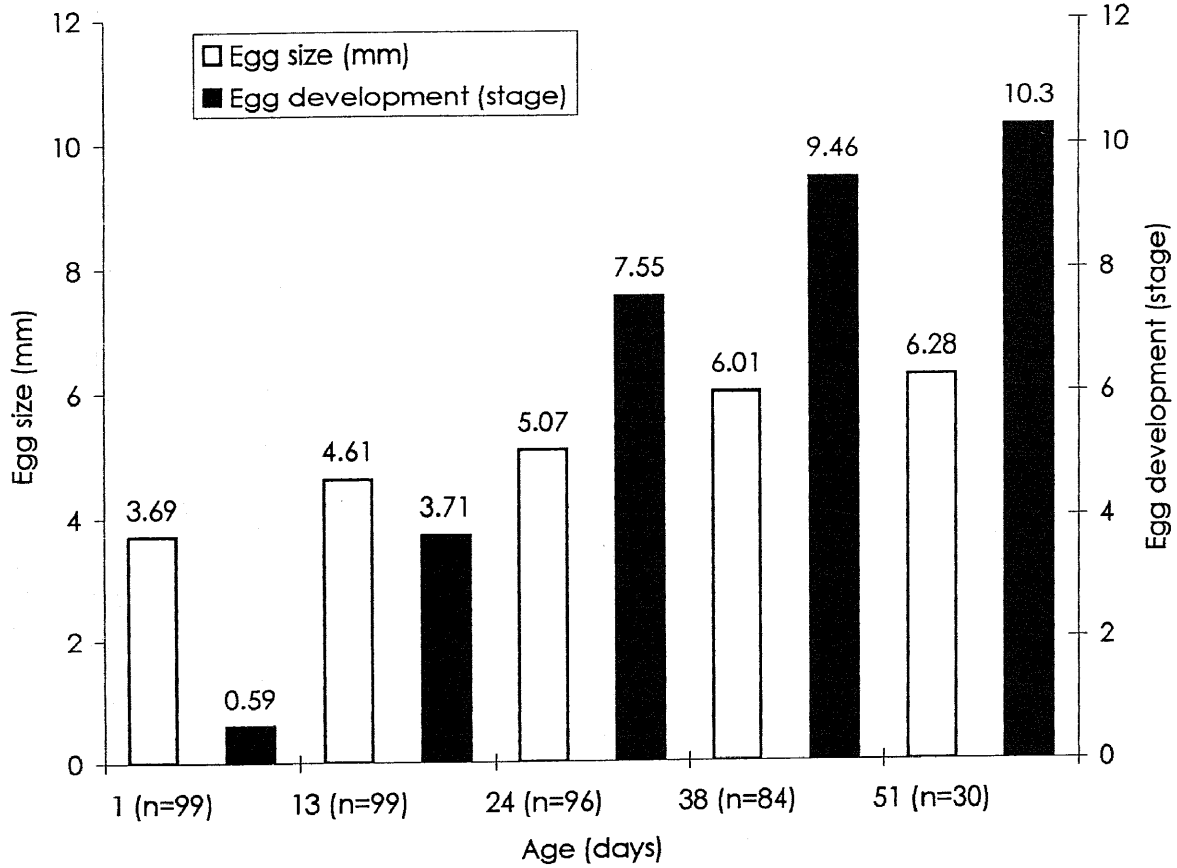

Egg size (mm)
Egg development (stage)
Egg size (mm)
Stage of development
Age (days)
3.7
0.5
3.76
4
4.84
6
4.84
8
5.66
9
5.66
10
5.66
10.5
4.5
11
1 (n=56)
8 (n=56)
14 (n=56)
20 (n=56)
26 (n=56)
29 (n=53)
38 (n=39)
41 (n=1)

Figure 23. Field development of *Hemidactylium* eggs (1996 only).


Egg size (mm)
Egg development (stage)
12
10
8
6
4
2
0
3.69
0.59
4.61
3.71
5.07
7.55
6.01
9.46
6.28
10.3
1 (n=99)
13 (n=99)
24 (n=96)
38 (n=84)
51 (n=30)
Age (days)
Egg size (mm)
Egg development (stage)

Larval Development

The first 1995 larvae were collected on 16 June 1995. Figure 24 shows 1995 larval development from time of hatching through transformation. Larvae hatched with a mean CW of 2.0 mm, a mean TL of 12.8 mm, a mean SVL of 8.9 mm, and an AG of 1.44 mm. Cranial width and SVL continued to increase through transformation. Significant gill reduction began 8 August 1995 and the gills were completely resorbed by 23 August 1995. Total length increased until gill resorption began, decreased sharply, and increased again after transformation was complete. Total transformation time in 1995 was 68 days.

Hatching began by 7 June 1996, the first 1996 larva was collected on 20 June 1996, and gill resorption began on 19 July 1996. No specimens were collected on 17 August 1996 suggesting that transformation had occurred. Transformation time in 1996 was 57 to 70 days.

Figure 25 shows 1996 larval development at all sites combined. Results from the ANOVA and DMRT ($p < 0.01$) suggested 3 distinct morphological stages in *Hemidactylium* larval development. Stage one was a post-embryonic period. Stage two was a growing period. Stage three represented gill resorption. A fourth stage, transformation, was observed in 1995 data.

Figure 24. Mean values for developmental stages of *Hemidactylium* larvae at Condon Run (1995 only).

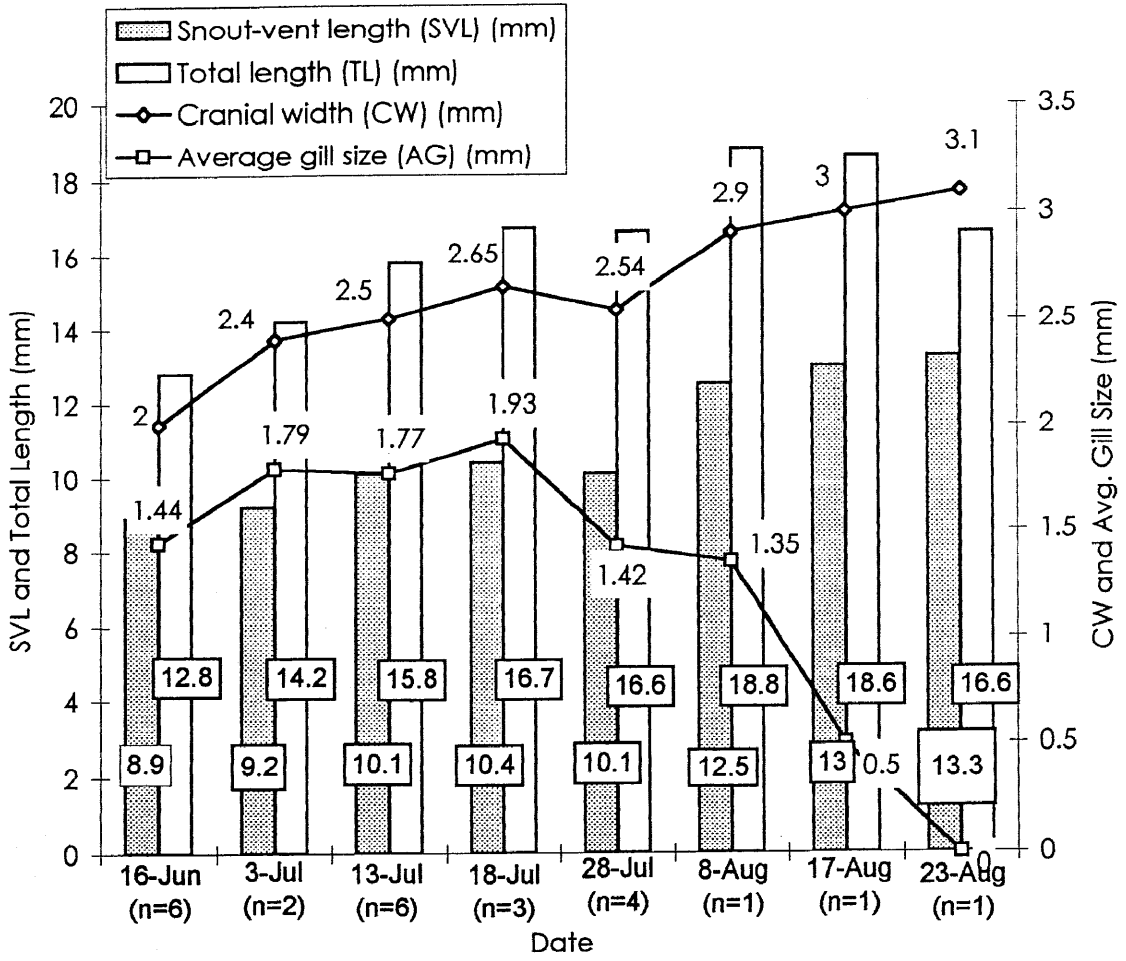
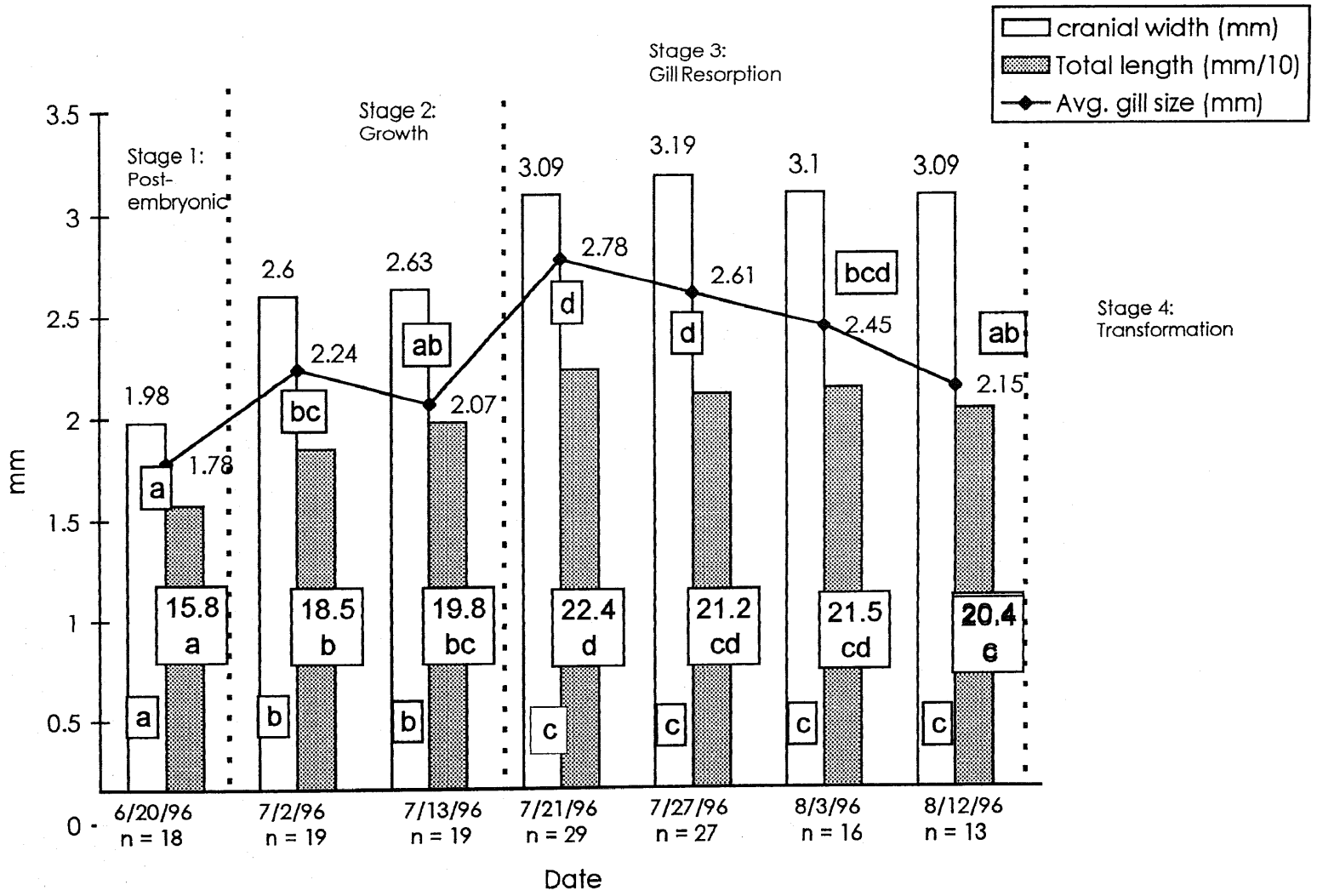

Snout-vent length (SVL) (mm)
Total length (TL) (mm)
Cranial width (CW) (mm)
Average gill size (AG) (mm)
SVL and Total Length (mm)
CW and Avg. Gill Size (mm)
Date
16-Jun (n=6)
3-Jul (n=2)
13-Jul (n=6)
18-Jul (n=3)
28-Jul (n=4)
8-Aug (n=1)
17-Aug (n=1)
23-Aug (n=1)
2
2.4
2.5
2.65
2.54
2.9
3
3.1
1.44
1.79
1.77
1.93
1.42
1.35
0.5
0
12.8
14.2
15.8
16.7
16.6
18.8
18.6
16.6
8.9
9.2
10.1
10.4
10.1
12.5
13
13.3

Figure 25. Mean morphological values for developmental stages of *Hemidactylium* larvae at all sites combined (1996 only).


cranial width (mm)
Total length (mm/10)
Avg. gill size (mm)
Stage 1: Post-embryonic
Stage 2: Growth
Stage 3: Gill Resorption
Stage 4: Transformation
mm
Date
3.5
3
2.5
2
1.5
1
0.5
0
1.98
2.6
2.63
3.09
3.19
3.1
3.09
1.78
2.24
2.07
2.78
2.61
2.45
2.15
a
bc
ab
d
d
bcd
ab
15.8 a
18.5 b
19.8 bc
22.4 d
21.2 cd
21.5 cd
20.4 e
a
b
b
c
c
c
c
6/20/96 n = 18
7/2/96 n = 19
7/13/96 n = 19
7/21/96 n = 29
7/27/96 n = 27
8/3/96 n = 16
8/12/96 n = 13

Discussion

My study provided the first detailed investigation of temperature and pH values associated with *Hemidactylium* development and showed that eggs and larvae developed in a wide range of pH values. Field observations revealed that eggs may be more tolerant than larvae to low pH conditions (see ch. III for more detailed information on acid tolerance) and that development is influenced by temperature. Hatching and gill resorption occurred during peaks in temperature which indicated that temperature was the main factor that affected *Hemidactylium* development.

Incubation required 38 field days in Michigan (Blanchard, 1923), 38 lab and 52 - 60 field days in New York (Bishop, 1918, 1941), 61 field days in Virginia (Wood, 1951), and 49 field days in West Virginia (Green, unpublished data). Incubation times of lab-reared and field-reared eggs in my study were comparable to those cited in the literature. *Hemidactylium* have a 50 to 60-day incubation period throughout their range. This period can vary seasonally with temperature and moisture (Blanchard, 1923; Bishop, 1941; Duellman and Trueb, 1986).

Lab-reared eggs were easily correlated with developmental stages defined by Bishop (1918). Differences noted in egg diameter during incubation of field and lab eggs can be explained by their different environments. Lab-reared eggs did not have the same opportunity as field-

reared eggs to absorb environmental moisture. My incubation data agreed with Bishop (1918, 1941) in that incubation time was longer for field-reared eggs as compared with lab-reared eggs. Field-reared eggs were subjected to a wide range of environmental conditions, and lab-reared eggs were kept in a stable environment. Duellman and Trueb (1986) stated that temperature and moisture affect length of incubation which explained developmental differences observed between lab and field nests.

Blanchard (1923) observed a 6-week larval period for *Hemidactylium* in southern Michigan. Duellman and Trueb (1986) cited this as the shortest larval period among salamanders. My research revealed a 9 - 10-week larval period in West Virginia. Duellman and Trueb (1986) stated that most pond type larvae, including *Hemidactylium,* have a sigmoidal growth curve. Wilder (1925) recognized the following 4 distinct morphological stages in development of *Eurycea bislineata* larvae: 1) post-embryonic stage; 2) typical feeding stage; 3)pre-metamorphic stage; and 4)metamorphic stage. Results in my study suggest similar stages in *Hemidactylium* larvae. Stage 1 began at hatching and probably lasted until larvae were in the water and acclimated to their new environment. Stage 2 was the period before gill resorption began and was marked by a sharp increase in all morphological features. Bishop (1918) stated that

Hemidactylium eggs show an early absorption of yolk which presupposes ability in the young larvae to take food independently soon after leaving the egg which supports the hypothesis that stage 2 represents a feeding stage where growth is maximal. Stage 3 marked the beginning of transformation characterized by minimal growth and gill resorption. Stage 4 included completion of transformation and resulted in fully transformed terrestrial individuals.

Morphological development in *Hemidactylium* eggs and larvae are closely related to that in *Eurycea bislineata* (Bishop, 1941). Brophy (1995) stated that incubation of *Eurycea bislineata* eggs occurred within 70 days and larval development took 1 - 3 years. Although the incubation and transformation times for *Eurycea bislineata* are greater than those for *Hemidactylium,* their similar developmental stages and larval skull morphology (Lombard and Wake, 1986; Rose, 1995) suggest a close phylogenetic relationship.

Summary

Hemidactylium development in West Virginia required 16 to 18 weeks for completion and was strongly influenced by temperature. A 7 to 8 week incubation period that began in mid-April was followed by a 9 to 10 week

larval period that ended in mid-August. Eggs were laid with a mean diameter of 3.7 mm and hatched with a mean diameter of 6.3 mm. Larvae averaged 8.9 mm SVL and 12 to 15 mm TL when they hatched and 13.3 mm SVL and 16.6 mm TL when they transformed. Larvae had the following 4 morphologically distinct developmental stages: 1) post-embryonic; 2) growth; 3) gill resorption; and 4)transformation. Eggs and larvae developed normally in both neutral and acid environments.

Chapter III. Acid Tolerance

Introduction

Researchers have noted that acidification of aquatic environments may be one cause for the worldwide decline in amphibian species. Studies on amphibian acid tolerance help the biological community better understand impacts of industrial acidification on the environment. *Hemidactylium* nests are often found in *Sphagnum* mosses (Bishop, 1918, 1941; Blanchard, 1923), which have a high capacity for water retention and a low pH (Conard and Redfearn, 1979; Smith, 1990). This suggests that *Hemidactylium* is tolerant of acid conditions. Wood (1951) is the only author that documents pH values for *Hemidactylium* nest sites. He sampled 5 sites and observed that eggs were laid adjacent to pools with an average water pH of 5.0. No long-term studies have documented the nature or degree of the relationship between *Hemidactylium* and low pH conditions.

Acid tolerance tests have been conducted on several amphibian species including *Rana sylvatica* and *Ambystoma maculatum*, and show that many species are fairly tolerant to acid conditions (Dale *et al.*, 1985; Pierce, 1985; Freda and Dunson, 1986; Ling *et al.*, 1986; Corn and Vertucci, 1992). This report attempts to define acid tolerance in *Hemidactylium* eggs and larvae. Morphological variance between field sites is noted in an attempt to

quantify effects of low pH on development.

Methods and Materials

Acid Tolerance

I tested *Rana sylvatica* embryos, *Hemidactylium* embryos and lab-reared and field-reared larvae in the laboratory to determine their acute tolerance to low pH conditions. *Rana sylvatica* were tested because their tolerance to acid conditions was well documented in the literature. The 96-hour Tl_m (median tolerance limit) test (A.P.H.A.I.,1965) was used as the measure of acute toxicity to low pH. This test was performed in triplicate for embryos and in duplicate for larvae to reduce experimental error. Fifty *Hemidactylium* embryos were collected from Moore Run and 60 *Rana sylvatica* embryos were collected from a small *Sphagnum* wetland near Otter Creek in the Otter Creek National Wilderness Area of the Monongahela National Forest for each test replication. *Rana sylvatica* embryos were collected during developmental stage 19 - 21 (Gosner, 1960) and *Hemidactylium* embryos were collected during developmental stage 8 - 9 (Bishop, 1918; Table 6) to ensure proper identification of death. Thirty-six larvae were collected from Moore Run Trail and 30 larvae were taken from laboratory collections for each replicate test. Field specimens were returned to the laboratory for

acclimation in a climate controlled environmental chamber over a 12-hr period. Temperature was held constant during each test, 20 to 21°C for embryos and 22.6 to 23.0°C for larvae. Different temperatures were used for embryos and larvae due to differences in ambient temperature at their collection sites. Animals were subjected to a 12:12 light:dark photoperiod.

Specimens were kept in 1 gallon glass bowls containing different pH values for 96 hours. *Hemidactylium* embryos were subjected to pH values of 1.0, 3.0, 3.5, and 4.0, *Hemidactylium* larvae were subjected to values of 1.85, 3.0, 3.5, 4.0, and 5.3, and *Rana sylvatica* embryos were subjected to pH values of 1.0, 3.0, 4.0, 5.0, and 6.4. These values were employed because each group of specimens was collected from a different ambient pH. Values of pH were established using dilutions of sulfuric acid. Every 4 hours during the test specimens were counted, pH was recorded and adjusted to compensate for fluctuations, and dead specimens were removed from the bowls. Readings showed that pH did not fluctuate more than 0.1 during the test.

Linear regression analyses were used to determine the pH value at which approximately 50 % of the specimens survived after 96 hours. Additional specimens were then tested at the calculated 50 percent survival value. Based on the initial regression analyses, *Hemidactylium* embryos were tested

at pH 2.25, *Rana sylvatica* embryos were tested at pH 3.6, and field-reared *Hemidactylium* larvae were tested at pH 3.3. This second test was not done for lab-reared larvae due to lack of specimens. Results from the second 96-hour TI_m were combined with those of the first and subjected to linear regression analyses to determine a more accurate value for 50 percent survival.

Field Development

Environmental and developmental data were collected during the 1996 field season (see Ch. II. Methods). These data were grouped by site and analyzed to ascertain developmental differences that occurred at sites with various pH values. Mean temperature and pH values were calculated for incubation and larval periods. Data were subjected to ANOVA and DMRT ($p < 0.01$). Embryonic development characteristics were grouped by date of observation and larval characteristics were grouped by developmental stage. Morphological data were subjected to ANOVA and NKMRT ($p < 0.05$) to show inter-site variance over time.

Results

Acid Tolerance

The 96-hour TI_m pH value was 3.51 for *Rana sylvatica* embryos (Fig. 26), while *Hemidactylium* embryos had a 96-hour TI_m pH value of 2.52 (Fig. 27).

Figures 28 and 29 show 96-hour Tl_m pH values of 3.67 and 3.45 for *Hemidactylium* lab-reared and field-reared larvae, respectively. Both *Rana sylvatica* and *Hemidactylium* embryos showed 100% survival at pH 4.0 and 100% mortality at pH 1.0. *Rana sylvatica* embryos had 100% mortality at pH 3.0, whereas *Hemidactylium* had 90% survival. Both lab-reared and field-reared *Hemidactylium* larvae showed 100% survival at pH 4.0 and 100% mortality at pH 3.0. At pH 3.5, field-reared larvae had 100% survival and lab-reared specimens showed only 16.7% survival. Table 7 lists embryonic mortality levels for several amphibian species based on accounts from literature.

Embryonic Development

Figure 30 shows results from analyses of environmental data collected during the incubation period. Moore Run (MT) had a significantly higher mean nest temperature than Bickle Knob (BK). There was no significant difference between mean temperature of nests from Condon Run (CR) and those from other sites. Bickle Knob had a significantly greater mean nest pH than CR , which had a significantly greater mean nest pH than MT. Table 8 lists the range for pH and temperature values recorded during the incubation and larval periods. The lowest nest pH (3.3) and highest nest temperature (33.0 °C) occurred at MT, while BK had the highest nest pH

Figure 26. Regression analysis for *Rana sylvatica* embryos.

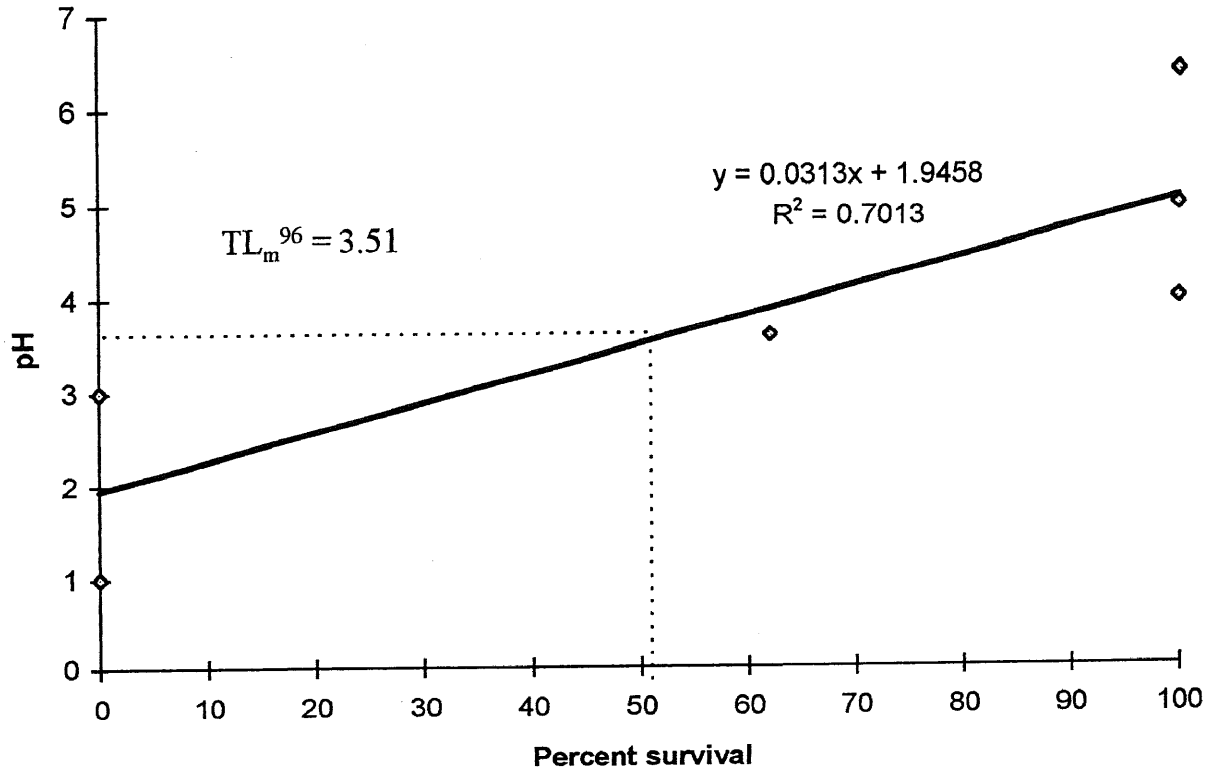

7
6
5
4
3
2
1
0
pH
y = 0.0313x + 1.9458
R2 = 0.7013
TLm96 = 3.51
0
10
20
30
40
50
60
70
80
90
100
Percent survival

Figure 27. Regression analysis for *Hemidactylium* embryos.

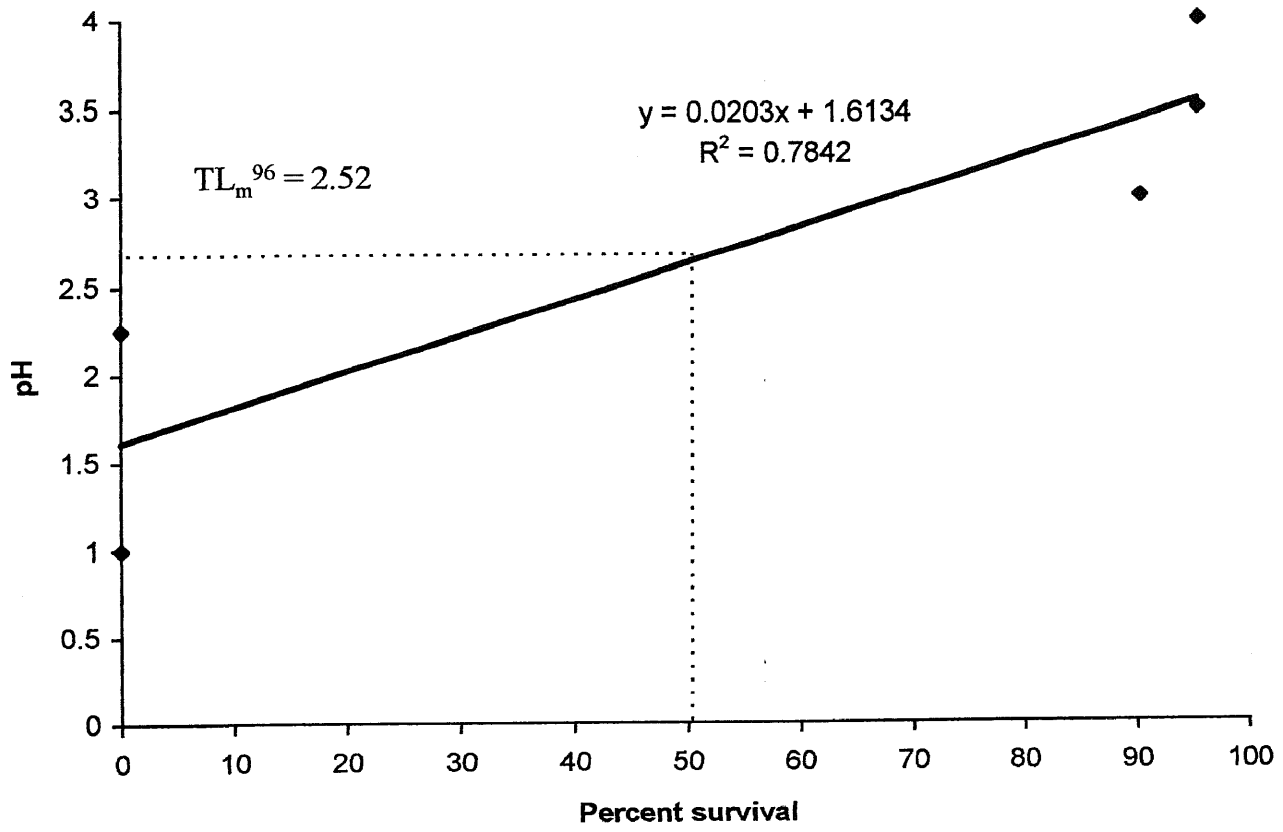

y = 0.0203x + 1.6134
R² = 0.7842
TLm96 = 2.52
pH
Percent survival
0
0.5
1
1.5
2
2.5
3
3.5
4
0
10
20
30
40
50
60
70
80
90
100

Figure 28. Regression analysis for lab-reared *Hemidactylium* larvae.

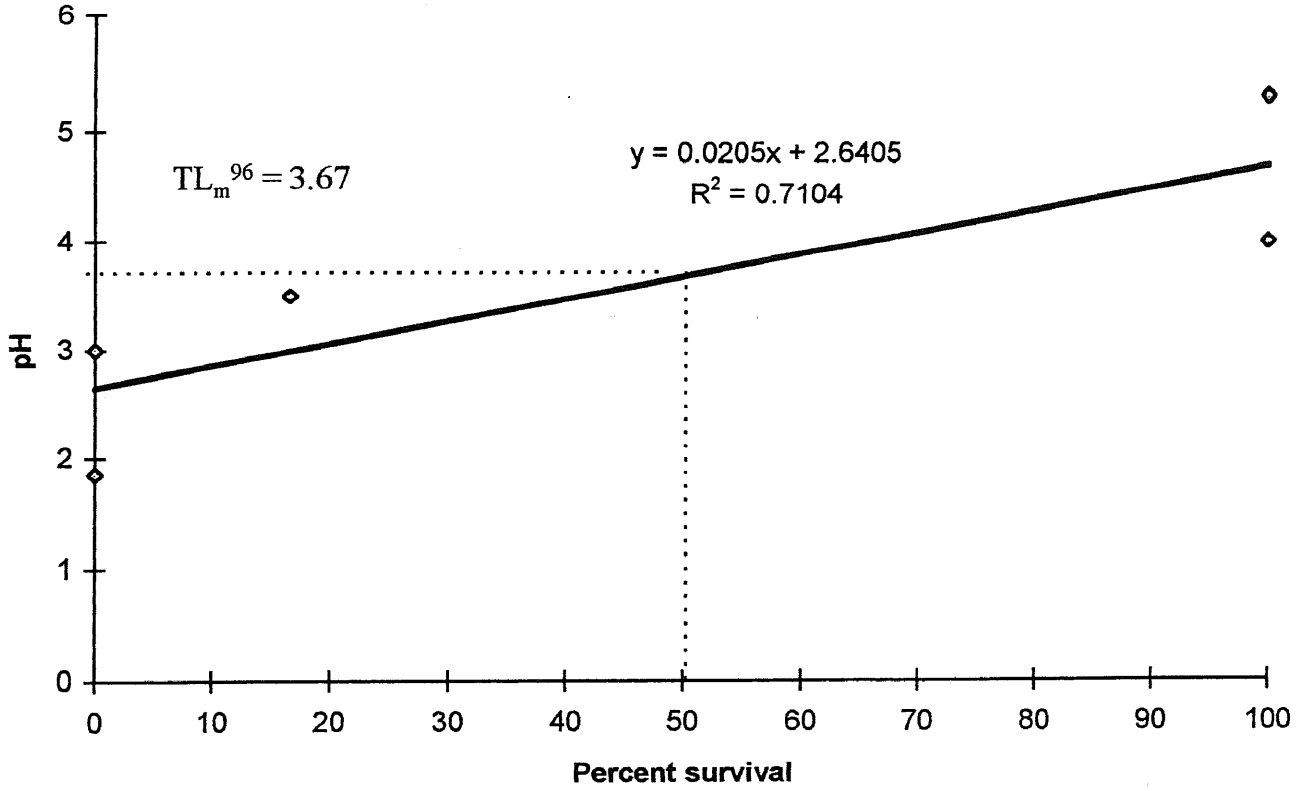

6
5
4
3
2
1
0
pH
$TL_m^{96} = 3.67$
y = 0.0205x + 2.6405
R^2 = 0.7104
0
10
20
30
40
50
60
70
80
90
100
Percent survival

Figure 29. Regression analysis for field-reared *Hemidactylium* larvae.

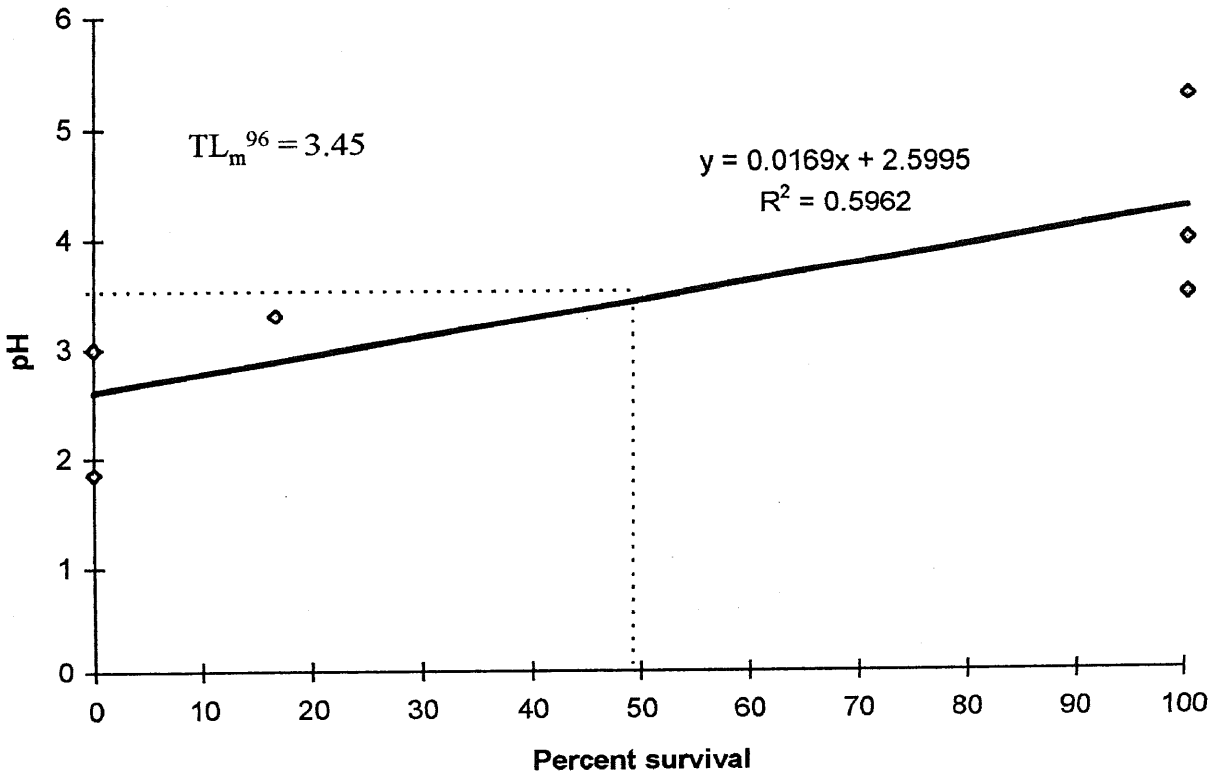

$TL_m^{96} = 3.45$
y = 0.0169x + 2.5995
R^2 = 0.5962
pH
Percent survival
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80
90
100

Table 7. Acid tolerance of amphibian embryos in acidified medium (modified from Pierce, 1985).

Species	pH		References*
	100% mortality	50% mortality	
FROGS			
Bufo a. americanus	3.5 - 3.9	4.0 - 4.5	6
Acris gryllus	4.1	4.2 - 4.6	1
Hyla andersoni	3.4	3.6 - 3.8	1
Hyla versicolor	3.8	4.0 - 4.2	1
Pseudacris c. crucifer	3.8	3.9 - 4.3	1
Pseudacris nigrita	3.8	3.9 - 4.1	1
Rana catesbeiana	3.9	4.1 - 4.3	1
Rana clamitans	3.8	3.8 - 4.1	1
Rana palustris	4.0	4.2 - 4.4	1
Rana pipiens	5.0	6.0	1
Rana sylvatica	? - 3.5	3.5 - 3.9	1,4,5,6
Rana sylvatica	3.0	3.51	This study
Rana sphenocephala	3.7	3.9 - 4.1	1
Rana virgatipes	3.4	3.6 - 3.8	1
Xenopus laevis	3.0 - 3.9	3.5 - 4.0	4,7
SALAMANDERS			
Ambystoma jeffersonianum	4.0 - 5.0	4.0 - 6.0	2,3
Ambystoma maculatum	4.0 - 5.0	5.0 - 7.0	2,3
Hemidactylium scutatum	2.25	2.52	This study

* References: (1) Gosner and Black 1957, (2) Pough and Wilson 1977, (3) Cook 1983, (4) Tome and Pough 1982, (5) Pierce et al. 1984, (6) Karns 1983, (7) Dunson and

Figure 30. Mean temperature and pH values collected during the 1996 incubation period.

Unlike letters represent significant differences between sites. Variance is ranked alphabetically from highest mean value to lowest.

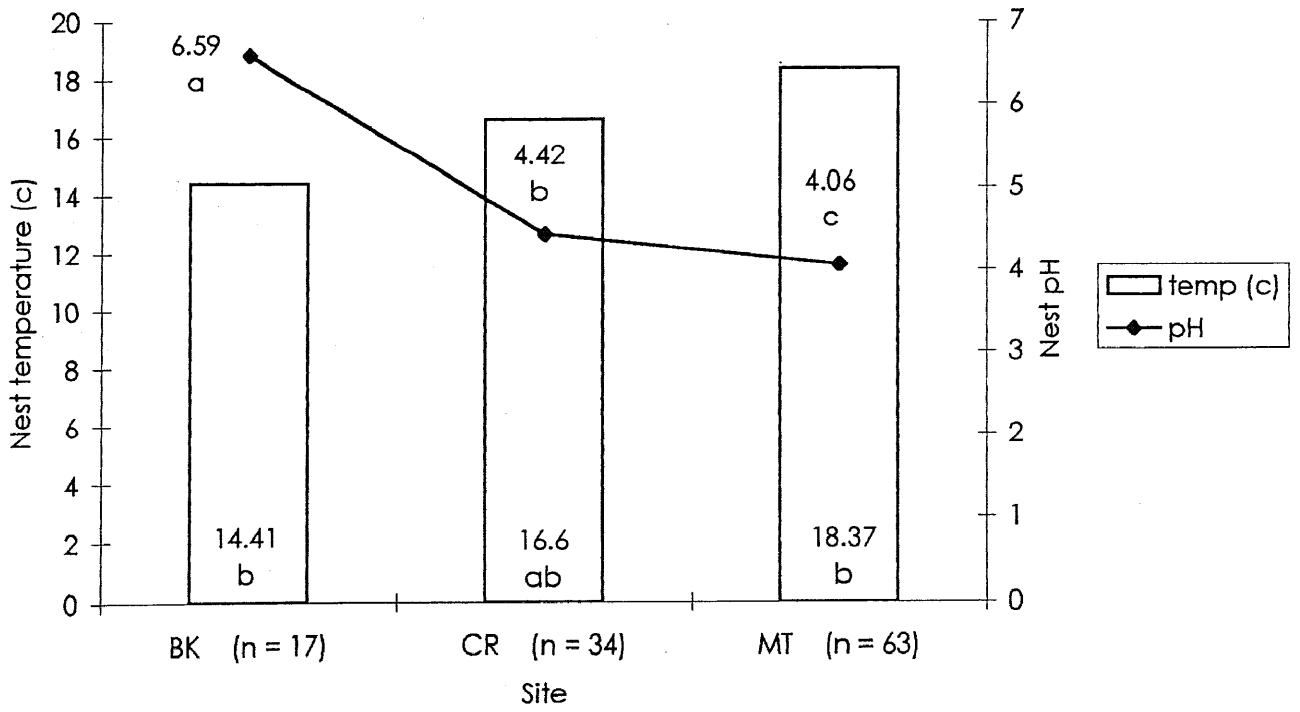

Nest temperature (c)
Nest pH
Site
BK (n = 17)
CR (n = 34)
MT (n = 63)
6.59
a
4.42
b
4.06
c
14.41
b
16.6
ab
18.37
b
temp (c)
pH
0
2
4
6
8
10
12
14
16
18
20
0
1
2
3
4
5
6
7

Table 8. Minimum, mean, and maximum values for environmental parameters collected during *Hemidactylium* development (1996 only).

Incubation Period

Site	Nest pH			Nest temperature (°c)		
	minimum	mean	maximum	minimum	mean	maximum
BK (n = 17)	5.60	6.59	7.45	5.5	14.4	25.0
CR (n = 34)	3.90	4.42	5.25	8.0	16.6	25.5
MT (n = 63)	3.30	4.06	5.20	7.5	18.4	33.0

Larval Period

Site	Water pH			Water temperature (°c)		
	minimum	mean	maximum	minimum	mean	maximum
BK (n = 42)	6.03	6.65	7.10	16.5	19.6	23.0
CR (n = 30)	4.4	5.01	6.05	13.5	15.8	22.0
MT (n = 68)	3.86	4.64	5.30	15.5	23.0	28.0

(7.45) and lowest nest temperature (5.5°C).

Table 9 shows embryonic development at each site over time. Egg diameter was generally smallest at BK and greatest at MT. Eggs at BK developed slower than those at other sites, but began hatching at a similar developmental stage. All nests at BK had hatched before those at other sites. Eggs at CR were at a significantly later stage of development than the other sites until hatching began, but it took longer for all eggs to hatch. Moore Run embryos developed faster than those at other sites as evidenced by the fact that they were laid later, and began hatching at the same time and in a similar stage of development.

Larval Development

Figure 31 displays results from analyses of environmental data collected during the larval period. Data analyzed by ANOVA and DMRT showed significant variance between the means of all 3 sites ($p < 0.01$) for pH and temperature values. Moore Run had the lowest mean water pH (4.64) followed by CR (5.01) and BK (6.65). Condon Run had the lowest mean water temperature (15.8°C) followed by BK (19.6°C) and MT (23.0°C). Table 8 lists ranges for environmental parameters collected during the *Hemidactylium* larval period. The lowest water pH (3.86) and highest temperature (28.0 °C) occurred at MT. The highest water pH (7.10) occurred at BK. Condon Run had the lowest water temperature (13.5°C).

Table 9. Morphological characters for *Hemidactylium* embryos by site and date of observation. Unlike letters represent significant differences between mean values of sites for each date observed. Variance is ranked alphabetically from highest mea value to lowest.

Date	Egg diameter (mm)			Mean developmental stage		
	BK	CR	MT	BK	CR	MT
04/30/96	3.4 ± 0.20 (n=6) b	3.7 ± 0.33 (n=27) a	no nests observed	0.5 ± 0.001 (n=6) b	1.0 ± 0.01 (n=27) a	no nests observed
05/13/96	4.7 ± 0.38 (n=15) ab	4.8 ± 0.42 (n=27) a	4.5 ± 0.65 (n=57) b	3.6 ± 1.55 (n=15) b	5.7 ± 1.18 (n=27) a	2.8 ± 2.05 (n=57) b
05/24/96	4.3 ± 0.34 (n=15) b	5.2 ± 0.56 (n=24) a	5.2 ± 0.83 (n=57) a	6.6 ± 0.76 (n=15) c	8.1 ± 0.34 (n=24) a	7.6 ± 0.90 (n=57) b
06/07/96	5.3 ± 0.45 (n=15) b	5.5 ± 0.50 (n=18) b	6.4 ± 0.51 (n=51) a	9.4 ± 0.51 (n=15) a	9.8 ± 0.77 (n=18) a	9.4 ± 0.68 (n=51) a
06/19/96	no nests ovbserved	5.9 ± 0.33 (n=6) b	6.4 ± 0.39 (n=24) a	no nests observed	9.8 ± 0.27 (n=6) b	10.4 ± 0.4 (n=24) a

Figure 31. Mean temperature and pH values collected during the 1996 larval period.

Unlike letters represent significant differences between sites. Variance is ranked alphabetically from highest mean value to lowest.

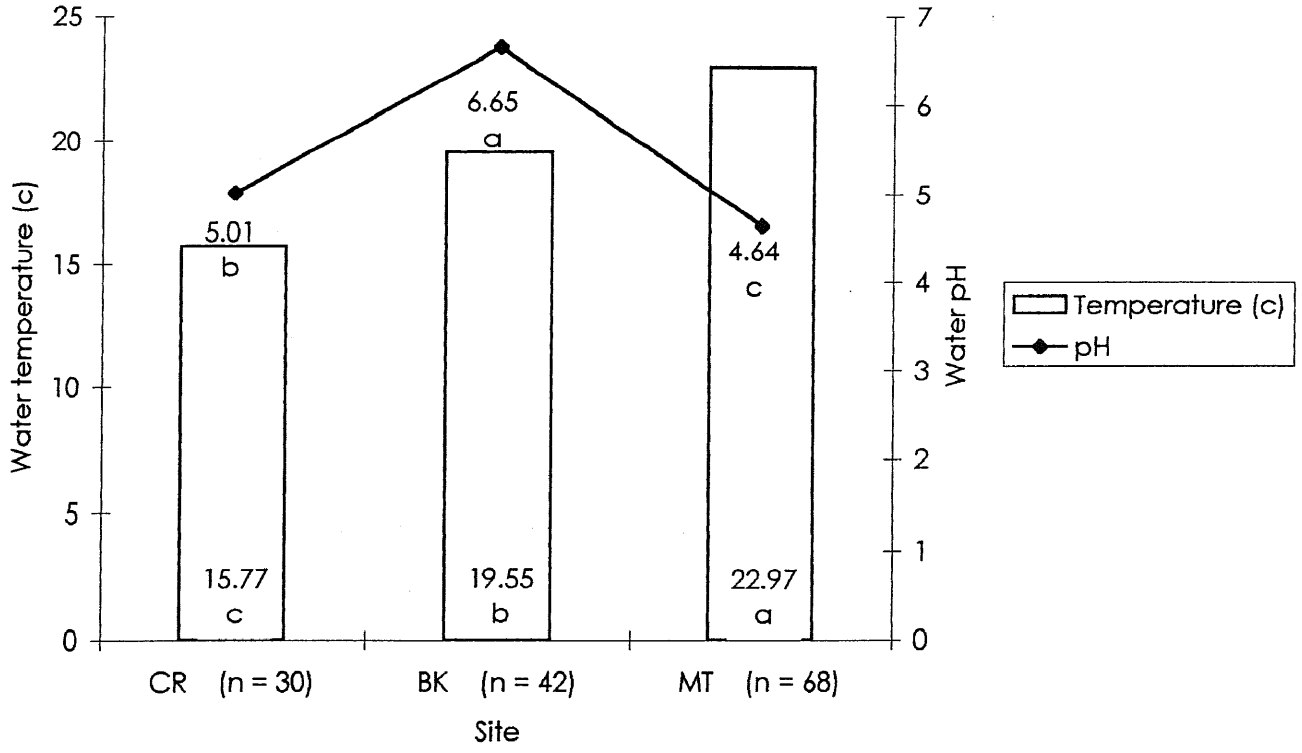

25
20
15
10
5
0
Water temperature (c)
7
6
5
4
3
2
1
0
Water pH
6.65
a
5.01
b
4.64
c
15.77
c
19.55
b
22.97
a
Temperature (c)
pH
CR (n = 30)
BK (n = 42)
MT (n = 68)
Site

Table 10 shows development at all sites during the larval period. Analysis of post-embryonic stage (1) data revealed that MT specimens had more advanced gills when they hatched. Condon Run larvae hatched at a smaller mean TL than specimens from other sites. Condon Run larvae had a smaller mean CW and MT specimens had a larger mean AG during growth stage (2). Analysis of gill resorption stage (3) data showed no significant variance in morphology between the means of all sites.

Discussion

Acid Tolerance

Amphibian larvae are reported to be more acid tolerant than embryos (Gosner and Black, 1957; Pough, 1976; Saber and Dunson, 1978; Pierce *et al.*, 1984; Clark and LaZerte, 1985; Pierce, 1985; Clark, 1986). This was not the case in my study. *Hemidactylium* embryos were more acid tolerant than both lab-reared and field-reared larvae. Tolerance to environmental conditions is generally related to acclimatization (Smith, 1990; Laws, 1993). Some studies have suggested that amphibians reared in acid environments are generally more acid tolerant than those reared under neutral conditions (Gosner and Black, 1957; Cook, 1983). *Hemidactylium* embryos and field-reared larvae were collected from the same sites, but not from the same ambient pH values. Mean ambient nest pH at Moore Run was 4.06 and

Table 10. Morphological characters for *Hemidactylium* larvae by site and stage of development. Unlike letters represent significant differences between mean values of sites for each date observed. Variance is ranked alphabetically from highest mean value to lowest.

Stage	Cranial Width (mm)			Total Length (mm)			Average gill length (mm)		
	BK	CR	MT	BK	CR	MT	BK	CR	MT
Stage 1: Post-embryonic	1.75 ± 0.37 (n=4) a	1.91 ± 0.23 (n=7) a	2.09 ± 0.19 (n=13) a	15.3 ± 1.80 (n=4) a	12.9 ± 0.68 (n=7) b	16.0 ± 2.05 (n=13) a	1.58 ± 0.29 (n=4) b	1.41 ± 0.19 (n=7) b	1.89 ± 0.28 (n=13) a
Stage 2: Growth	2.68 ± 0.40 (n=14) a	2.29 ± 0.46 (n=7) b	2.70 ± 0.19 (n=17) a	19.6 ± 2.15 (n=14) a	17.9 ± 3.80 (n=7) a	19.3 ± 1.58 (n=17) a	1.97 ± 0.35 (n=14) b	1.86 ± 0.45 (n=7) b	2.43 ± 0.49 (n=17) a
Stage 3: Resorption of gills	3.10 ± 0.37 (n=25) a	3.13 ± 0.36 (n=22) a	3.12 ± 0.27 (n=33) a	21.6 ± 2.62 (n=25) a	21.1 ± 2.50 (n=22) a	21.6 ± 1.75 (n=33) a	2.65 ± 0.67 (n=25) a	2.30 ± 0.62 (n=22) a	2.65 ± 0.39 (n=33) a
Stage 4: Transformation (n = 0)	-	-	-	-	-	-	0	0	0

mean water pH was 4.64. *Hemidactylium* eggs may have evolved a greater acid tolerance than larvae because they are laid in more acidic environments.

Pierce and Harvey (1987) demonstrated that embryonic acid tolerance was not correlated with habitat acidity, however larval acid tolerance was correlated with habitat acidity. They showed that larvae whose parents came from an acidic environment were more tolerant to acid, suggesting that larval tolerance is associated with genetics. Gravid females were collected from BK (pH = 6.65) in my study and their eggs and larvae were reared under neutral laboratory conditions. Field-reared larvae were collected from MT (pH = 4.64). Results from regression analyses in my study showed little difference between field-reared and lab-reared larvae. However, the raw data suggests that field-reared specimens were more tolerant to acid conditions than lab-reared specimens. Both groups had 100 percent survival at pH 4.0 and 100 percent mortality at pH 3.0. At pH 3.5 there was a noticeable difference between groups, 100 percent survival for field reared larvae and only 16 percent survival for lab-reared larvae. These results suggest both a critical lethal limit at pH 3.0 for *Hemidactylium* larvae and the adaptation of some populations to acidic environments. Although lab-reared specimens were laid and reared in the laboratory, it is possible that stress due to captivity influenced the results of my study.

Geographically diverse studies have suggested that *Rana sylvatica* demonstrate a generally high tolerance to acidic environments (Gosner and Black, 1957; Dale *et al.*, 1985; Freda and Dunson, 1986; Pierce and Harvey, 1987). Results for *Rana sylvatica* embryos in West Virginia are similar to those reported in the literature (Gosner and Black, 1957; Tome and Pough, 1982; Karns, 1983; Pierce *et al.*, 1984). A comparison of my results with those listed in Table 7 suggests that *Hemidactylium* is more tolerant to low pH conditions than other amphibian species. This high level of tolerance is probably a result of long-term exposure to acid conditions. Several studies have examined geographic and genetic variation in amphibian acid tolerance (Pough, 1976; Pough and Wilson, 1977; Cook, 1983; Pierce and Sikand, 1985; Pierce and Harvey, 1987) and have shown that embryonic acid tolerance is not directly influenced by genetics. However, maternal factors such as size or composition of the egg sacs may influence acid tolerance (Pierce and Sikand, 1985). There is a strong, but unexplained tendency for *Hemidactylium* females to have communal nests and remain in attendance with embryos through hatching (Blanchard, 1934b; Bishop, 1941; Wood, 1951; Harris and Gill, 1980; Breitenbach, 1982; Harris *et al*, 1995). Whether or not this behavior influences embryonic acid tolerance can not be answered from my results as the study was not designed to address communal nesting behavior.

Preliminary observations in my study showed that BK, the only site with a neutral pH value, was also the only site without communal nests.

It is not clear how much of the observed variation in acid tolerance between my study and others listed in Table 7 is due to actual physiological factors and how much is due to differences in experimental design. *Hemidactylium* habitat is often associated with acid environments (Bishop, 1918, 1941; Blanchard, 1923,1931; Green and Pauley, 1987; Conant and Collins, 1991). It is reasonable to assume that they are well adapted to low pH conditions. Grant (1955) suggested that *Hemidactylium* were less aggressive than other plethodontid salamanders when defending their territory. *Hemidactylium* may have a selective advantage in their use of acidic environments that may provide a refuge from competitive and predatory factors present in more accessible habitats.

Development

Research has noted that amphibian eggs shrink when exposed to acidic environments (Pough, 1976; Pough, 1977; Dunson and Connell, 1982; Pierce, 1985). In my study, this was not the case for *Hemidactylium* development in acid environments. Bickle Knob had the highest pH values and the smallest egg sizes. Differences observed in egg diameter in my study can be accounted for by examination of moisture levels at each site. Moore Run

eggs were laid in a wet habitat in *Sphagnum* moss substrate. Bickle Knob eggs were laid surrounding a vernal pool in non-*Sphagnum* moss substrate. Embryo diameter was the same for all field and lab nests during each stage of development. This further supports moisture as the cause of inter-site differences of egg diameter.

The role of pH in *Hemidactylium* embryo development remains questionable. Research on chronic effects of acid on amphibian development suggest that development is inhibited and often terminated as a result of low pH conditions (Pough and Wilson, 1977; Dunson and Connell, 1982; Pierce *et al.*, 1984). Results from my study showed that embryonic development was completed at all sites studied. Bickle Knob eggs were laid in a neutral environment and showed the slowest development. Moore Run eggs were laid in an acidic environment and showed the fastest development. Condon Run eggs were between the other 2 sites in both habitat acidity and developmental rates. These results might suggest that pH plays an important role in *Hemidactylium* embryonic development. However, a closer look reveals that development is probably more strongly affected by other factors. Temperature and moisture are 2 key factors that influence incubation time in amphibians (Bishop, 1918; Blanchard, 1923; Duellman and Trueb, 1986; Green and Pauley, 1987). Bickle

Knob had the lowest mean nest temperature and its substrates had a lower capacity for water retention than the other sites. It must be more than coincidence that BK also had the slowest development during incubation. Moore Run, which showed the fastest development, had the highest mean nest temperatures and its substrate had a high capacity for water retention. Several distinct deformations, including curling of the embryo, shrinking of the perivitelline space and hardening of the egg capsule, are associated with development in acidic conditions (Pough, 1976; Pough and Wilson, 1977; Dunson and Connell, 1982; Pierce *et al.*, 1984;). Embryos measured in the field in my study showed no distinct signs of acid related deformations as noted in the literature. This suggests that *Hemidactylium* embryos are either not affected in the same manner as other amphibians or were not suffering stress related to ambient pH conditions. Normal development is suggested by the fact that incubation time was similar at all sites and comparable to reports from the literature (Bishop, 1918, 1941).

It is interesting that MT larvae hatched at a slightly more advanced stage and developed faster than specimens from other sites. This was the only site where large numbers of other species including *Ambystoma maculatum* and *Rana sylvatica* were captured. Perhaps increased competition and predation played a significant role in development (Duellman and Trueb,

1986). Transformation time was similar at all sites. Results suggested that although environmental factors contributed to developmental rates, they did not affect the overall length of transformation. Results were similar to those from embryonic development in that sites with highest temperatures and lowest pH had the fastest developmental rates. Once again, the role of pH is obscured by other environmental factors. Research suggested that larval development was slowed when specimens were reared in acid environments (Pough, 1976; Freda and Dunson, 1984, 1985; Clark and LaZerte, 1985; Karns, 1992;). Although no accurate developmental rates for *Hemidactylium* were available for comparison, larvae observed in the field did not appear stressed from exposure to acid conditions. Freda and Dunson (1984, 1985) have determined that interspecific variation in larval acid tolerance is related to differences in body sodium content. Larvae with lower body sodium content are more acid tolerant because they lose less sodium when placed in an acid environment.

It is difficult to determine how pH affects *Hemidactylium* development in field situations. It may contribute to differences between sites, but it is probably not the main factor influencing development. These data are based on 1 season of field observations. As ambient conditions change from year to year it is unlikely that these data represent the full scope of

developmental variation in *Hemidactylium.* Environmental factors not measured such as aluminum concentration and other water chemistry elements, small sample sizes, and study design may have affected the results. These data are intended to present an introduction to *Hemidactylium* development in acid environments and provide a basis for future experiments. Detailed studies should be performed under laboratory conditions to determine chronic effects of pH on *Hemidactylium* development.

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