1999

Ecology and natural history of Rana clamitans melanota in West Virginia

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Ecology and Natural History of *Rana clamitans melanota* in West Virginia

Thesis submitted to
The Graduate School of
Marshall University

In partial fulfillment of the
Requirements for the Degree of
Master of Science
Biological Sciences

by

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March 5, 1999

This thesis was accepted on 20 January 1999

as meeting the research requirements for the master's degree.

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Acknowledgments

I wish to thank my advisor, Dr. Thomas Pauley, for the opportunity to learn from him. He has been a great instructor and friend. My committee members Drs. Donald Tarter, Dan Evans, and Mark Ford, have made a significant contribution to my research through their critical review of the manuscript. Additionally, I would like to thank Dr. Suzanne Strait whose suggestions, support, and friendship have been a tremendous help.

Thanks to my lab mates for their help in the field, and in the laboratory. I especially want to thank Sandie Kilpatrick, Jennifer Piascik, and Beth Pauley for their camaraderie and encouragement. Special thanks to Bart Paxton for helping me chisel through rock to get those pitfall traps into the ground. Brian Lindley deserves special recognition for helping me measure at least half of the 2800 tadpoles that were captured in 1997. Finally, without Paul Marcum, Sandy Raimondo, and Shawn Clark of the West Virginia Department of Agriculture, I would still be keying the contents of froglet stomachs.

My family, especially my mom and dad, deserve a heart felt thanks for their support and encouragement during the course of my graduate work. I know that everyone is very proud of my accomplishment. Special thanks to my sister, Alisha Rogers, and Paul Marcum for being there through some very tough times.

I collectively thank Pam Bailey, Kecia Gerlach, Lisa Gatens, Sonya and Scott Jones, Chris Gatens, Greg Hunter, Warren Reuschel, and Brian Cottrill. All of you have made me laugh when the pressure got the best of me!

I want to thank the Westvaco Corporation, the Department of Biological Sciences at Marshall University, and the Marshall University Graduate Student Council Research Committee for partial funding of my research. All animals were collected under scientific collection permit number 86-1997 issued by the West Virginia Division of Natural Resources.

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Abstract

Fourteen study sites were sampled with aquatic funnel traps and D-frame net sweeps in the plateau and mountain region of West Virginia to determine differences in larval period. Larval stage class abundance and larval total length and mass measurements were recorded from February to December 1997. Larval stage classes 25 and 26, the first free living stages, were absent from the plateau region from February to April, but were present in the mountain region. Pre-transformation larval stages, 40 through 44, were found from May through October, and peaked in July. Significant relationships were found with simple linear regression analysis between larval stage and total length, and larval stage and mass during all months except for the summer period (May to August). Stage class abundance shifted from stages 27 and 34 in the May/June data set to stages 25 and 46 in the July/August data set. Breeding activity in this species was found to be a month later in the mountain regions than previously reported. Data suggests that in the mountain regions, this species may have a two year larval period. Additionally, froglet dispersal began in June, and peaked in August. Changes in stomach contents over a two month period suggest dietary shifts in froglets due to prey availability.

Water pH and water temperature data were analyzed for differences in elevation, habitat, and monthly groups with a Kruskal-Wallis ANOVA ($P<0.05$). In spring (March and April) water pH was significantly lower at high elevation sites (4000ft) and in smaller habitat types (roadside ditch, oxbow, and temporary pool). Water pH results during the summer (May to August) and autumn (September and October) periods showed no clear trend in elevation and habitat groups. Water temperature results were significantly lower in the oxbow during the summer and autumn periods. Significant differences in water temperature between the months of May and June, July and August, and September and October show the activity of *R. c. melanota* is affected by water temperature.

Introduction

Frogs are the most successful extant amphibians, with over 4000 known species which occupy broad geographical ranges (Stebbins and Cohen 1995). All frogs species are in Superorder Salientia, and extant frog species are in Order Anura (Duellman and Trueb 1994). Natural history studies are necessary to understand the ecological and reproductive diversity within this large amphibian group (Zug 1993; Duellman and Trueb 1994).

This study focuses on the natural history of the green frog, *Rana clamitans*. *Rana clamitans* is a member of family Ranidae and subfamily Raninae (Duellman and Trueb 1994). The genus *Rana* is composed of over 200 species, and is the only genus of Ranidae that occurs in North America (Conant and Collins 1991; Zug 1993). Most ranid frogs exhibit direct development, but ranines such as *R. clamitans* have a free living larval stage (Zug 1993). Ranid frogs appear in the fossil record during the Oligocene (Zug 1993). In the northern panhandle of Texas, a *R. clamitans* scapula from the Middle Pleistocene marks the first appearance of this species in the fossil record (Stewart 1983).

Taxomony

Rana clamitans was first described by Latreille in 1802, and the type locality is Charleston, South Carolina (Mecham 1954; Stewart 1983). In 1820, Rafinesque described a similar frog from Lake Champlain, Vermont and from Lake George, New York as *Ranaria melanota;* however, no type specimen exists (Mecham 1954; Stewart 1983). Rhoads (1895) suggested there were two "races" of

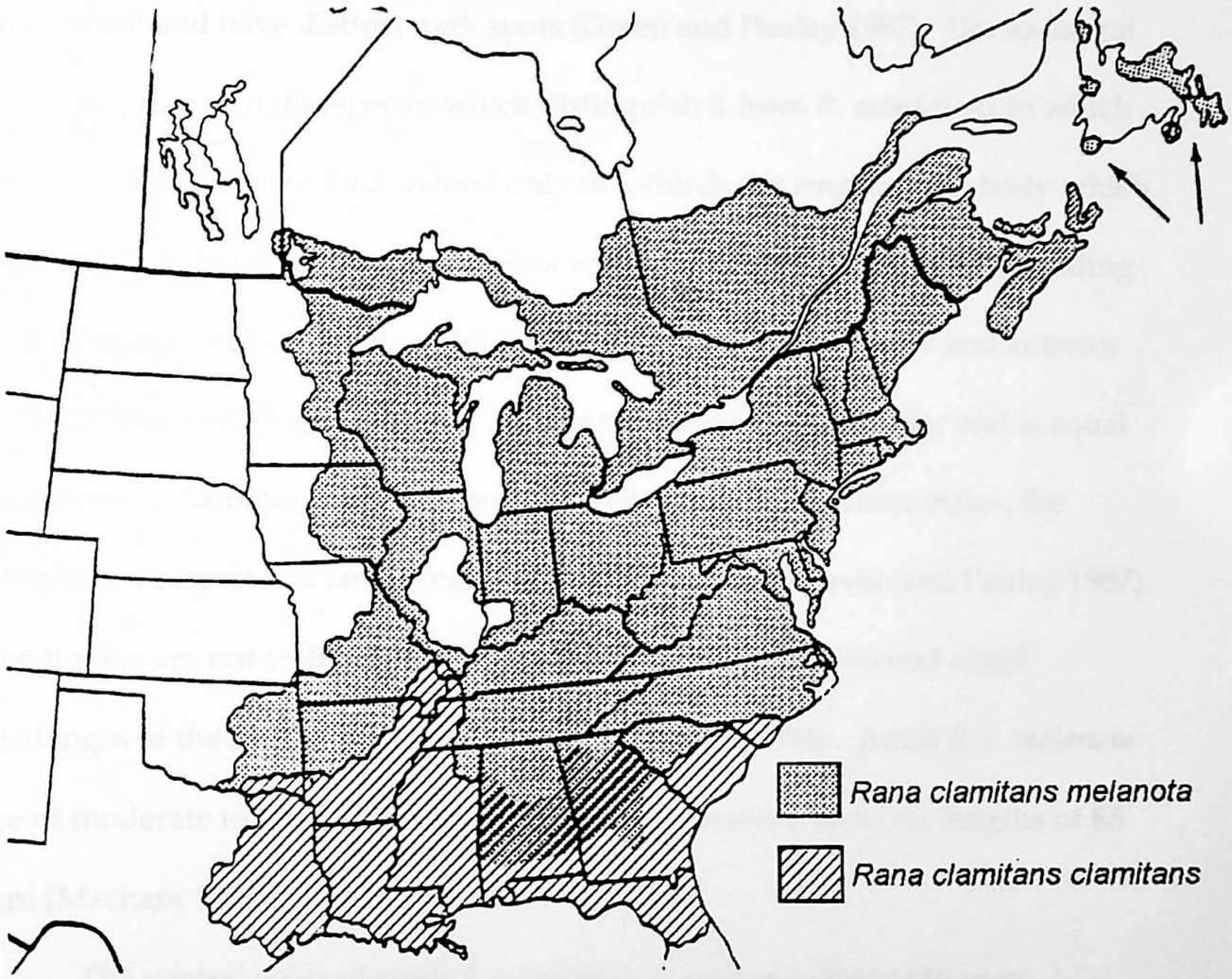
R. clamitans. He called the northern geographic race *R. c. melanota* and the southern geographic race *R. c. clamitans* (Mecham 1954). In 1954, Mecham used snout-to-urostyle length, tibia length, head length, tympanum diameter, dorsal spotting, skin characteristics, and color to definitively separate the subspecies of *R. clamitans* as Rhoads (1895) had suggested. Rafinesque was the first to describe *R. c. melanota*; hence he is given authorship of this subspecies (Mecham 1954).

Distribution

Rana clamitans is found throughout eastern North America (Figure 1). *Rana c. clamitans* is found in the coastal plain from southern North Carolina to north central Florida, and west to central Texas. Populations occur in the Mississippi River Valley to the mouth of the Ohio River (Conant and Collins 1991). *Rana c. melanota* is found from eastern Canada to North Carolina, and west to Minnesota and eastern Oklahoma. Populations are absent from a large part of Illinois and Indiana. *Rana c. melanota* has been introduced in Newfoundland, western Canada, Washington, and Utah (Conant and Collins 1991).

Green and Pauley (1987) reports the subspecies *R. c. melanota* throughout West Virginia. However, the subspecies *R. c. clamitans* does not occur in West Virginia. *Rana c. melanota* is found in permanent aquatic habitats such as lakes, ponds, permanent pools, swamps, marshes, and streams (Green and Pauley 1987). Additionally, this frog can successfully breed in vernal pools and roadside ditches which consistently hold water (Pauley pers. comm.).

Figure 1: Map showing the range of the *R. clamitans* (modified from Conant and Collins 1991).


Rana clamitans melanota
Rana clamitans clamitans

Description

Adult and immature frogs

The dorsal color of adult and immature *R. c. melanota* is dark green, brown or grayish with black blotches (Figure 2). *Rana palustris* and *R. pipiens* are brown to greenish and have distinct dark spots (Green and Pauley 1987). Dorso-lateral folds are present in this species which distinguish it from *R. catesbeiana* in which they are absent. These folds extend only two-thirds the length of the body which distinguish this species from *R. sylvatica* which has dorso-lateral folds extending to the groin (Green and Pauley 1987). The sides of the head below and anterior to the eye are bright green (Figure 2). The tympanum is olive color and is equal to the eye in diameter in females and immature frogs. In mature males, the tympanum diameter is larger than the eye (Walker 1946; Green and Pauley 1987). The fingers are not webbed, but all toes except the first and second distal phalanges of the fourth toe are fully webbed (Walker 1946). Adult *R. c. melanota* are of moderate to large size, usually exceeding snout-to-urostyle lengths of 85 mm (Mecham 1954).

The ventral color of adult *R. c. melanota* is creamy white with gray mottling on the lower sides. Male frogs have a bright yellow throat. Immature frogs have gray reticulate patterns marking the white ventral side (Walker 1946).

Larvae

The overall size of larval *R. c. melanota* is variable (Figure 2). Tadpoles can attain total body lengths of 80 mm or more (Walker 1946). The dorsal color is

Figure 2: Photographs of the life stages of *R. c. melanota*
a) larvae; b) froglet; c) subadult; d) adult.

a

b

c

d

deep green with various dark spots, while the ventral color is creamy white, and the sides are slightly iridescent pink. The tail is long, the dorsal tail crest low, and the tail fin and musculature are mottled with dark blotches (Walker 1946; Green and Pauley 1987). The eyes are dorsal, and the labial tooth-row formula is 2/3. The tooth-row immediately above the beak consists of two short lateral sections which may be reduced making the labial tooth-row formula 1/3 (Walker 1946). The beak is narrowly pigmented, and there is a marginal fringe of papillae at the sides of and below the beak (Walker 1946; Green and Pauley 1987). *Rana catesbeiana* larvae have similar characteristics except the top tail fin and adjacent tail musculature have distinct black spots (Figure 3). Additionally, *R. palustris* and *R. pipiens* larvae appear similar, but can be distinguished by a heavily pigmented beak (Figure 3).

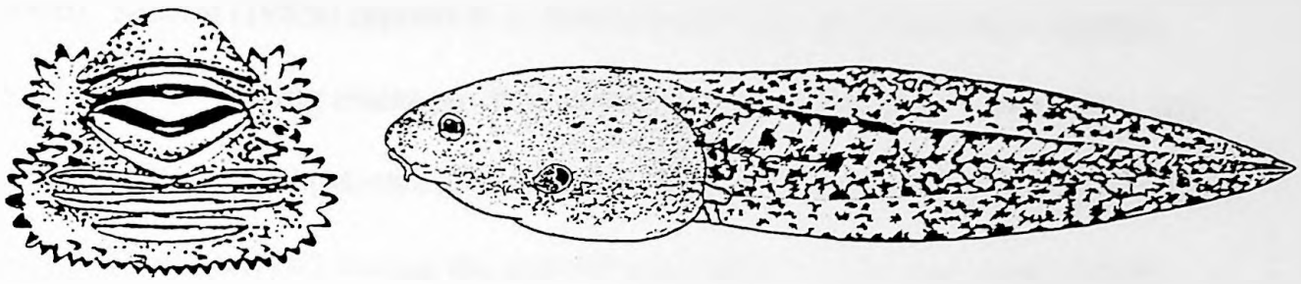
Ecology and Natural History

The life history and ecology of *R. clamitans* in the northern part of its range have been studied by Wright (1914), Wright and Wright (1949), Martof (1953a, 1953b, 1956a, 1956b), Jenssen (1967), Stewart and Sandison (1972), Wells (1976, 1977, 1978), Breven et al. (1979), McDonald et al. (1984), and McAlpine and Dilworth (1989). Although much is known about this species, no studies have focused on West Virginia populations.

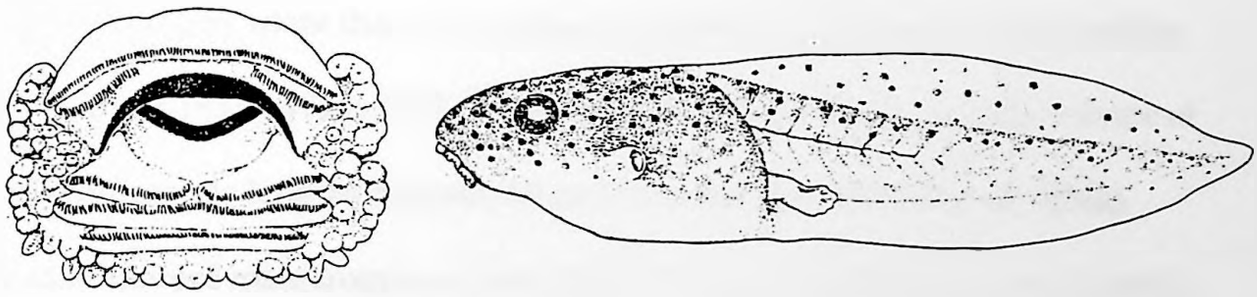
Adult and immature frogs

In late March and early April, male *R. c. melanota* emerge from hibernation when mean ambient temperatures are above 15.6 C (Martof 1953b). Warm

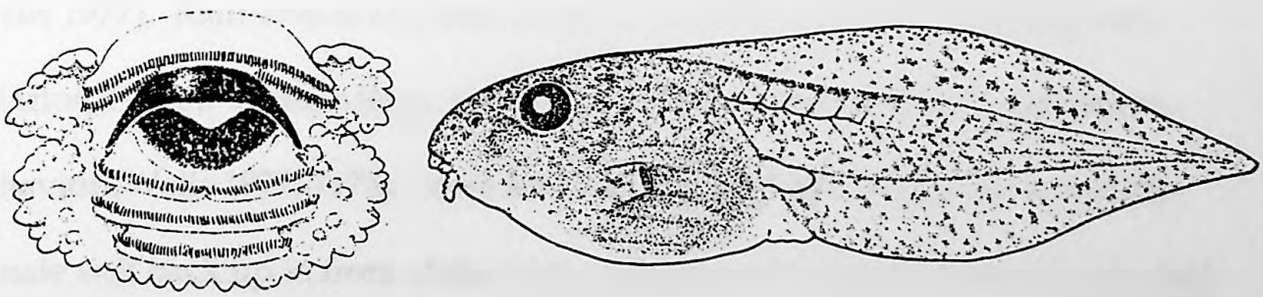
Figure 3: Line drawings of *R. c. melanota*, *R. catesbeiana*, and *R. palustris* larvae with emphasis on pigmentation, tail fin, beak, and labial morphology.



Rana clamitans melanota
(modified from Conant and Collins 1991)



Rana catesbeiana
(modified from Walker 1946)



Rana pipiens
(modified from Walker 1946)

spring rains will also bring male frogs out of their hibernacula (Martof 1953b, 1956b). Martof (1953a) reports *R. c. melanota* calling males from mid-April to mid-October. Calling males perch in areas of dense vegetation around the edge of the breeding pool (Martof 1953a; Wells 1977, 1978). Male frogs are more active at night, but often call during the day (Wright 1914; Wright and Wright 1949; Martof 1953a, 1953b; Wells 1977). Furthermore, warm rains increase breeding activity in males (Martof 1956b). Territories are established and defended by male frogs (Martof 1953a; Schroeder 1968; Wells 1977, 1978). However, male frogs will occupy more than one territory and may use more than one breeding pool (Martof 1953a; Wells 1977, 1978). Male *R. c. melanota* are sexually mature at snout-to-urostyle lengths between 60 and 65 mm (Ryan 1953; Martof 1956a). Sexually mature male frogs are found perched 1 to 4 m from one another on the bank of the breeding pool (Martof 1953; Wells 1977, 1978).

Female *R. c. melanota* spend most of the time away from breeding sites, only coming to the breeding area when ready to deposit eggs (Martof 1953a; Wells 1977). Rain events will also stimulate females to move to breeding sites (Martof, 1956b). Female frogs are not territorial and do not exhibit aggressive behavior (Wells 1977, 1978). Male frogs call female frogs to territories, and the female will back up in front of the male. Amplexus occurs for several hours, and eggs are laid as surface films attached to vegetation in shallow water (Wright 1914; Wells 1977). *Rana c. melanota* females are sexually mature between 65 and 75 mm snout-to-urostyle length, and can lay two clutches of eggs in one breeding

season (Ryan 1953; Martof 1956a,1956b; Wells 1976). Eggs are found in Michigan and New York from late May/early June to mid-August (Martof 1956a, 1956b; Wells 1977).

During the day, *R. c. melanota* are usually in water, or about 1 m from water, in areas of dense vegetation (Wright and Wright 1949; Martof 1953a, 1953b; Wells 1977, 1978; McAlpine and Dilworth 1989). Definite home ranges of about 3 and 4 m^2 are exhibited by adult and subadult frogs. However, during rain events frogs utilize home ranges between 20 and 200 m^2 (Martof 1953b). Movements outside the home range are attributed to growth and maturation, breeding activity, and overwintering (Martof 1953b).

Large dispersal events occur at times of larval metamorphosis. Newly transformed *R. c. melanota* have a snout-to-urostyle length between 28 and 38 mm with a mean of 32 mm (Martof 1956a; Wells 1977). Sexual maturity is reached at varying times depending upon when transformation occurs. Generally, *R. c. melanota* froglets are sexually mature the year following transformation (Ryan 1953). *Rana c. melanota* froglets move large distances, and do not exhibit a home range (Martof 1953b; Schroeder 1976). Heinen and Hammond (1997) show that *R. c. melanota* froglets are vulnerable to *Thamnophis s. sirtalis* while moving on land because of sluggish escape behavior when encounters with potential predators occur.

The diet of *R. c. melanota* adults varies greatly, and is dependent upon geographic location, habitat, and season (Stewart and Sandison 1972). The most

prominent invertebrates in the diet of *R. c. melanota* are coleopterans, dipterans, hymenopterans, larval lepidopterans, spiders, and snails (Hamilton 1948; Whitaker 1961; Stewart and Sandison 1972; Werner et al. 1995).

Larvae

The prolonged breeding season of *R. c. melanota* creates many questions about larval development and transformation in this species. Most accounts report the larval period to be 370 to 400 days with the larvae overwintering one season before transformation (Walker 1946, Wright and Wright 1949, Barbour 1971, Mitchell and Anderson 1995). However, laboratory reared *R. c. melanota* larvae develop in 92 days (Ting 1951). Martof (1952, 1956a) suggests *R. c. melanota* larvae from eggs laid early in the season transform the same season and do not overwinter. Furthermore, eggs laid before July transform in August and September, and eggs laid after June transform the following year around early June and mid-July (Martof 1956a). Additionally, Richmond (1964) reports some *R. c. melanota* larvae in an artificial pond developing in one season.

Jenssen (1967) reports the diet of larval *R. c. melanota* consists of algae and diatoms. During metamorphosis, larvae do not feed due to drastic cellular changes in the digestive tracts. Feeding rates increase in areas where the water temperature is above 22 C, and differences between feeding rates during the day and at night may be attributed to water temperature differences between areas of the pool (Warkentin 1992a, 1992b).

Many studies have shown that *R. c. melanota* larvae can tolerate acidic environments (McDonald et al. 1984, Dale et al. 1985, Freda and Taylor 1992). This species occurs in many different habitat types including roadside ditches, small and large pools, ponds, and lakes (Walker 1946; Wright and Wright 1949; Martof 1953a, 1953b, 1956; Barbour 1971; Dale et al. 1985; Green and Pauley 1987; Pearman 1993). Many smaller habitat types are seasonally acidic, and *R. c. melanota* can survive in water pH as low as 3.5 (Dale et al. 1985; Freda and Taylor 1992).

Rana c. melanota larvae are found most abundantly in roadside ditches, small and large pools, and temporary pools; however, these larvae are found less frequently in marshes (Dale et al. 1985). *Rana c. melanota* larvae attain larger sizes in larger habitats, but show a decrease in survival in large, deep pools (Pearman 1993).

Purpose of Study

The natural history of *R. c. melanota* in West Virginia has not been the subject of past studies of this common frog. Pauley and Barron (1995) provide limited insight on *R. c. melanota* breeding habits at Green Bottom Wildlife Management Area in the plateau region of the state. Little is known about this species in the mountain region of West Virginia. Amphibians are ectothermic; hence, activity patterns, breeding periods, growth, and development of a single species can vary depending upon latitude, longitude, elevation, and climatic variation (Stebbins and Cohen 1995). Therefore, the objectives of my study are 1)

to determine the larval period and transformation of *R. c. melanota* in plateau and mountain habitats, 2) to document the breeding season of *R. c. melanota,* 3) to determine size, dispersal, and basic dietary habitats of *R. c. melanota* froglets in mountain habitats, and 4) to determine elevation and habitat differences in water pH and water temperature of *R. c. melanota* habitats.

The first three objectives address potential differences in *R. c. melanota* larval period, breeding activity, and transformation in two geographic regions of West Virginia. Predicting amphibian metamorphosis is difficult because life histories will vary with different environments (Brattstrom 1962; Wilbur and Collins 1973; Breven et al. 1979; Smith-Gill and Breven 1979). Larval growth, cell differentiation, and metamorphosis is in part affected by temperature and other environmental factors (Smith-Gill and Breven 1979). Larval development can be described at a given collection time with stage tables (Gosner 1960). Stage-specific body size differences and differentiation rates of *R. c. melanota* larvae from different breeding seasons can be used to determine the length of the larval period (Breven et al. 1979; Smith-Gill and Breven 1979). Threshold temperatures for cessation of larval growth and cell differentiation can be used with stage classified larvae to pinpoint beginning and ending times of metamorphosis (Breven et al. 1979; Smith-Gill and Breven 1979).

The final objective addresses the potential differences in various aquatic habitat types in two geographic areas. West Virginia has an elevation range between 240 ft (73 m) and 4862 ft (1482 m) and is characterized by great relief

and diverse terrestrial and aquatic habitats (Lee et al. 1973). The state is located between the northern latitudes and southern latitudes of eastern North America. Latitude, longitude, and elevation allow areas in northern West Virginia to be similar to northeastern North America; likewise, areas in southern West Virginia are similar to southeastern North America. Many parts of West Virginia have been timbered, strip mined, developed for commercial use, or altered to meet mitigation requirements. Alteration of the landscape can create new habitat for non-specialist species such as *R. c. melanota* to exploit.

Materials and Methods

Description of Study Sites

Fourteen study sites were established in plateau and mountain regions of West Virginia to address the study objectives. Three sites were located in Green Bottom Wildlife Management Area, Cabell County. These sites were sampled from February to April. Eight sites were located in the Westvaco Wildlife and Ecosystem Research Forest, Randolph County. Seven of these sites were sampled from February to December. The temporary pool was sampled from February to April. Three study sites were located near the Barton Knob fire tower on Cheat Mountain, Monongahela National Forest, Randolph County. These sites were sampled from May to December.

Green Bottom Wildlife Management Area (GBWMA)

Green Bottom Wildlife Management Area is a 338 ha area 26.7 km north of Huntington. Most of this area lies between State Rt. 2 and the Ohio River in Cabell County with a small portion in Mason County. It has an extensive land use history dating back to the late Archaic Period, 5000 years ago, and recent history includes many periods of agricultural development (Allen et al. 1995). In 1980, a mitigation plan was submitted to Congress by the U. S. Army Corps of Engineers to restore the area to its natural state (WVDNR 1991). In 1989, the West Virginia Department of Natural Resources began a twenty-five year lease from the U. S. Army Corps of Engineers to manage the area for public hunting, fishing, recreation, and scientific use (WVDNR 1991). Fifty-eight hectares of

GBWMA are wetland areas surrounded by a narrow bottomland hardwood forest (Stark 1993). Figure 4 shows specific locations of the study sites at GBWMA. Figure 5 illustrates the typical habitat of the study sites at GBWMA.

Site 1: Elevation: 560 ft (171 m) Water Temperature Range: 2.0-17.5 C

Habitat Type: Ditch Mean Water pH: 7.21

This site is located in the new swamp area created by mitigation efforts of the U. S. Army Corps of Engineers. It is approximately 85 cm wide and 3.5 m long. In winter and early spring, the water level ranges from 50 to 120 cm. Throughout the summer, the water level drops and plants begin to fill in the adjacent bottomland field. Plant species found include *Leersia oryzoides* (L.) Sweet, *Polygonum amphibian* L., and *Hibiscus moscheutos* L. Other amphibian species observed were *Ambystoma maculatum* (Shaw), *Notophthalmus v. viridescens* (Rafinesque), *Pseudacris c. crucifer* (Weid), *Rana catesbeiana* Shaw, and *Rana sylvatica* LeConte. The central mudminnow, *Umbra limi* (Kirkland), was captured at this site. Various species of aquatic insects, leeches, and crayfish were also found in this ditch.

Site 2: Elevation: 560 ft (171 m) Water Temperature Range: 2.5-17.0 C

Habitat Type: Large pool Mean Water pH: 7.74

This pool was created by a weir installed by the U. S. Army Corps of Engineers as part of mitigation efforts at GBWMA. This site is in the new swamp

Figure 4: Map showing locations of study sites 1-3 at Green Bottom Wildlife Management Area, Cabell Co., WV (modified from DeLorme 1997).

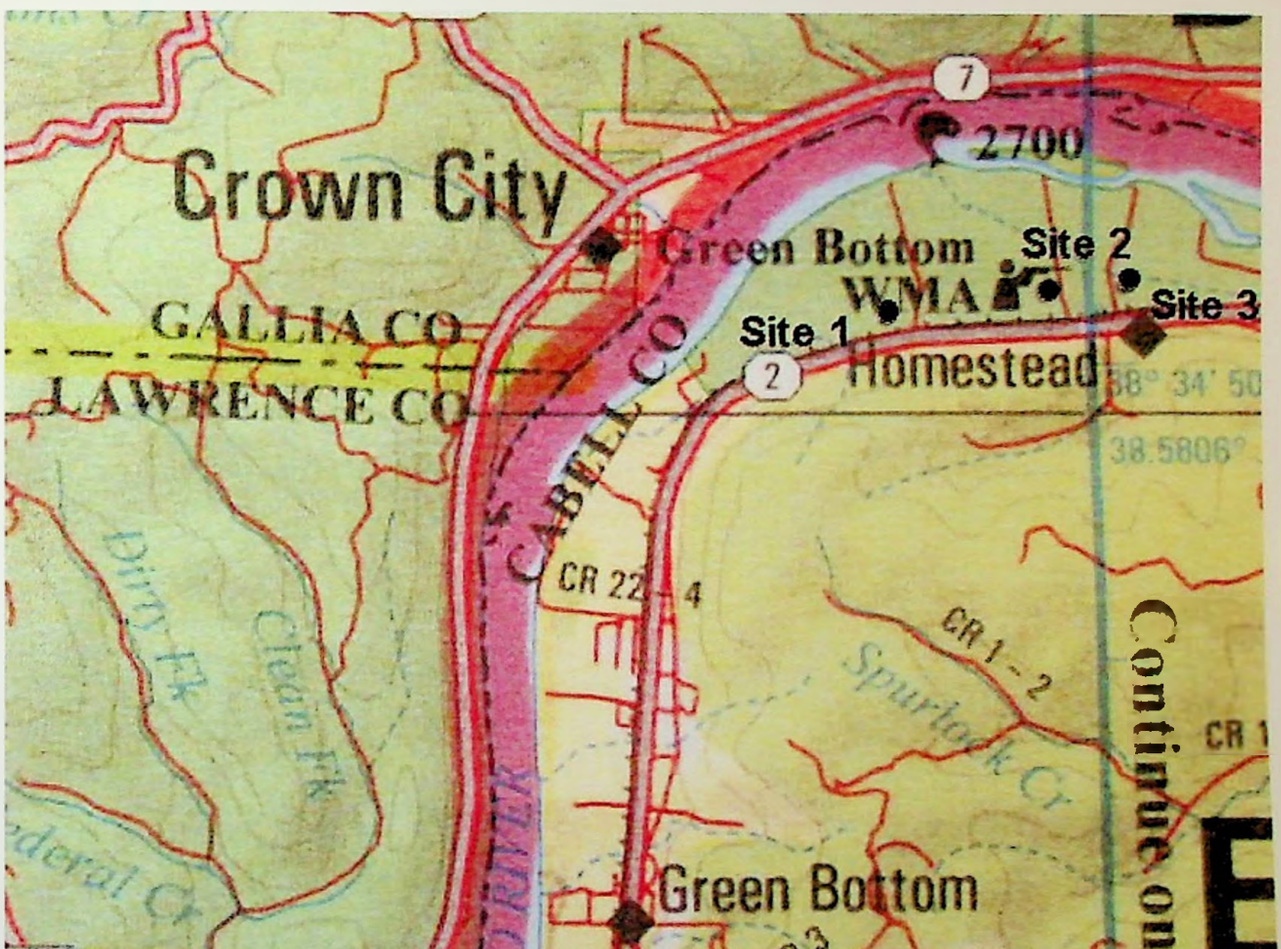

Crown City
GALLIA CO
LAWRENCE CO
CABELL CO
Green Bottom
WMA
Site 1
Site 2
Site 3
Homestead
2700
7
2
CR 22
CR 1-2
Spurlock Cr
Dirty Fk
Clean Fk
Green Bottom
Continue on
38.5806°

Figure 5: Photographs of study sites at Green Bottom Wildlife Management Area a) Site 3; b) Site 2.

a

b

section of GBWMA, and is approximately 30 m wide and 400 m long. The area sampled was about 50 cm deep, but in some areas this pool can be as deep as 1 to 1.5 m depending upon rainfall. Plants found at this site include *Typha latifolia* L., *L. oryzoides, Spirodela polyrhiza* (L.) Schleid, *Lemna minor* L., *Wolffia brasiliensis* Wedell, *Acer negundo* L, and *H. moscheutos*. Other amphibians found at this site include *A. maculatum, N. v. viridescens, Bufo a. americanus* Holbrook, *Hyla chrysoscelis* Cope, *P. c. crucifer, R. catesbeiana, R. sylavtica, Rana pipiens* Schreber, and *Rana palustris* LeConte. Reptile species observed at this site include *Chelydra s. serpentina* (Linnaeus), *Chrysemys picta marginata* Agassiz, and *Thamnophis s. sirtalis* (Linnaeus). Fish species observed were the bowfin, *Amia calva* Linnaeus and the grass pickeral, *Esox americanus vermiculatus* Lesueur. Additionally, many species of aquatic insects were captured at this site.

Site 3: Elevation: 560 ft (171 m) Water Temperature Range: 1.5-17.0 C

Habitat Type: Large pool Mean Water pH: 7.54

This site is located in the original or old swamp area of GBWMA. It is approximately 200 m wide and 300 m long. At times of high water levels it is much longer as it merges with the old swamp area. Water depth at this site is variable. Deeper areas in the middle range from 1to 2 m, while edge areas are more shallow. The sampling area was along the edge of this pool, and water levels ranged from 12 to 50 cm. The plant community consists of *Sparganium eurycarpum* Engelm ex. Gray, *L. oryzoides, S. polyrhiza, L. minor, W. brasiliensis,*

P. amphibian, *H. moscheutos*, and *Cornus amomum* Mill. Other amphibians recorded at this site include *P. c. crucifer*, *R. catesbeiana*, *R. sylvatica*, *R. pipiens*, and *R. palustris*. Reptile species sited include *C. s. serpentina* and *T. s. sirtalis*. Various species of aquatic insects were also captured at this site.

Westvaco Wildlife and Ecosystem Research Forest (WWERF)

This research forest was established in January 1994 by the Westvaco Corporation to study the impacts of silvicultural practices on the terrestrial and aquatic communities within its boundaries (Ford pers. comm.). This 3,413 ha area is located near Adolph, West Virginia with entrances on County Rt. 46 and County Rt. 42/2 in Randolph County. It ranges in elevation from 2400 ft (732 m) to 3860 ft (1177 m), and is drained by four streams which are part of the Middle Fork watershed. The coniferous/mixed deciduous forest landscape is everchanging due to various methods of timbering. There are many areas that collect water temporarily or permanently along roadsides, along streams, in open areas, and along haul roads created by timber harvesting. Figure 6 shows specific locations of the study sites on the WWERF. Figures 7 and 8 illustrate the various habitat types of the study sites on the WWERF.

Site 4: Elevation: 2560 ft (780 m) Water Temperature Range: 2.0-29.5 C

Habitat Type: Ditch Mean Water pH: 6.66

This site is a ditch along one of the gravel roads in the research forest. It is approximately 55 cm wide and 1.2 m long. Runoff from a steep bank created by

Figure 6: Map showing locations of study sites 4-11 at Westvaco Wildlife and Ecosystem Research Forest, Randolph Co., WV (modified from DeLorme 1997).

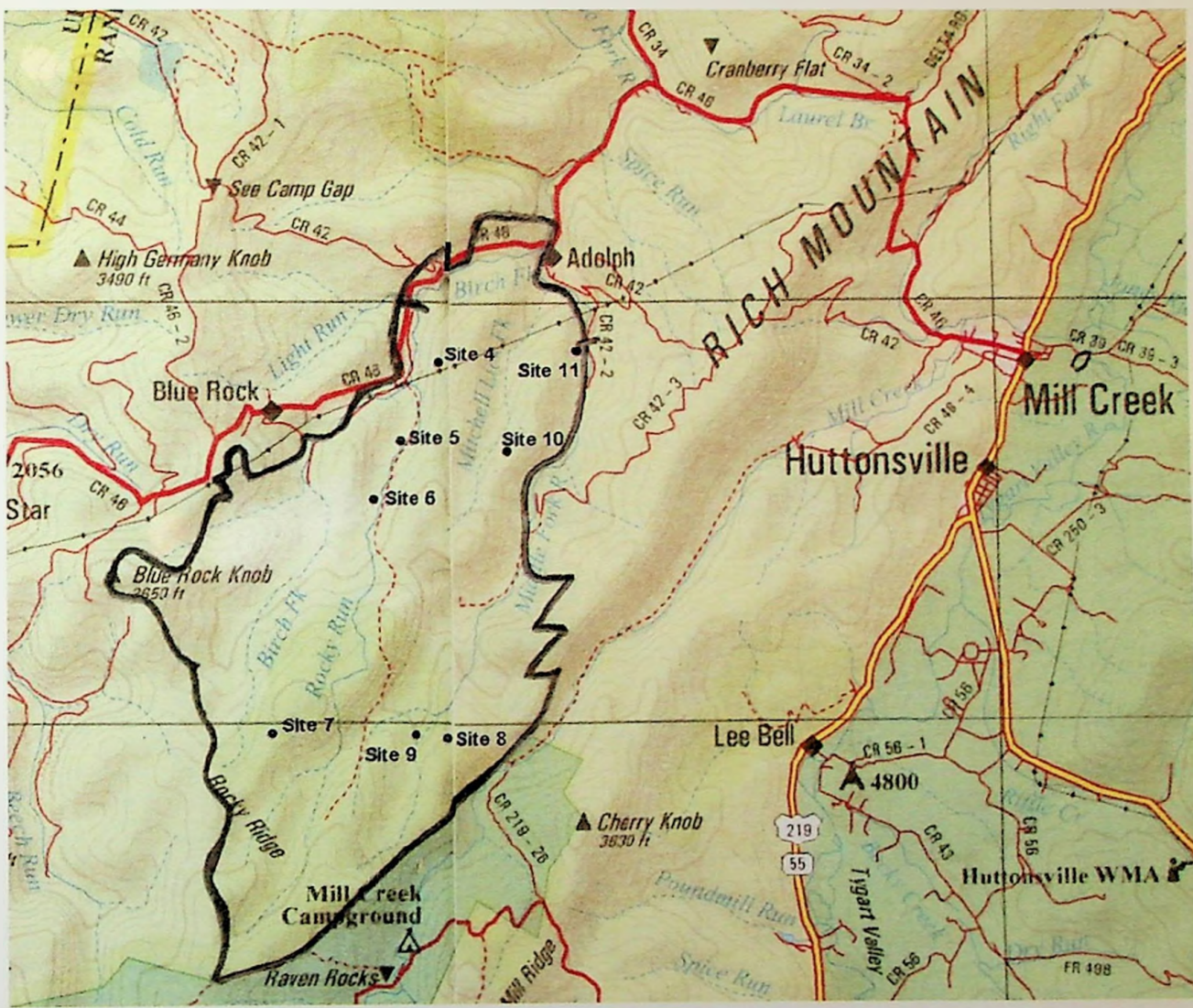

Cranberry Flat
See Camp Gap
High Germany Knob
3490 ft
Adolph
RICH MOUNTAIN
Blue Rock
Site 4
Site 11
Site 5
Site 10
Site 6
Mill Creek
Huttonsville
2056
Star
Blue Rock Knob
2650 ft
Site 7
Site 9
Site 8
Lee Bell
4800
Cherry Knob
3630 ft
Mill Creek Campground
Raven Rocks
Rocky Ridge
Tygart Valley
Huttonsville WMA
CR 42
CR 42 - 1
CR 44
CR 46
CR 46 - 2
CR 48
CR 42 - 2
CR 42 - 3
CR 34
CR 34 - 2
CR 46 - 4
CR 39
CR 39 - 3
CR 250 - 3
CR 56
CR 56 - 1
CR 43
CR 219 - 28
219
55
FR 498

Figure 7: Photographs of study sites at Westvaco Wildlife and Ecosystem Research Forest a) Site 6; b) Site 8.

a

b

Figure 8: Photographs of study sites at Westvaco Wildlife and Ecosystem Research Forest a) Site 4; b) Site 9.

a

b

the road feed this ditch which is 30 cm deep. The bottom is very silty, and plants found at the water's edge included *Scirpus cyperinus* (L.) Kunth, *Carex baileyi* Britton, *Juncus effusus* L., and *Juncus brevicaudatus* (Engelm) Fernald. Other amphibians found at this site include *N. v. viridescens* and *P. c. crucifer*. One species of reptile, *Lampropeltis t. triangulum* (Lacepede), was captured at this site. Aquatic insects in orders Odonata, Hemiptera, and Megaloptera were captured in this ditch.

Site 5: Elevation: 2570 ft (783 m) Water Temperature Range: 1.0-39.4 C

Habitat Type: Ditch Mean Water pH: 6.74

This ditch is situated between a steep hillside and a flat area with a natural gas storage tank. Runoff from the hillside feed this ditch which is 62 cm deep. The main part of the ditch is approximately 80 cm wide and 1.5 m long; however, at times of heavy precipitation, a depression near the gas storage tank fills nearly doubling the area of this ditch. Plants found in this area include *T. latifolia*, *L. oryzoides*, *S. cyperinus*, *Eleocharis obtusa* (Willd.) Schultes, *C. baileyi*, *Juncus tenuis* Willd., *J. brevicaudatus*. Other amphibians species recorded include *A. maculatum*, *N. v. viridescens*, *P. c. crucifer*, and *R. sylvatica*. Reptile species found at this site include *Nerodia s. sipedon* (Linnaeus), *Diadophis punctatus edwardsii* (Merrem), *Opheodrys vernalis* (Harlan), and *L. t. triangulum*. Aquatic insects in orders Odonata, Hemiptera, Coleoptera, and Trichoptera were captured at this site.

Site 6: Elevation: 2840 ft (866 m) Water Temperature Range: 0.5-27.8 C

Habitat Type: Large pool Mean Water pH: 7.25

This large pool is a small sandstone borrow pit created by road construction that is filled by runoff from snow melt (Ford pers. comm.). It is approximately 2.2 m wide and 2.3 m long. When completely filled with water, it is approximately 2.1 m deep. During unseasonably dry periods, the water level of this pool drops, but fills again by spring. There are small shallow areas of this pool which range from 20 cm to 86 cm deep. Plants found along the edge of this pool include *L. oryzoides*, *S. cyperinus*, *E. obtusa*, *C. baileyi*, *J. effusus*, *J. tenuis*, *J. brevicaudatus*, and *Rubus* sp. Other amphibians found at this site include *A. maculatum*, *N. v. viridescens*, *B. a. americanus*, *H. chrysoscelis*, *P. c. crucifer*, and *R. sylvatica*. One adult *R. palustris* was pulled from pitfall traps placed around this pool; however, no calling male frogs were recorded and no larvae were captured. One species of reptile, *N. s. sipedon*, was sighted at this pool. Aquatic insects in the orders Odonata, Hemiptera, Coleoptera, Megaloptera, and Trichoptera were captured.

Site 7: Elevation: 3440 ft (1049 m) Water Temperature Range: 2.0-35.6 C

Habitat Type: Temporary pool Mean Water pH: 6.03

This site was a depression resulting from a tank trap placed in an old haul road. It was approximately 60 cm wide and 70 cm long, and 52 cm deep. The

bottom of this pool was covered with fallen leaves and silt. It was considered a temporary pool because during dry periods in the summer, it became a small mud puddle. During the summer of 1996, this pool did not drastically change water levels because of increased precipitation that year. In spring 1997, the pool began to dry and was nearly dry before it was destroyed by timber harvesting in August. *Juncus effusus* was the only plant species found along the edge of this pool. Other amphibian species found were *N. v. viridescens*, *H. chrysoscelis*, *P. c. crucifer*, and *R. sylvatica*. The only aquatic insects captured were those in order Odonata.

Site 8: Elevation: 3160 ft (963 m) Water Temperature Range: 0.5-33.9 C

Habitat Type: Small pool Mean Water pH: 6.49

This site is a depression along the side of the road which filled with water. It is one of two sites that is partially covered by the surrounding forest canopy. This pool is approximately 58 cm wide and 66 cm long. The bottom of this pool is covered with fallen leaves and silt, and is approximately 70 cm deep. Plant species found along the water's edge include *E. obtusa*, *J. effusus*, and *J. brevicaudatus*. Other amphibian species captured were *N. v. viridescens*, *P. c. crucifer*, and *R. sylvatica*. Aquatic insects in the orders Odonata and Trichoptera were also captured.

Site 9: Elevation: 3000 ft (914 m) Water Temperature Range: -0.6-25.0 C

Habitat Type: Stream oxbow Mean Water pH: 6.11

This small oxbow is the result of periodic stream flooding and the construction of a road crossing the stream. A small seep area surrounds a depression that is approximately 87 cm wide, 1.5 m long, and 76 cm deep. This site is the second of two sites partially covered by the surrounding forest canopy. Plant species include *C. baileyi*, *J. effusus*, and *J. brevicaudatus*. *N. v. viridescens* and *H. chrysoscelis* were two amphibians found at this site with *R. c. melanota*. Aquatic insects found were in the orders Odonata and Trichoptera.

Site 10: Elevation: 2880 ft (878 m) Water Temperature Range: -5.5-35.0 C

Habitat Type: Large pool Mean Water pH: 7.52

This large pool has one steep side that slopes into a large depression that is filled by snow melt and precipitation runoff. The overall size of this pool varies seasonally. In the winter and spring this pool is approximately 3.0 m wide, 3.1 m long, and 89 cm deep. During the summer and autumn months, it is approximately 1.5m wide, 2.5 m long, and 47 cm deep. The most dominant plant species is *T. latifolia* which helps hold water during the summer and autumn months. Other plant species found include *Scirpus rubricosus* Frenald, *E. obtusa*, and *J. effusus*. Other amphibian species include *A. maculatum*, *N. v. viridescens*, *B. a. americanus*, *H. chrysoscelis*, *P. c. crucifer*, and *R. sylvatica*. One *R. palustris* male

was heard calling in May, but no larvae were found. Aquatic insects in the orders Odonata, Hemiptera, Coleoptera, and Trichoptera were also sighted.

Site 11: Elevation: 2400 ft (732 m) Water Temperature Range: 2.0-28.9 C

Habitat Type: Small pool Mean Water pH: 7.89

This site consists of a slope that becomes deep at one end. It is 76 cm wide and 89 cm long. The water depth of the deep portion is approximately 1 m, while the water depth of the shallow area is 20 cm. Plant species found include *Equisetum arvense* L., *S. cyperinus*, *E. obtusa*, *C. baileyi*, and *Spiranthes cernua* (L.) Richard. *E. arvense* and *S. cernua* were confined to the shallow end of the pool that remained wet, but did not consistently hold water. Other amphibian species included *A. maculatum*, *N. v. viridescens*, *H. chrysoscelis*, and *P. c. crucifer*. One species of reptile, *C. s. serpentina*, was sited in the deep portion of this pool. Aquatic insects in the orders Odonata, Hemiptera, and Coleoptera were also captured.

Monongahela National Forest, Cheat Ranger District, Barton Knob (MNFBK)

The Monongahela National Forest encompasses a large portion of the mountains of northeastern West Virginia. Study sites were established on Cheat Mountain near the Barton Knob fire tower in Randolph County. Access to the Cheat Mountain, Barton Knob study sites is off of U. S. Rt. 250/State Rt. 92 onto Forest Service Rt. 227. This area is considered part of the Mower Tract that was timbered, then strip mined for coal before USDA Forest Service acquisition in

1977 (Pauley pers. comm.). During reclamation, many large and small pools were created. Approximately 1.1 km on Forest Service Rt. 227 there is an old strip mine shelf directly across from the Barton Knob fire tower. On this shelf approximately 700 m from Forest Service Rt. 227, three pools served as study sites. This part of Cheat Mountain is above 3800 ft (1158 m), and these pools are at an elevation of 4000 ft (1219 m). Figure 9 shows specific locations of the study sites. Figure 10 illustrates the habitat types of study sites located on this portion of Cheat Mountain.

Site 12: Elevation: 4000 ft (1219 m) Water Temperature Range: 1.1-26.1 C

Habitat Type: Small pool Mean Water pH: 6.43

This small pool is approximately 60 cm wide, 76 cm long, and 56 cm deep. It is connected to a larger pool (Site 13) by a small wetland area composed of various species of *Sphagnum* moss. This pool also drains through a steel pipe into another pool on the opposite side of an old gravel mine road. Plant species found at this site include *S. cyperinus*, *C. baileyi*, and *J. effusus*. Other amphibian species found include *N. v. viridescens*, *Eurycea bislineata* (Green), *B. a. americanus*, *P. c. crucifer*, and *R. sylvatica*. Various species of aquatic insects in the orders Odonata, Hemiptera, and Trichoptera were also captured.

Figure 9: Map showing locations of study sites 12-14 at Monongahela National Forest, Barton Knob, Randolph Co., WV (modified from DeLorme 1997).

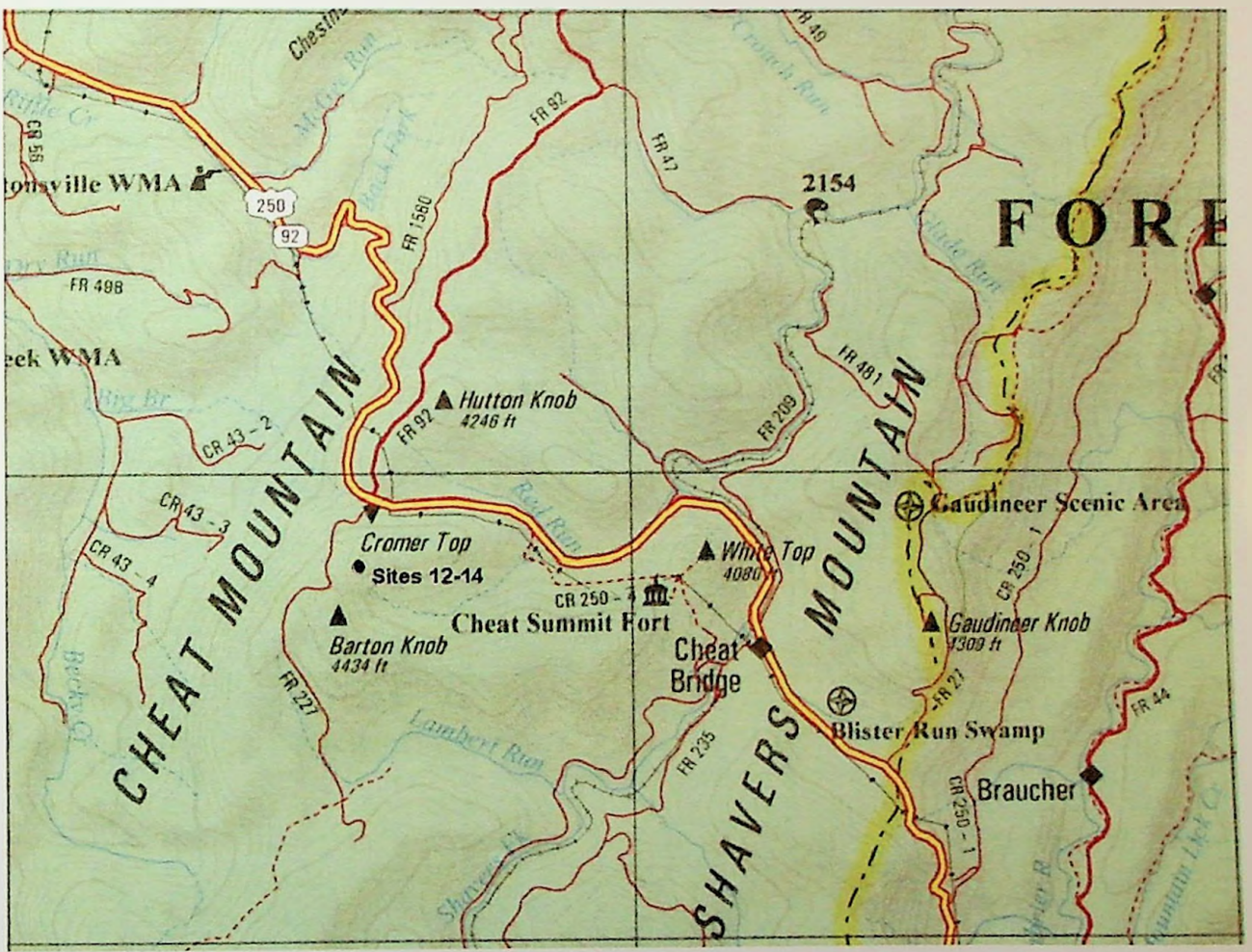

CHEAT MOUNTAIN
SHAVERS MOUNTAIN
Cromer Top
Sites 12-14
Hutton Knob
4246 ft
Barton Knob
4434 ft
Cheat Summit Fort
Cheat Bridge
White Top
4080 ft
Gaudineer Scenic Area
Gaudineer Knob
4309 ft
Blister Run Swamp
Braucher
2154
FR 92
FR 1560
FR 498
FR 47
FR 491
FR 209
FR 227
FR 235
FR 21
FR 44
CR 43 - 2
CR 43 - 3
CR 43 - 4
CR 250 - 4
CR 250 - 1
CR 56
250
92

Figure 10: Photographs of study sites at Monongahela National Forest, Barton Knob a) Site 13; b) Site 14.

a

b

Site 13: Elevation: 4000 ft (1219 m) Water Temperature Range: -0.6-30.0 C

Habitat Type: Large pool Mean Water pH: 6.74

This site is approximately 2.8 m wide, 3.5 m long, and 74 cm deep. It is a large depression fed by runoff from a steep wall typically associated with strip mines. As stated previously, this pool is connected by a wetland area to Site 12. The shallow area of this pool adjacent the wetland area is 15 cm deep. In addition to *S. cyperinus, C. baileyi*, and *J. effusus*, this site has small populations of *Carex squarrosa* L. and *S. cernua*. Other amphibian species found at this pool are the same as Site 12 since they move from one site to the other via the wetland area. Aquatic insects found were members of the orders Odonata, Hemiptera, Coleoptera, and Trichoptera.

Site 14: Elevation: 4000 ft (1219 m) Water Temperature Range: 1.1-32.8 C

Habitat Type: Small pool Mean Water pH: 8.12

This site is located approximately 5 m from the previous two sites. It is 56 cm wide, 74 cm long, and 39 cm deep. Plant species found at this small pool include *S. cyperinus, C. baileyi*, and *J. effusus*. Other amphibian species collected include *N. v. viridescens* and *R. sylvatica*. Aquatic insects found at this site were in the orders Odonata and Hemiptera.

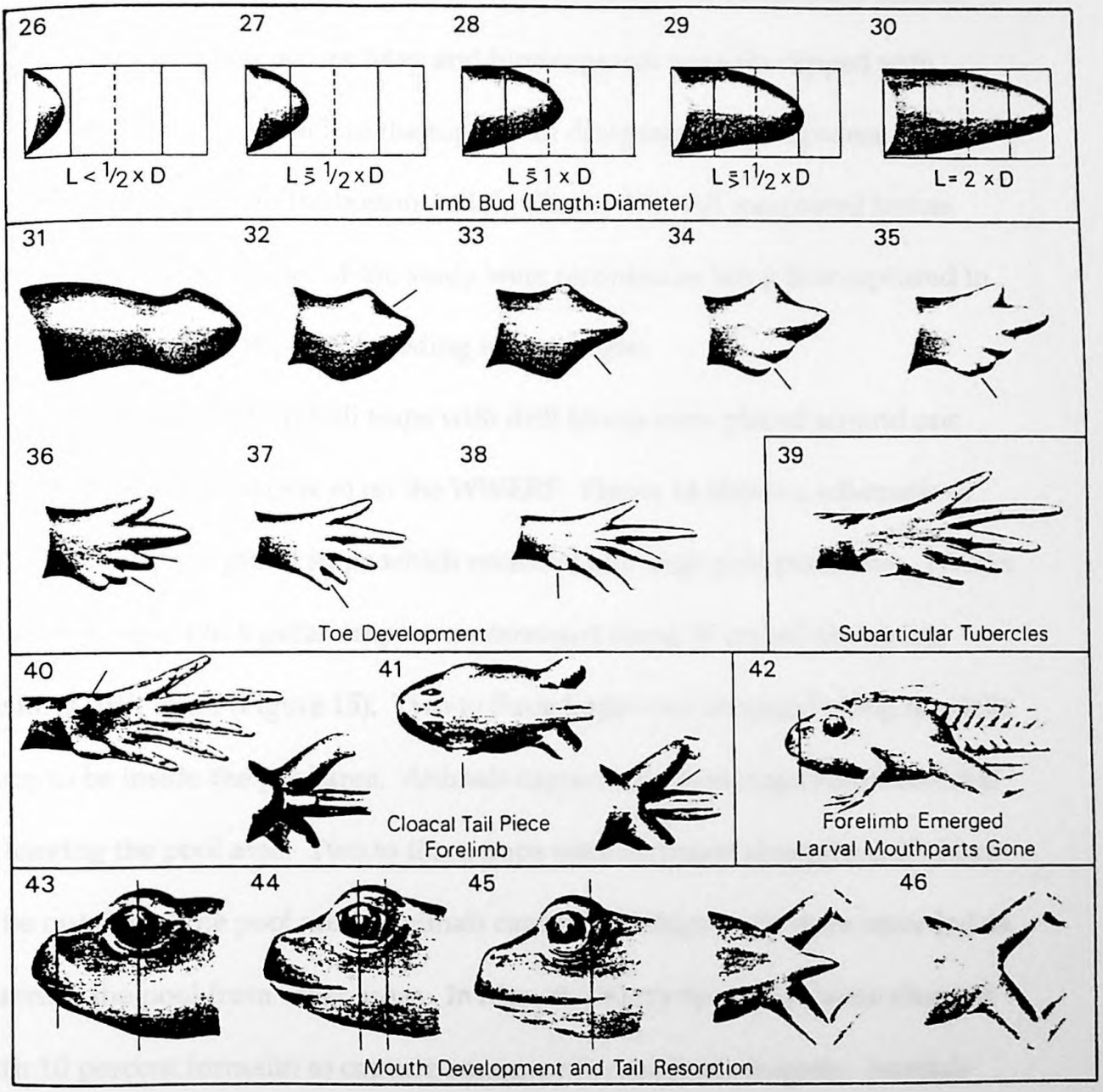
Biological Data Collection

To capture *R. c. melanota* larvae, aquatic funnel traps were placed in the water at each study site. These traps were checked once a month from February to December. From May until August, these traps were checked twice a month. Additionally, dip net sweeps with a D-frame dredge were employed at all study sites from May to December to standardize methods and increase sampling success. Sets of 10 dip net sweeps in 5 m^2 area were used (Shaffer et al. 1994). Large permanent pools required three sets of dip net sweeps. Small permanent pools, roadside ditches, and the stream oxbow required one to two sets depending on water level.

Anuran larvae captured in funnel traps were identified to species. From February to April, approximately 25 percent of all *R. c. melanota* larvae collected were anesthetized with chlorotone and fixed in 10 percent formalin for stage classification. Stage classification followed Gosner (1960) described in Table 1 and illustrated in Figure 11. Leg bud and toe formation was magnified with a 10X dissecting microscope. In May, all *R. c. melanota* larvae captured by funnel traps and dip net sweeps were placed in stage classes with a 10X hand lens to magnify leg bud and toe formation. This method reduced the number of animals sacrificed as only 10 to 40 *R. c. melanota* larvae were sacrificed per month to complete an annual series. These specimens were deposited in the West Virginia Biological Survey collection housed in the N. B. Green Museum of Natural History at Marshall University. Upon stage classification, total length of each

Table 1: Description of larval stages 26-46 according to Gosner 1960.	
Stage	**Description**
26	Hind limb bud just visible
27	Hind limb bud length greater than or equal to half the diameter
28	Hind limb bud length greater than or equal to the diameter
29	Hind limb bud length greater than or equal to one and one half the diameter
30	Hind limb bud length equal to twice the diameter
31	Formation of foot; foot is paddle shape
32	First indentation of the foot
33	Second indentation of the foot
34	Third indentation of the foot
35	Fourth indentation of the foot
36	Indentations on foot further develops into toes
37	Five distinct toes on foot present
38	Metatarsal tubercle present on foot; further development of toes
39	Appearance of subarticular tubercles on toes (light patches); further development of toes
40	Further development of toes; tubercles present
41	Cloacal tail piece disappears; tail begins to diminish; internal anatomical changes begin
42	Forelimbs appear; mouth parts begins to breakdown
43	Mouth just half way to middle of eye; tail length shortens
44	Mouth extending to middle of eye; tail length shorter than stage 43
45	Mouth fully developed; tail only a stub
46	Metamorphosis complete; froglet stage

Figure 11: Drawings of Gosner (1960) larval stage classes 26-46 (modified from Duellman and Trueb 1994).


26
27
28
29
30
L < 1/2 x D
L ≷ 1/2 x D
L ≷ 1 x D
L ≷ 1 1/2 x D
L = 2 x D
Limb Bud (Length:Diameter)
31
32
33
34
35
36
37
38
Toe Development
39
Subarticular Tubercles
40
41
Cloacal Tail Piece
Forelimb
42
Forelimb Emerged
Larval Mouthparts Gone
43
44
45
46
Mouth Development and Tail Resorption

larvae was measured with veriner dial calipers to the nearest 0.1 mm as described in Table 2. Mass of all living larvae captured between May and December was measured with an analytical balance to the nearest 0.01 g (Figure 12).

To determine if *R. c. melanota* larvae over winter more than one season before transformation occurs, May and June captures were fin clipped with fingernail clippers. A notch in the top tail fin designated May captures. June captures had a notch in the bottom tail fin (Figure 13). All recaptured larvae throughout the remainder of the study were recorded as being first captured in May or June, before the 1997 breeding season began.

In January 1997, pitfall traps with drift fences were placed around one large permanent pool (Site 6) on the WWERF. Figure 14 shows a schematic of the nine groups of pitfall traps which encircled one large pool perimeter. Within these 9 groups, 4 to 6 pitfall traps were arranged along 35 cm tall aluminum flashing drift fence (Figure 15). Two to three traps were arranged along the drift fence to be inside the pool area. Animals captured in these traps were recorded as leaving the pool area. Two to three traps were arranged along the drift fence to be outside of the pool area. Animals captured in these traps were recorded as entering the pool from other areas. In May, the 30 cm deep traps were charged with 10 percent formalin to capture emergent *R. c. melanota* froglets. Animals were collected from pitfall traps twice a month to document the transformation of *R. c. melanota* larvae and dispersal time of froglets. Biological data collected on

Table 2: Description of morphological measurements made on eggs, embryos, larvae, froglets, and frogs.

Character	Description
Egg Total Length	Diameter of egg; excluding the vitellus
Embryo Total Length	Tip of posterior end of embryo to tip of anterior end; excluding the vitellus
Larval Total Length	Straightened larva laid on its side; tip of nose to tip of tail fin
Snout-to-Urostyle Length (SUL)	Frog laid on dorsal side; straightened back; tip of snout to posterior projection of the urostyle
Right Tibia Length (RTL)	Posterior end of right tibia (knee) to anterior end of tibia (heel)
Cranial Width (CW)	Behind eyes on either side of head at widest point

Figure 12: Photograph of the larval measurement set up.

Figure 13: Photograph of fin clipped *R. c. melanota* larvae.

Figure 14: Schematic of drift fence and pitfall trap arrangement around Site 6 on the Westvaco Wildlife and Ecosystem Research Forest, Randolph Co., WV.

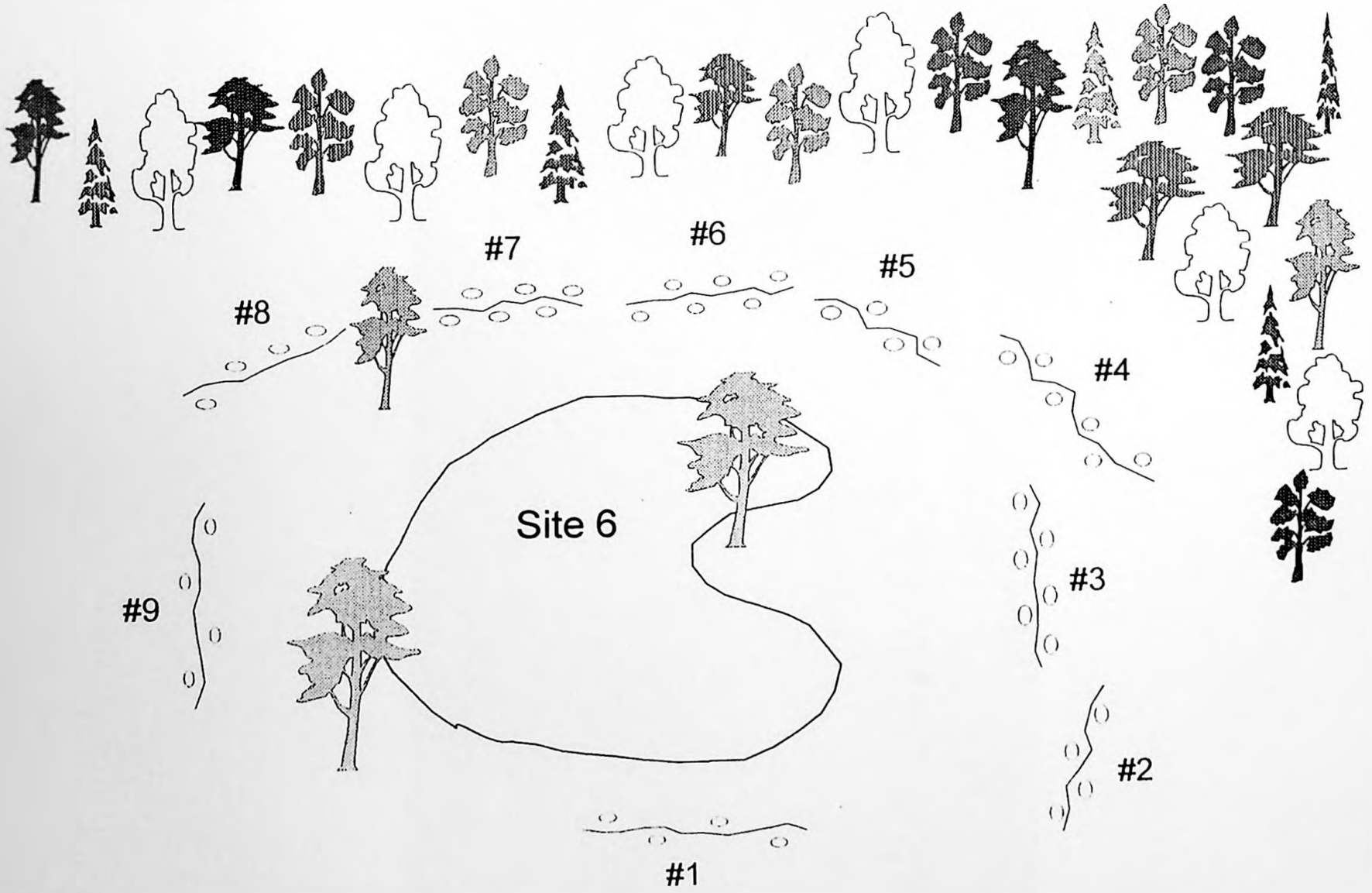

#7
#6
#8
#5
#4
Site 6
#9
#3
#2
#1
Road

Figure 15: Photograph of drift fence and pitfall trap at Site 6 on the Westvaco Wildlife and Ecosystem Research Forest, Randolph Co., WV.

these specimens included the morphological measurements of snout-to-urostyle length (SUL), right tibia length (RTL), and cranial width (CW) as described in Table 2. Measurements were made to the nearest 0.1 mm with vernier dial calipers. Any other life stages (juvenile, subadult, and adult) of *R. c. melanota* captured by pitfall traps were measured in the manner described for the froglets.

Ten to fifteen *R. c. melanota* froglets per collection date were dissected to remove the stomach to assess food habits. Each stomach was emptied and the contents were identified to class, order, or family dependent upon condition of the food item. Identification followed Borror et al. (1989) and Merritt and Cummins (1996). Percentage of each class present and percentage of each order in the dominant class were calculated. Numbers of individuals within the dominant taxa were graphed according to collection time. Terrestrial and aquatic designations were given to animals found in the froglet stomachs. Percentages based on these designations were calculated.

From mid-May to early September, a calling male census was conducted at the WWERF sites. Calling males were identified by listening to calls at night for 3 to 5 minutes at least once a month. One permanent pool and one roadside ditch at the WWERF were searched once a month at night with a flashlight for calling male locations and breeding activity. While sampling study sites for larvae, all sites were visually searched for eggs and embryos and any calling male activity was recorded. A small portion of any egg mass and/or embryos found were collected and preserved in 10 percent formalin. Egg and embryo

total length measurements were made as described in Table 2 with 150 mm Mitutoyo dial calipers to the nearest 0.01 mm. Egg and embryos were placed in a stage class with Gosner (1960) described in Table 3 and illustrated in Figure 16. Mean values of measurements made on collections from all study sites on the WWERF were calculated.

Environmental Data Collection

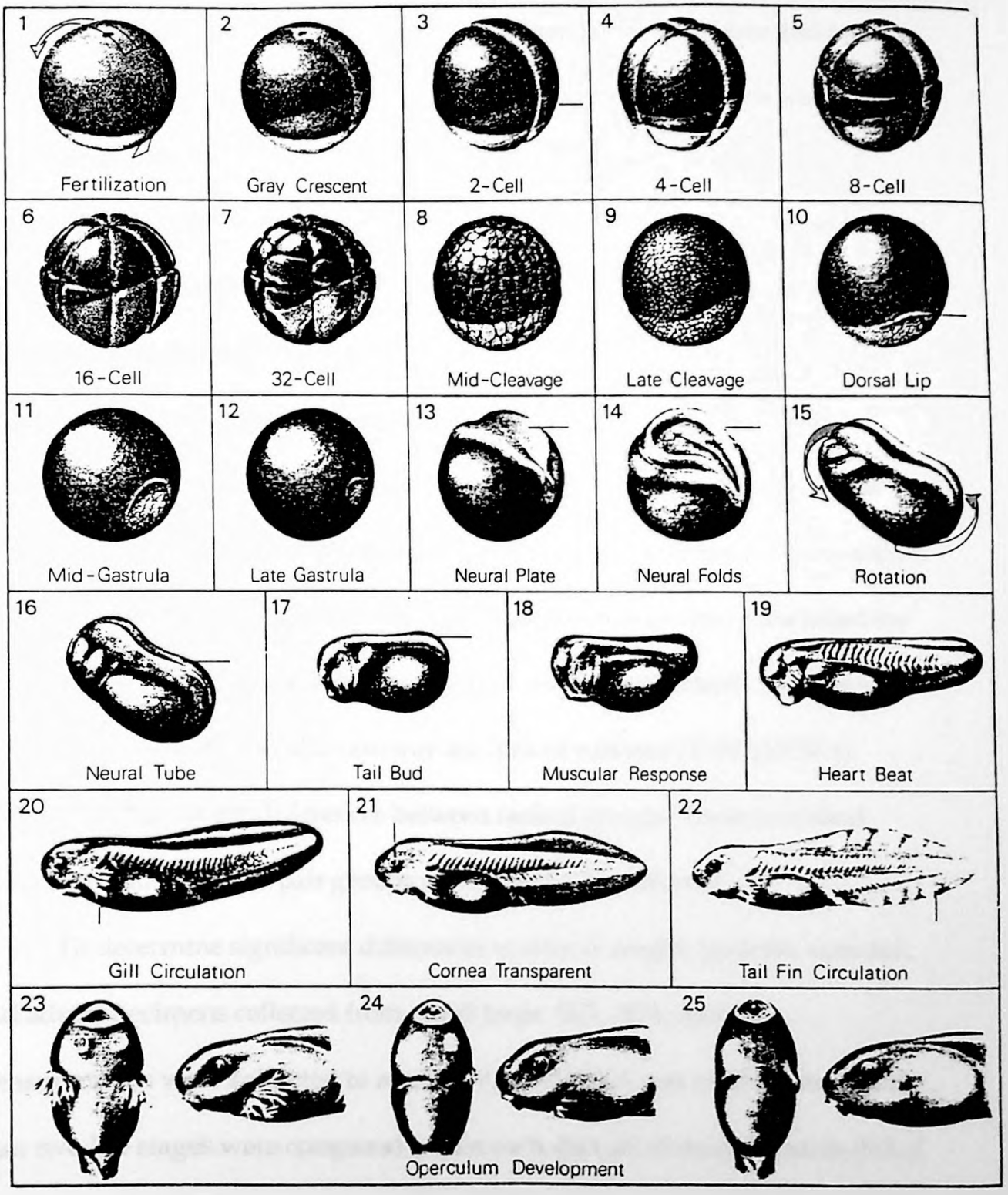
Water pH and water temperature data were recorded during each visit to all fourteen sites. Water pH was measured with an Oakton pH Testr 2 to the nearest 0.1 standard unit. These values were later used to calculate *M* hydrogen ion concentration. Water temperature was measured with an armored thermometer to the nearest 0.5 C. Minimum and maximum thermometers were placed in the water at each site. Temperature values were recorded and reset each time the sites were visited.

Statistical Analysis

All biological and environmental data collected during this period were divided into season designations as follows: late winter (February), spring (March and April), summer (May through August), autumn (September and October), and early winter (December). Each seasonal data set was analyzed for significant differences with various tests at the 95 percent ($P<0.05$) confidence level with Sigma Stat 2.0 (1997) statistical software package.

Table 3: Description of egg and embryonic stages 1-25 according to Gosner 1960.	
Stage	**Description**
1	Fertilization; embryo rotation until animal pole is upright
2	Second polar body expulsion; appearance of gray cresent
3	2-cell cleavage
4	4-cell cleavage
5	8-cell cleavage
6	16-cell cleavage
7	32-cell cleavage
8	Cell cleavage; smaller blastomeres than stage 7
9	Cell cleavage; expansion of darker hemisphere
10	Beginning of gastrulation; appearance of dorsal lip
11	Mid gastrula; blastopore ventral
12	Late gastrula; blastopore shifts
13	Neural plate development
14	Neural folds; elongation of embryo
15	Neural fold narrows; rotation of embryo
16	Neural tube develops; gill plates appear
17	Development of tail bud
18	Arch in embryo pronounced; lengthening tail bud; divisions in gill plates; oral suckers appear
19	Arch in embryo less pronounced; lengthening tail bud; further development of gills; oral suckers pronounced
20	Embryo body straight; lengthening tail; oral suckers present; hatching occurs
21	Oral suckers begin to disappear; cornea transparent
22	Tail fin becomes transparent
23	Beginning development of the operculum; oral suckers barely visible
24	Right gill disappears; oral suckers not visible
25	Left gill disappears; spiracle present; first free-living stage

Figure 16: Drawings of Gosner (1960) egg and embryonic classes 1-25 (modified from Duellman and Trueb 1994).


1
Fertilization
2
Gray Crescent
3
2-Cell
4
4-Cell
5
8-Cell
6
16-Cell
7
32-Cell
8
Mid-Cleavage
9
Late Cleavage
10
Dorsal Lip
11
Mid-Gastrula
12
Late Gastrula
13
Neural Plate
14
Neural Folds
15
Rotation
16
Neural Tube
17
Tail Bud
18
Muscular Response
19
Heart Beat
20
Gill Circulation
21
Cornea Transparent
22
Tail Fin Circulation
23
24
25
Operculum Development

Biological Data

All larval measurements were subjected to simple linear regression analysis (SLR) to determine a relationship between larval stage class and total length, and larval stage class and mass. Stage frequency histograms were used to illustrate stage class abundance.

Total length and mass measurements for larval stages 26, 32, 36, and 38 collected from the WWERF sites between May and October were analyzed with an one way analysis of variance (ANOVA) for significant monthly differences. These larval stages represent major morphological hind leg development changes, and an ANOVA was used to compare more than two months (Sokal and Rohlf 1981). If data failed the Kolmogrov-Smirnov (K-S) test for normality, it was logarithmically transformed. If logarithmically transformed data failed the K-S normality test, the raw data were ranked, and non-parametric tests were performed. A Kruskal-Wallis one way analysis of variance (K-WANOVA) determined significant differences between ranked groups. Dunn's method (DM) determined which pair groups were statistically different .

To determine significant differences in sizes of froglet, juvenile, subadult, and adult specimens collected from pitfall traps, SUL, RTL, and CW measurements were subjected to an ANOVA. ANOVA was used because more than two life stages were compared within each data set of measurements (Sokal and Rohlf 1981). RTL mean values between life stage were significantly different; a Tukey's test (TT) was used to determine statistically different pair

groups. SUL and CW data failed the K-S test for normality, and logarithmically transformed data also failed the K-S normality test. SUL and CW median values were ranked, and analyzed with a K-WANOVA for significant differences between life stages. DM was used to determine which pair groups were statistically different.

SUL, CW, and RTL measurements of froglets captured in pitfall traps between May and October were analyzed for significant monthly differences. The raw and logarithmically transformed data failed the K-S normality test. All three morphological measurements were ranked and subjected to a K-WANOVA to determine significant monthly differences. K-WANOVA was used because more than two months were compared within each measurement set (Sokal and Rohlf 1981). DM determined which ranked groups were statistically different.

Environmental Data

All environmental data were grouped by elevation and habitat then subjected to an ANOVA to determine significant differences between elevation group means and habitat groups means. ANOVA was used because more than two elevations or habitats were compared in each data set (Sokal and Rohlf 1981). Environmental data were also grouped by month for the periods February through April and May through October. If mean values between groups were significantly different, a TT was used to determine statistically different pair groups. Data that failed the K-S test for normality and could not be normalized by logarithmic transformation were ranked. K-WANOVA was used

to determine significant differences between groups in each data set. DM was used to determine which pair groups were statistically different.

Results

Late Winter and Spring (January 1997 - April 1997)

Larvae Data

Sampling for late winter 1996-97 yielded 164 *R. c. melanota* larvae from study sites on the WWERF and GBWMA. Stage 25 (n=92) was the most abundant stage, and all larvae of this stage were captured on the WWERF (Figure 17). Stage 28 was the earliest stage found on the GBWMA. Minimum, mean, and maximum total length values for larval stages from WWERF and GBWMA are listed in Table 4. Figure 18 shows the winter SLR of larval stage class vs. larval total length. A significant relationship existed between stage class and total length (R^2=0.854; P<0.001).

The spring *R. c. melanota* sample set showed stage 37 (n=40) as the most abundant stage class from study sites on the WWERF (Figure 19). As with the late winter data set, study sites on the WWERF yielded the greatest number of *R. c. melanota* larvae (253/330). The earliest stage from the GBWMA sites was 27, and the most abundant stage was 28 (n=14). Larval total length minimum, mean, and maximum values for *R. c. melanota* larvae collected from WWERF and GBWMA are listed in Table 5. Figure 20 shows the larval stage class vs. larval total length SLR for spring. Again, a significant relationship existed between stage class and total length (R^2=0.777; P<0.001).

Table 6 shows the total *R. c. melanota* larvae captured during this period at study sites located on GBWMA and WWERF. In winter, most *R. c. melanota*

Figure 17: Green Bottom WMA (GB) and Westvaco WERF (WV) late winter (February 1997) larval stage frequency histogram

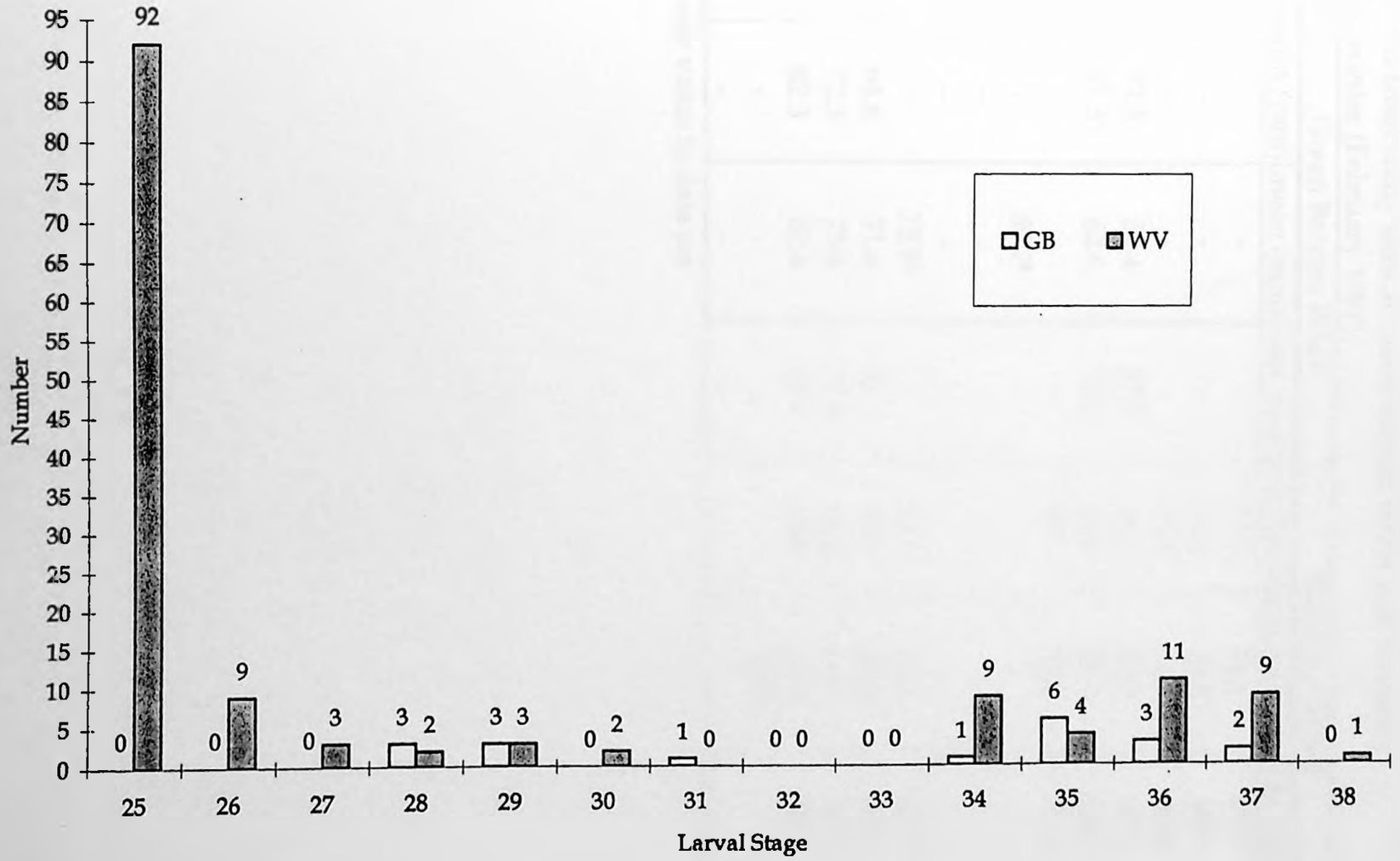


Table 4: Minimum, mean, and maximum total length values for *R. c. melanota* larvae captured from study sites at Green Bottom WMA and Westvaco WERF in late winter (February 1997).

Larval Stage	Green Bottom WMA			Westvaco WERF		
	min (mm)	mean (mm)	max (mm)	min (mm)	mean (mm)	max (mm)
25	-	-	-	21.9	34.6	49.7
26	-	-	-	32.0	41.8	48.4
27	-	-	-	41.8	43.8	46.0
28	52.3	54.4	57.3	46.8	47.0	47.2
29	57.3	62.6	72.2	45.8	50.0	52.2
30	-	-	-	50.3	51.8	53.2
31	-	59.7*	-	-	-	-
32	-	-	-	-	-	-
33	-	-	-	-	-	-
34	-	75.9*	-	53.1	60.1	67.7
35	64.8	71.6	81.3	56.9	61.8	67.5
36	72.3	73.4	74.3	56.5	65.7	73.1
37	82.3	87.4	92.4	65.8	74.0	82.5
38	-	-	-	-	92.6*	-
39	-	-	-	-	-	-

*indicates only value in data set

Figure 18: Green Bottom WMA and Westvaco WERF late winter (February 1997) larval stage vs. total length linear regression graph

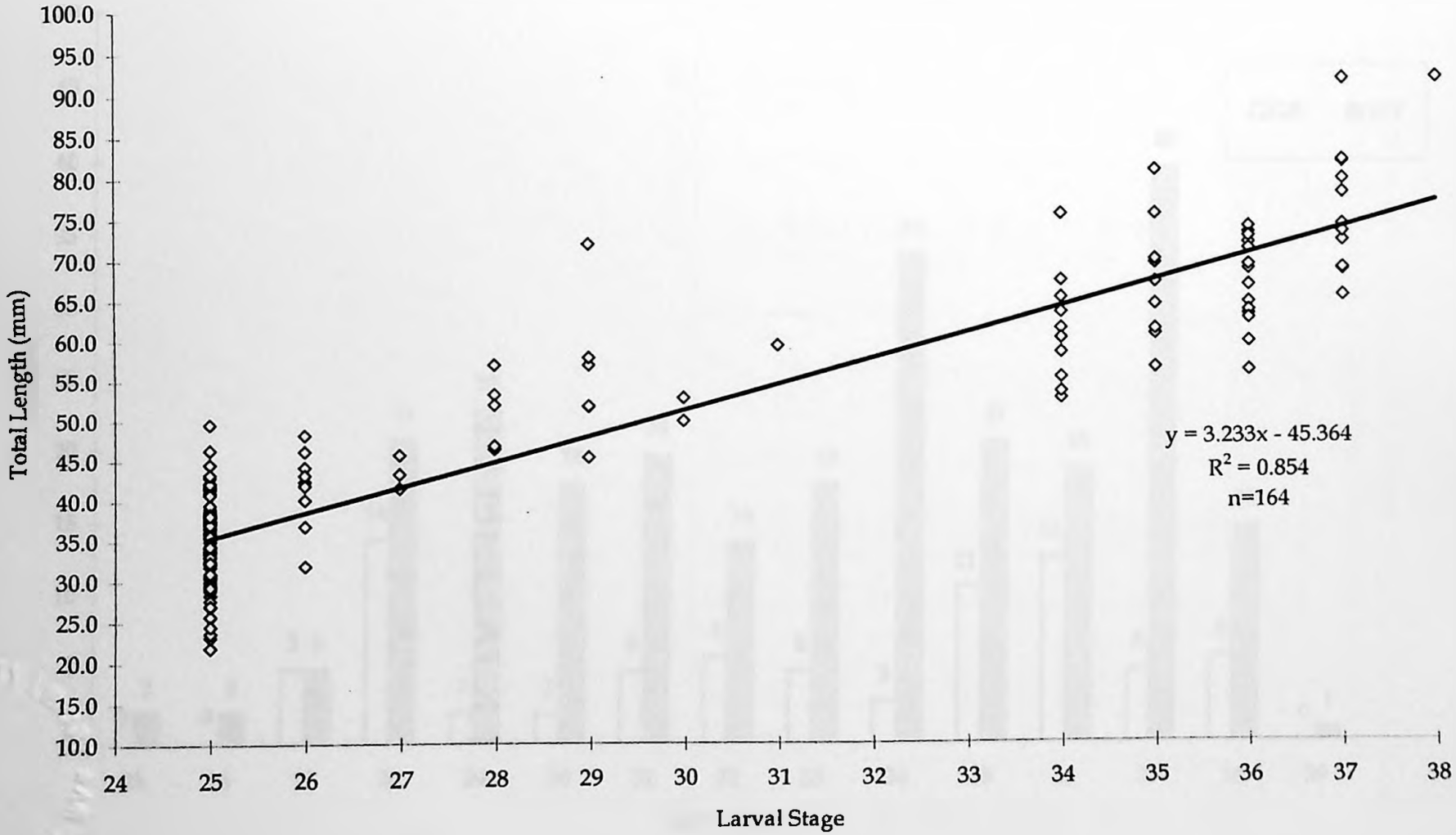


Figure 19: Green Bottom WMA (GB) and Westvaco WERF (WV) spring (March - April 1997) larval stage frequency histogram

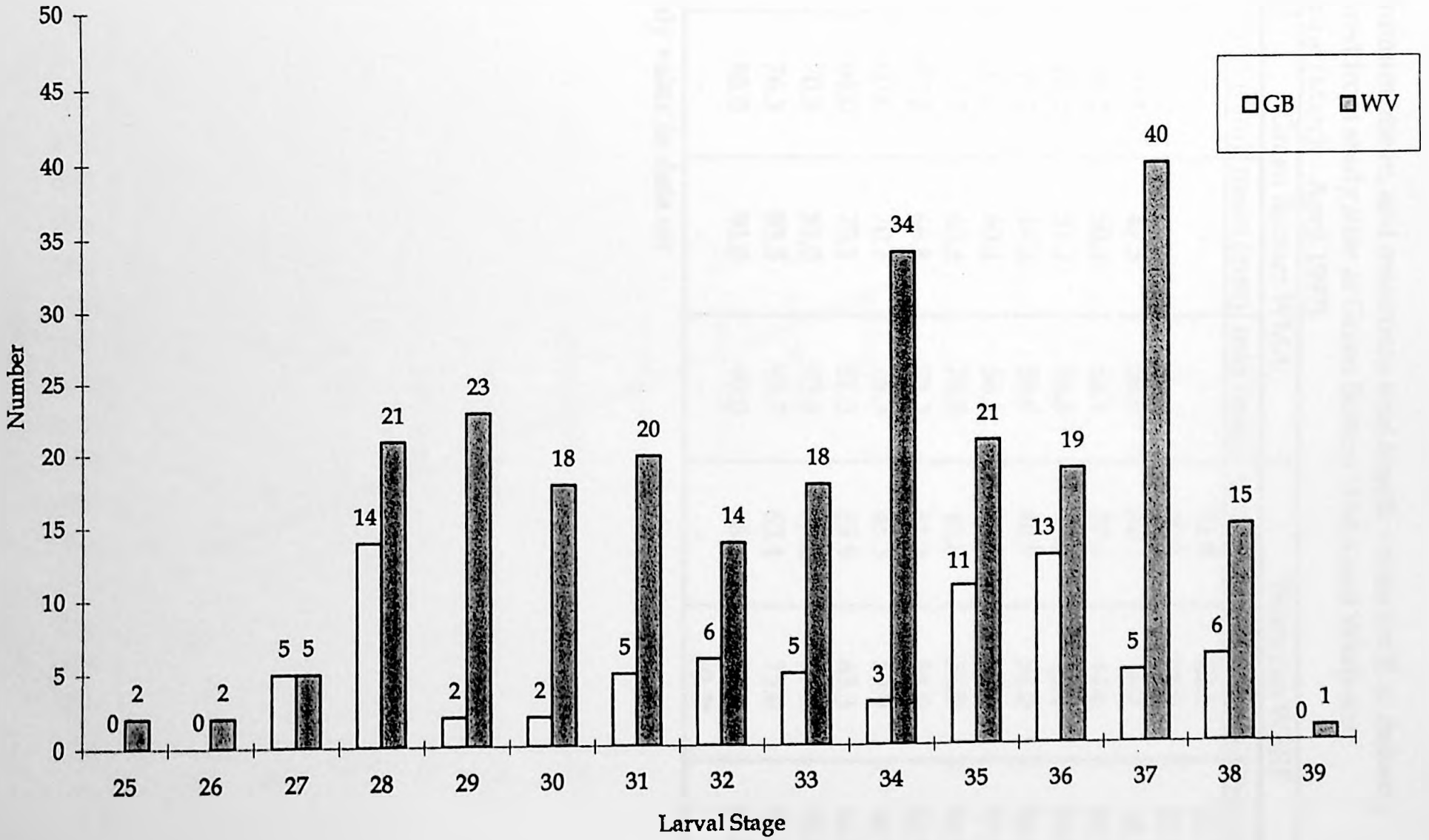


Table 5: Minimum, mean, and maximum total length values for *R. c. melanota* larvae captured from study sites at Green Bottom WMA and Westvaco WERF in spring (March - April 1997).

Larval Stage	Green Bottom WMA			Westvaco WERF		
	min (mm)	mean (mm)	max (mm)	min (mm)	mean (mm)	max (mm)
25	-	-	-	21.8	22.6	23.3
26	-	-	-	30.5	35.9	41.2
27	39.1	47.5	58.9	34.7	40.7	45.5
28	38.2	50.4	62.1	37.9	44.6	49.7
29	45.5	51.2	56.8	37.9	46.1	56.0
30	38.4	47.2	56.0	40.9	51.0	59.9
31	52.8	60.4	66.1	45.5	52.4	57.9
32	57.2	63.6	70.5	46.0	53.9	60.9
33	57.2	66.8	71.2	51.3	56.9	62.7
34	60.6	70.7	78.3	45.3	59.9	68.2
35	64.0	75.1	81.3	55.9	63.3	68.0
36	70.3	81.0	93.8	63.2	69.4	78.6
37	76.3	83.5	93.7	63.4	75.0	86.6
38	85.0	91.0	99.0	68.3	80.1	89.7
39	-	-	-	-	80.2*	-

*indicates only value in data set

Figure 20: Green Bottom WMA and Westvaco WERF Spring (March - April 1997) larval stage vs. total length linear regression graph

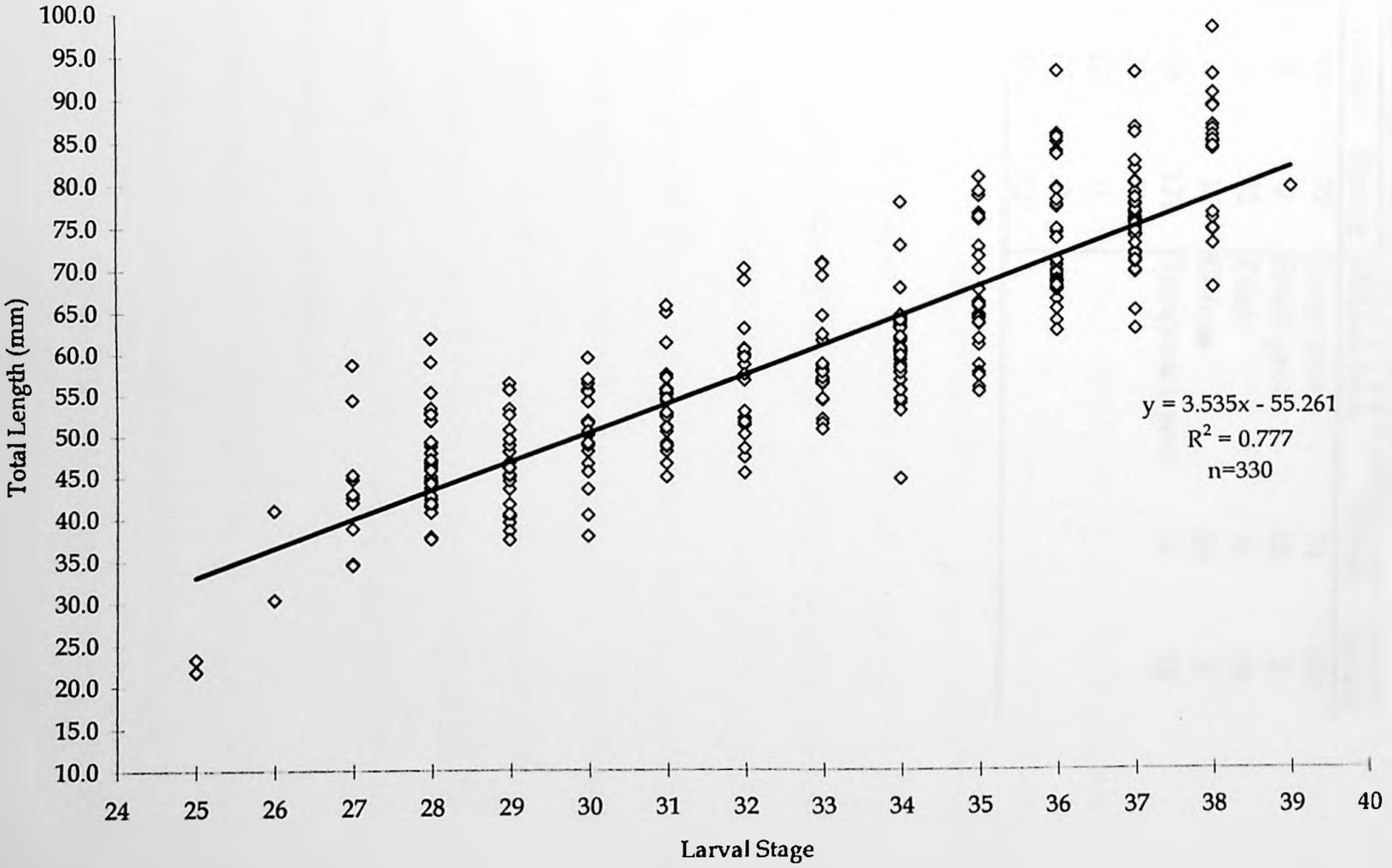


Table 6: Total number of *R. c. melanota* larvae captured February to April 1997 from study sites located at all elevations and habitat types at Green Bottom WMA and Westvaco WERF.

Elevation	Winter	Spring	Habitat type	Winter	Spring
560	19	77	Large pool	74	239
2400	3	0	Small pool	80	0
2560	-	32	Ditch	0	79
2570	0	16	Oxbow	10	0
2840	26	121	Temporary pool	0	12
2880	29	72			
3000	10	0			
3160	77	0			
3440	0	12			

larvae were collected from the study site at 3160 ft (n=77). In spring most larvae were captured from the study site at 2840 ft (n=121). The GBWMA sites at 560 ft yielded low capture numbers in winter (n=19). In spring, the numbers increased (n=77), but capture numbers were low considering there were three sites at this elevation.

Environmental Data

February water pH and water temperature median values grouped by elevation are illustrated in Figure 21. There were no significant differences in median water temperature values between elevation groups (H=10.168; 7 df; P=0.179). Significant differences were found in median water pH values (H=35.629; 7df; P<0.001). Median water pH values at 3000 ft (F), 3160 ft (G), and 3440 ft (H) were significantly lower than those taken at 560 ft (A) and 2400 ft. (B). Late winter environmental data grouped by habitat is shown in Figure 22. There were no significant differences in median water temperature values between habitat groups (H=3.293; 4 df; P=0.510). Median water pH values were significantly different (H=27.703; 4 df; P<0.001). The ditch (B), oxbow (D), and temporary pool (E) habitats had significantly lower median water pH values than those of the large pool (A) habitat.

Spring median water temperature values were significantly different (H=16.450; 8 df; P=0.036), between elevations (Figure 23). The median water temperature at 3000 ft (G) was significantly lower than 2560 ft (C). Median water pH values showed differences between most elevations (H=54.775; 8 df;

Figure 21: Green Bottom WMA and Westvaco WERF late winter (February 1997) environmental data (grouped by elevation)

Data labels with letters matching elevational group designations indicate significant differences in median values (K-WANOVA, P<0.05).

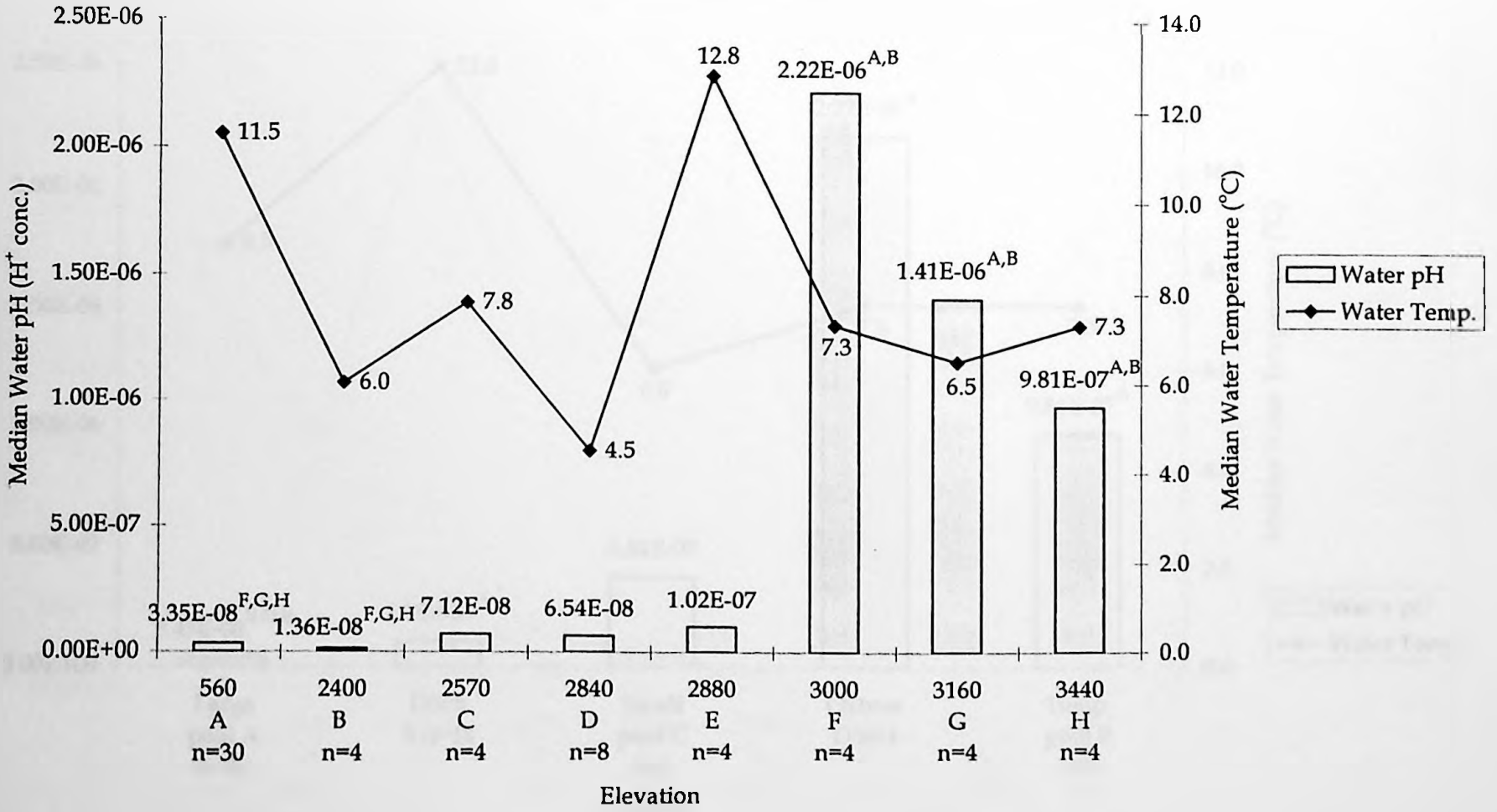


Figure 22: Green Bottom WMA and Westvaco WERF late winter (February 1997) environmental data (grouped by habitat)

Data labels with letters matching habitat group designations indicate significant differences in median values (K-WANOVA, P<0.05).

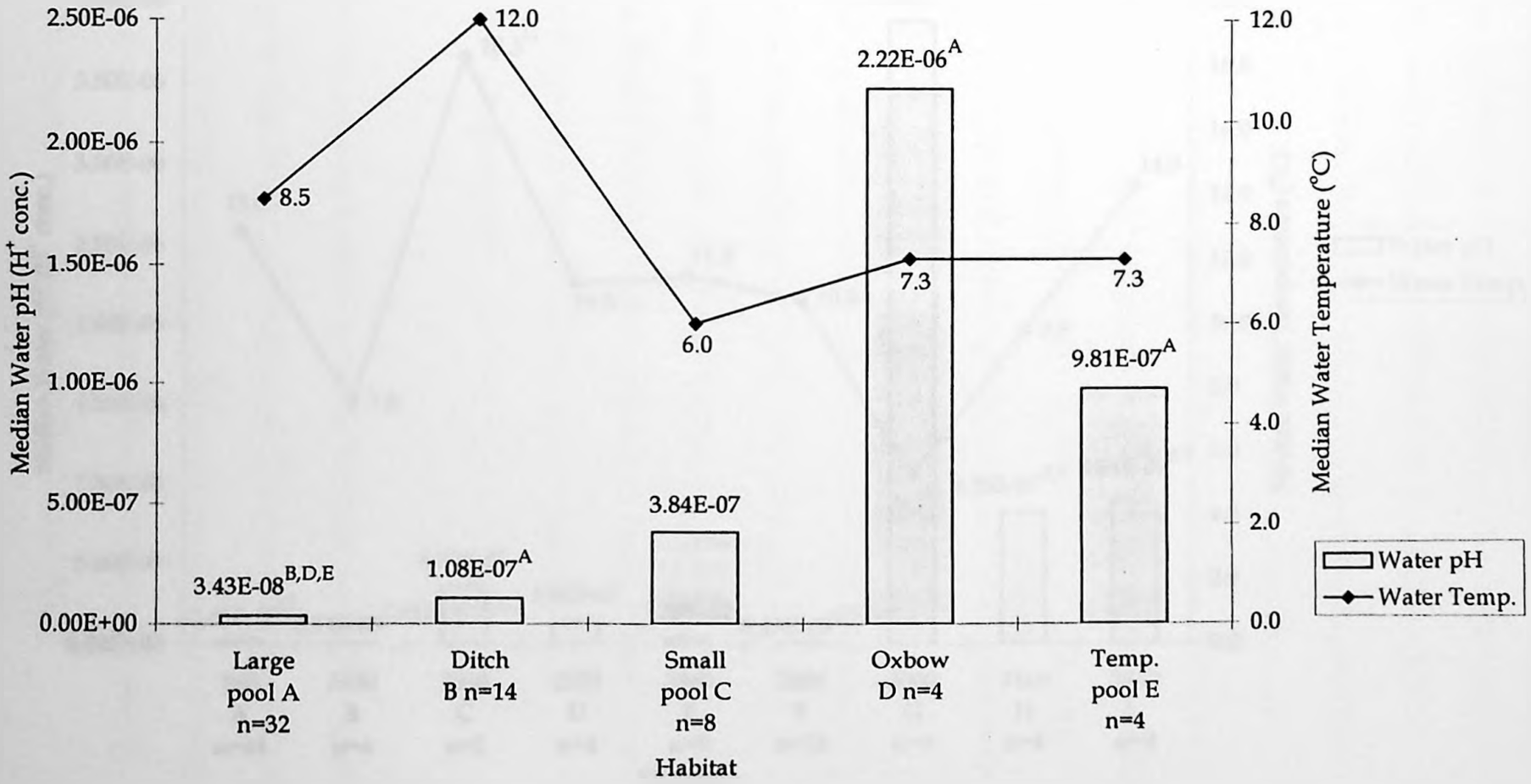
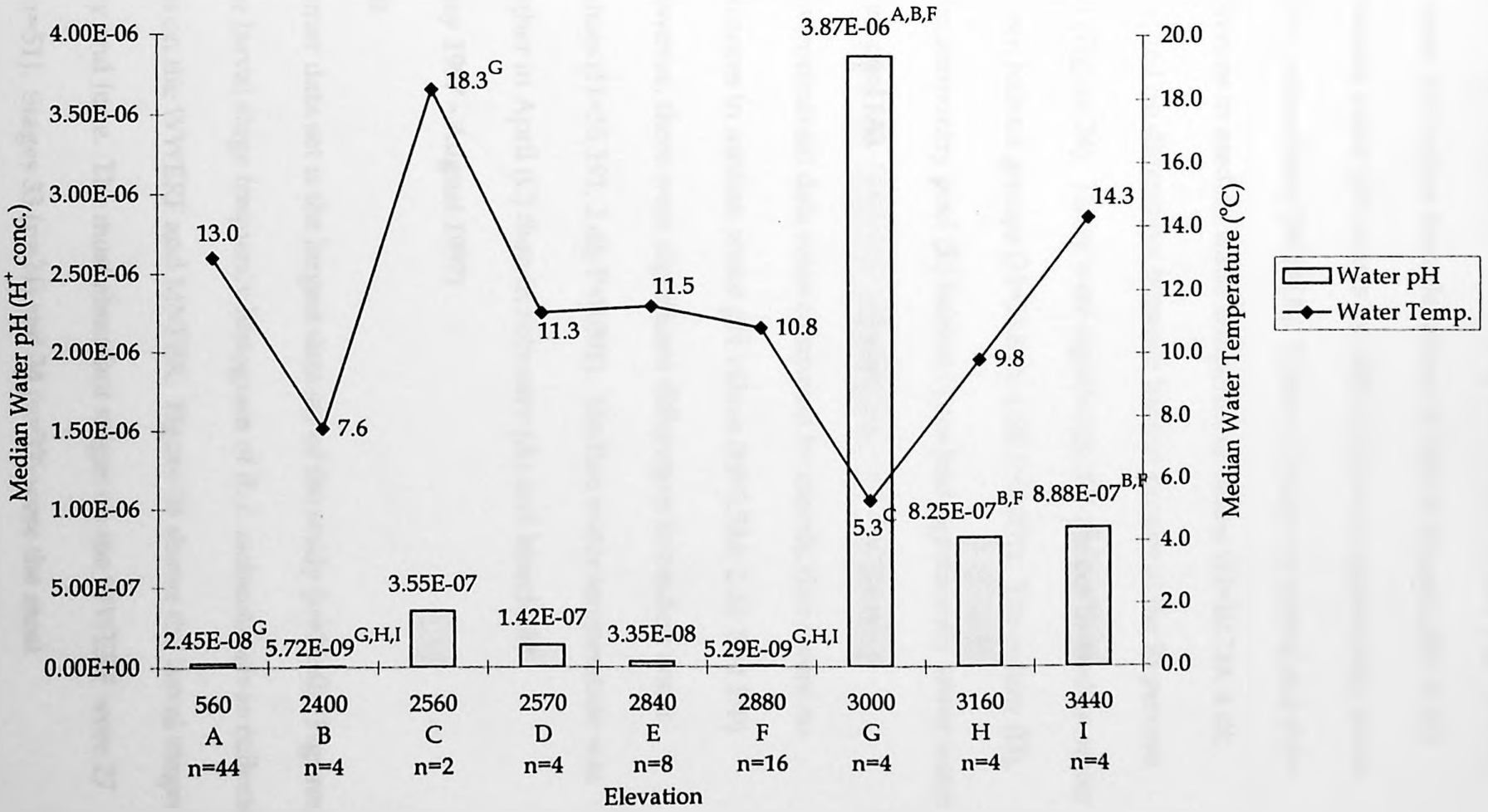


Figure 23: Green Bottom WMA and Westvaco WERF spring (March - April 1997) environmental data (grouped by elevation)

Data labels with letters matching elevational group designations indicate significant differences in median values (K-WANOVA, P<0.05).



P<0.001). Elevations of 3000 ft (G), 3160 ft (H), and 3440 ft (I) had significantly lower median water pH values than elevations of 2400 ft (B) and 2880 ft (F). Additionally, median water pH values for 3000 ft (G) were significantly lower than those median values from 560 ft (A). Habitat groups for spring did show significant differences in median water temperature values (H=10.718; 4 df; P=0.030), but yielded no differences between habitat groups at the 95 percent confidence level (Figure 24). There were significant differences in median water pH values between habitat groups (H=33.813; 4 df; P<0.001). The oxbow (D), ditch (B), and the temporary pool (E) habitat types had significantly lower water pH than the large pool (A).

When environmental data were compared by month, there were no significant differences in median water pH values (H=3.544; 2 df; P=0.170) (Figure 25). However, there were significant differences in median water temperature values (H=35.191; 2 df; P<0.001). Median water temperature was significantly higher in April (C) than in February (A) and March (B).

Summer (May 1997 - August 1997)

Larvae Data

The summer data set is the largest data set of the study (n=1660). Figures 26 and 27 show the larval stage frequency histogram of *R. c. melanota* larvae collected from study sites on the WWERF and MNFBK. Figure 26 shows the larval stages captured in May and June. The most abundant stages on the WWERF were 27 (n=44) and 34 (n=51). Stages 33 (n=24) and 34 (n=32) were the most

Figure 24: Green Bottom WMA and Westvaco WERF spring (March - April 1997) environmental data (grouped by habitat)

Data labels with letters matching habitat group designations indicate significant differences in median values (K-WANOVA, P<0.05).

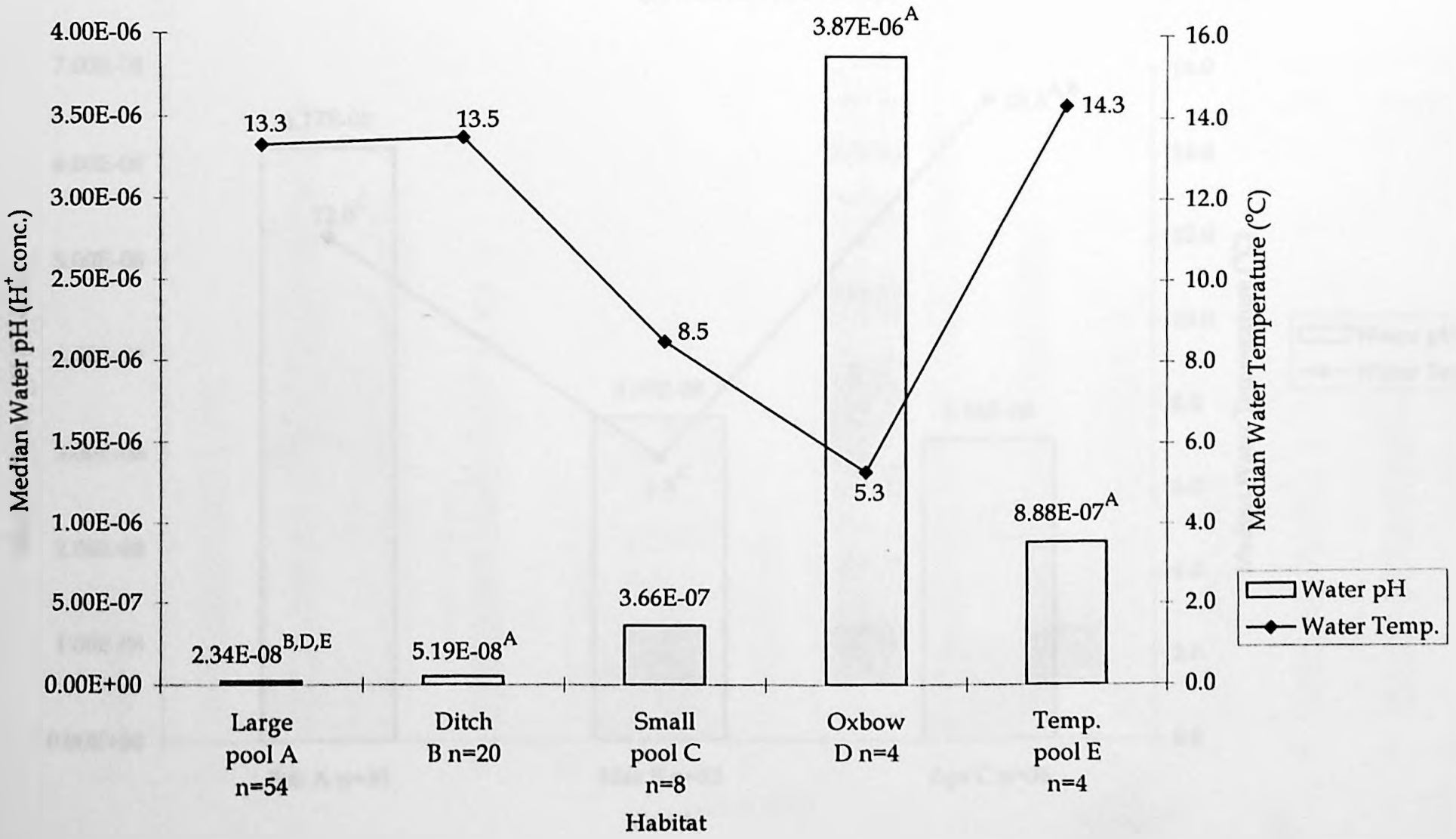


Figure 25: Green Bottom WMA and Westvaco WERF environmental data from February to April 1997 (grouped by month)

Data labels with letters matching month designations indicate significant differences in median values (K-WANOVA, P<0.05).

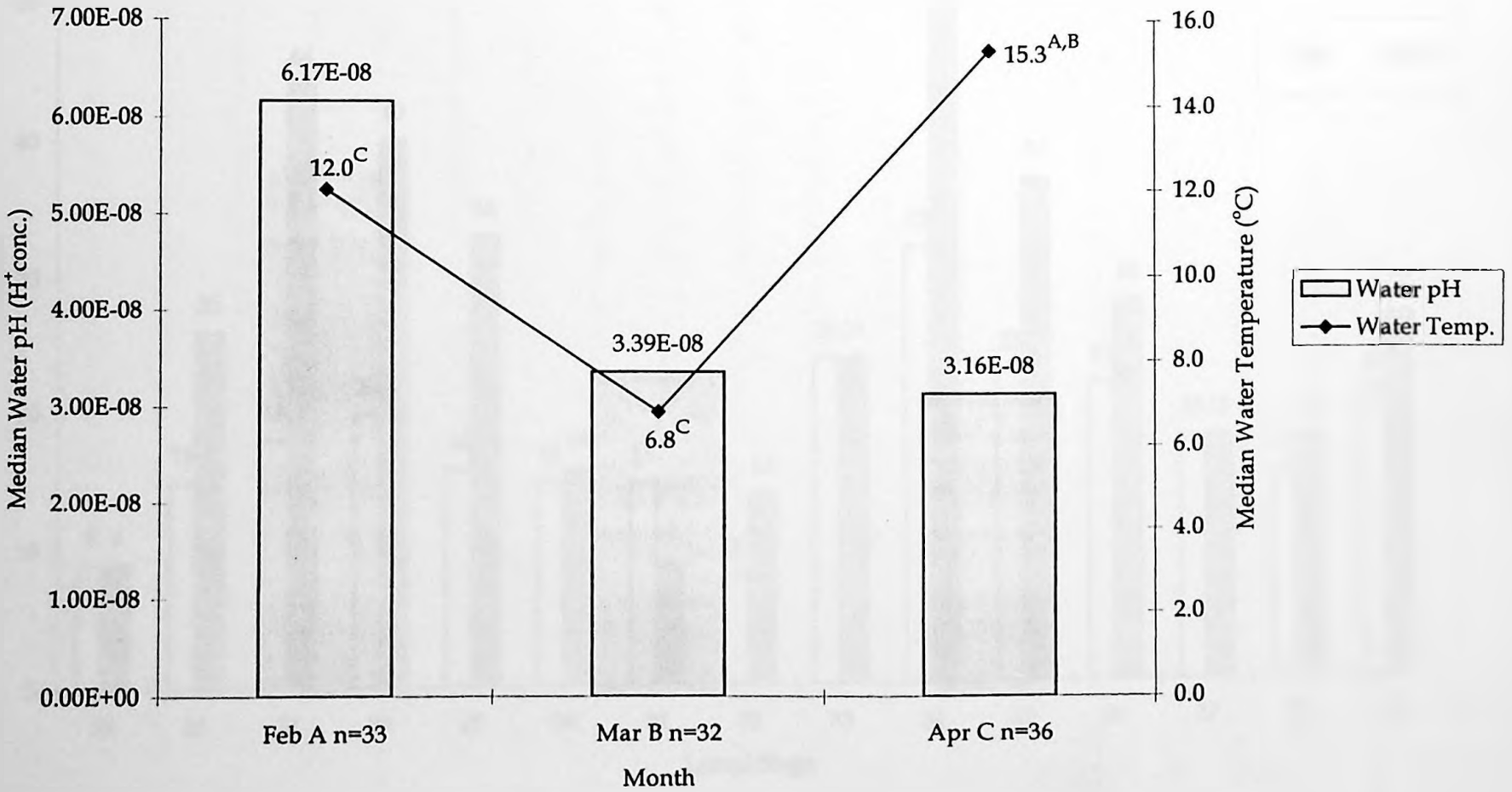
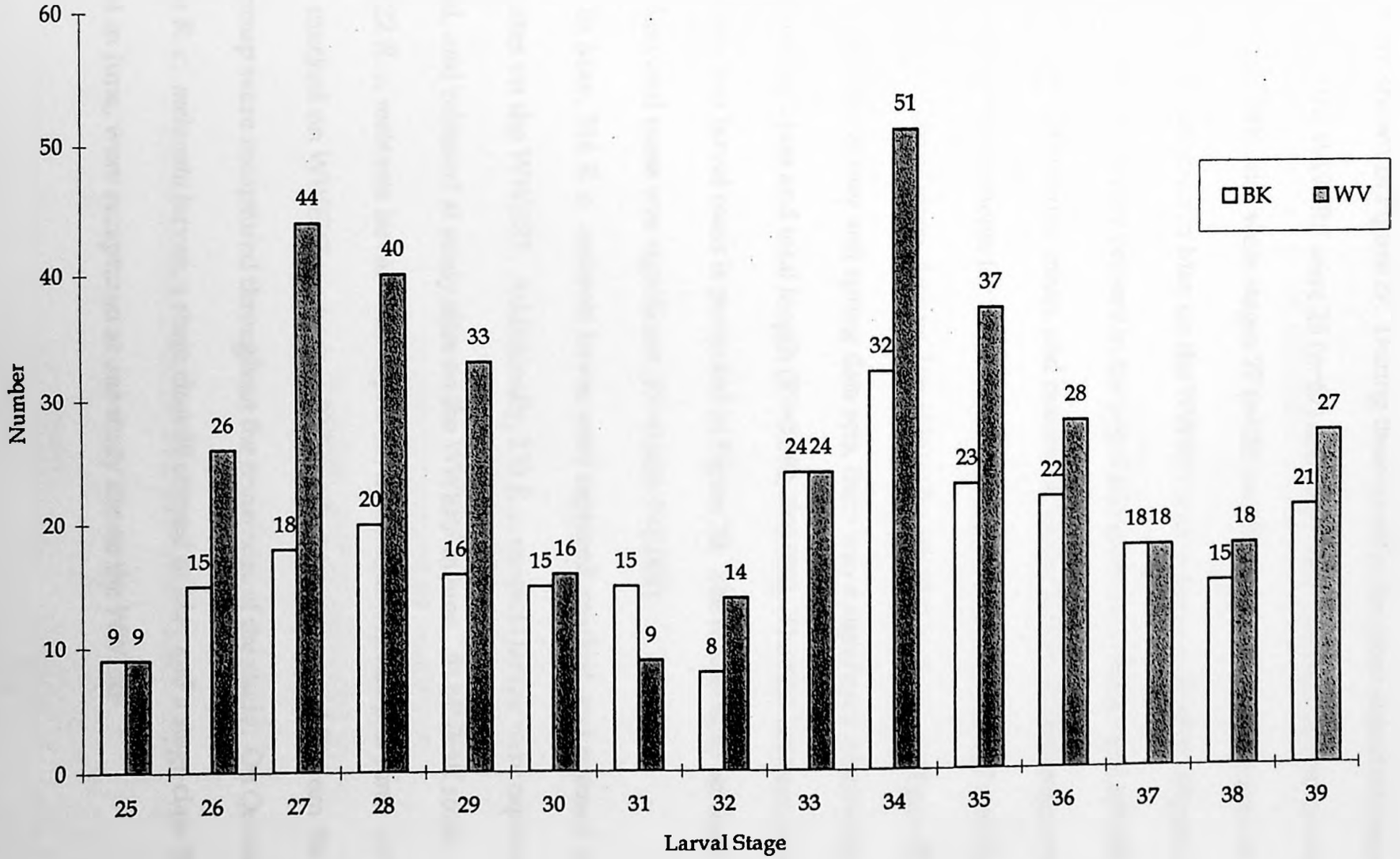


Figure 26: MNF Barton Knob (BK) and Westvaco WERF (WV) May and June 1997 larval stage frequency histogram



abundant stages from the MNFBK sites. Larval stages captured in July and August are shown in Figure 27. During these months, the most abundant stages captured on the WWERF were 25 (n=67) and 39 (n=46). The most abundant stages from the MNFBK sites were stages 27 (n=20) and 28 (n=27). The appearance of stage class 40 occurred in May on the WWERF and in June on MNFBK (Figure 28). These stage classes were present in the populations on the WWERF and MNFBK until October. Minimum, mean, and maximum values for larval total length and larval mass measurements for the summer data set are listed in Tables 7 and 8, respectively. Larval stage class vs. larval total length SLR is shown in Figure 29. As with the late winter and spring data sets, there was a significant relationship between stage class and total length (R^2=0.692; P<0.001). The SLR between larval stage class and larval mass is presented in Figure 30. The relationship between stage class and mass was significant (R^2 =0.660; P<0.001).

In May, 316 *R. c. melanota* larvae were captured, marked, and released at study sites on the WWERF. Additionally, 135 *R. c. melanota* larvae were captured, marked, and released at study sites on the WWERF in June. At MNFBK study sites, 122 *R. c. melanota* larvae were captured, marked, and released in June. Of the larvae marked on WWERF study sites, 23 from the May group and 20 from the June group were recaptured throughout the remainder of the study. On October 17, two *R. c. melanota* larvae, a stage class 39 clipped in May and a stage class 28 clipped in June, were recaptured at one study site on the WWERF

Figure 27: MNF Barton Knob (BK) and Westvaco WERF (WV) July and August 1997 larval stage frequency histogram

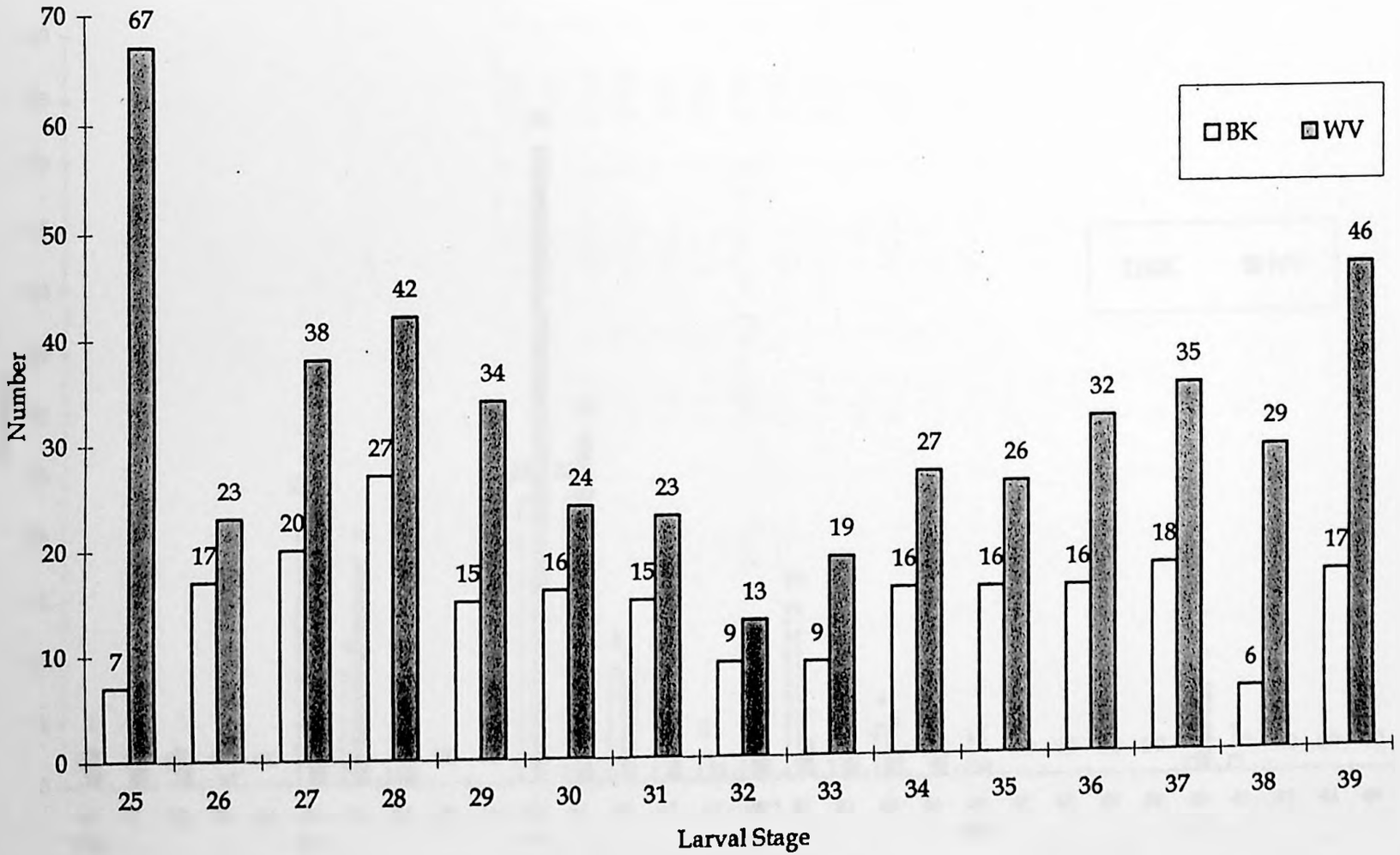


Figure 28: Histogram of larval stages 40-44 captured from May through October 1997 at MNF Barton Knob (BK) and Westvaco WERF (WV) study sites

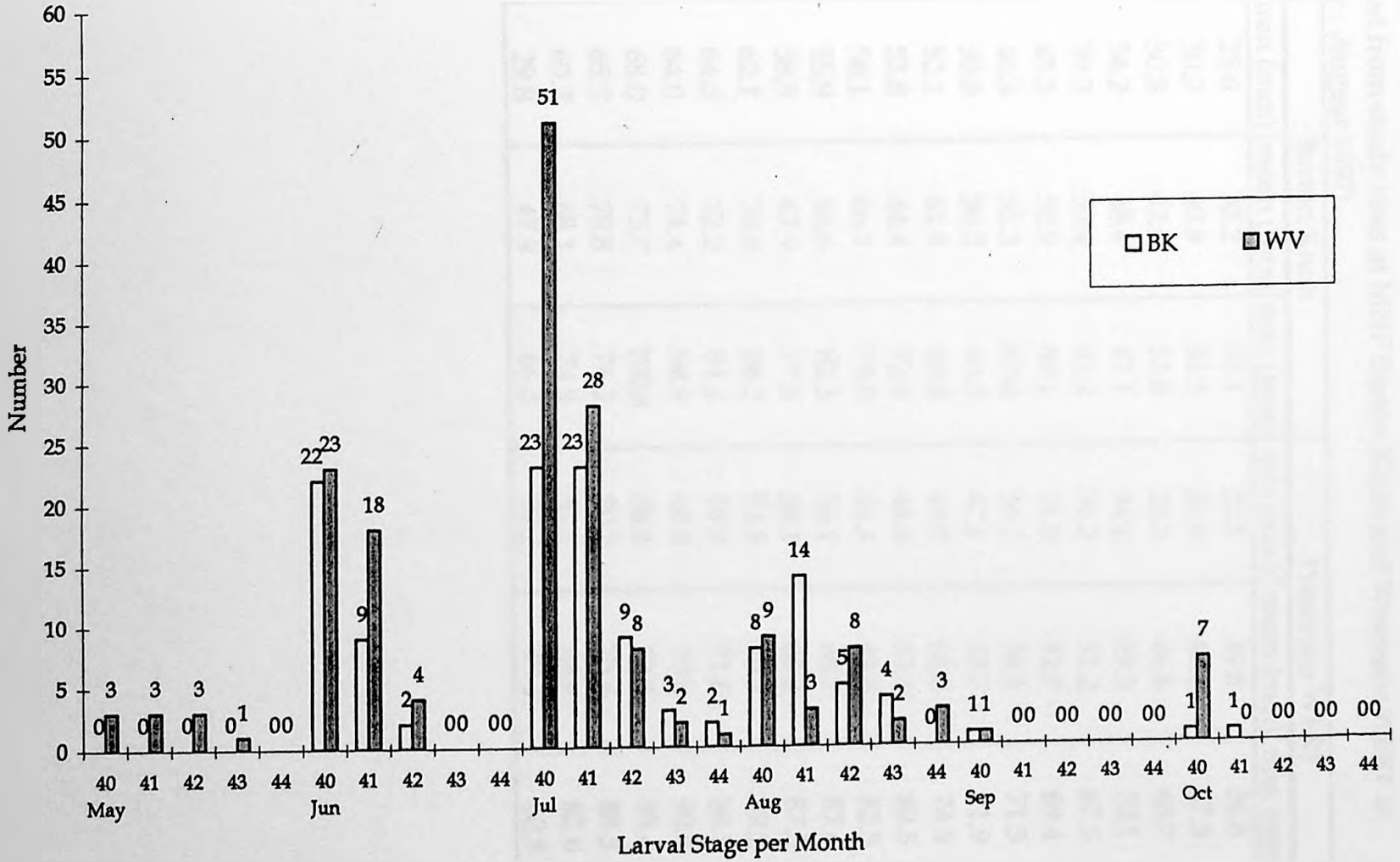


Table 7: Minimum, mean, and maximum total length values for *R. c. melanota* larvae captured from study sites at MNF Barton Knob and Westvaco WERF in summer (May - August 1997).

Larval Stage	Barton Knob			Westvaco WERF		
	min (mm)	mean (mm)	max (mm)	min (mm)	mean (mm)	max (mm)
25	25.6	32.2	38.1	22.5	39.8	56.6
26	30.2	40.9	51.5	26.8	40.3	57.5
27	30.3	42.6	53.8	22.0	46.8	68.7
28	34.2	48.4	67.1	34.4	49.3	70.1
29	39.3	50.4	61.2	36.2	51.2	67.5
30	43.3	52.9	60.1	31.5	52.7	69.4
31	46.3	55.3	63.4	36.2	54.3	71.5
32	50.0	56.2	63.3	47.1	57.7	71.9
33	52.1	61.8	69.8	49.0	61.0	74.9
34	53.8	64.4	72.6	48.0	63.4	80.5
35	56.1	66.1	79.9	51.3	65.3	82.5
36	55.9	66.6	82.3	56.1	66.7	87.6
37	58.3	67.9	77.5	58.1	68.3	87.5
38	62.1	70.9	88.2	54.3	70.7	89.2
39	64.5	72.2	84.4	59.9	71.6	86.5
40	64.0	74.4	98.9	60.3	70.8	90.4
41	68.0	75.7	100.8	58.4	72.3	93.4
42	65.7	70.8	76.2	50.2	73.6	88.5
43	63.7	68.1	74.9	51.1	66.9	82.6
44	29.8	47.9	66.0	33.2	49.4	60.5

Table 8: Minimum, mean, and maximum mass values for *R. c. melanota* larvae captured from study sites at MNF Barton Knob and Westvaco WERF in summer (May - August 1997).

Larval Stage	Barton Knob			Westvaco WERF		
	min (g)	mean (g)	max (g)	min (g)	mean (g)	max (g)
25	0.17	0.38	0.72	0.11	0.75	1.91
26	0.26	0.69	1.43	0.28	0.75	1.57
27	0.30	0.83	1.48	0.20	1.14	3.38
28	0.48	1.09	1.85	0.37	1.30	2.72
29	0.65	1.26	2.19	0.60	1.43	2.75
30	0.92	1.48	2.06	0.76	1.63	2.90
31	1.03	1.67	2.26	0.84	1.60	3.50
32	1.26	1.74	2.53	0.92	1.82	3.05
33	1.43	2.40	3.45	1.00	2.20	3.83
34	1.31	2.60	3.75	1.06	2.48	4.55
35	1.59	2.91	4.83	1.20	2.56	4.98
36	1.59	2.93	4.87	1.39	2.79	4.82
37	1.91	3.08	4.64	1.65	3.01	5.50
38	2.07	3.31	5.56	1.74	3.30	5.89
39	2.58	3.70	6.30	1.75	3.37	5.43
40	2.81	4.00	6.50	2.16	3.34	5.85
41	2.77	3.94	7.12	2.20	3.54	6.40
42	2.02	3.07	4.18	1.67	3.42	5.83
43	2.15	2.96	4.06	1.83	3.29	4.31
44	2.24	3.44	4.64	2.00	2.78	3.49

Figure 29: MNF Barton Knob and Westvaco WERF summer (May - August 1997) larval stage vs. total length linear regression graph

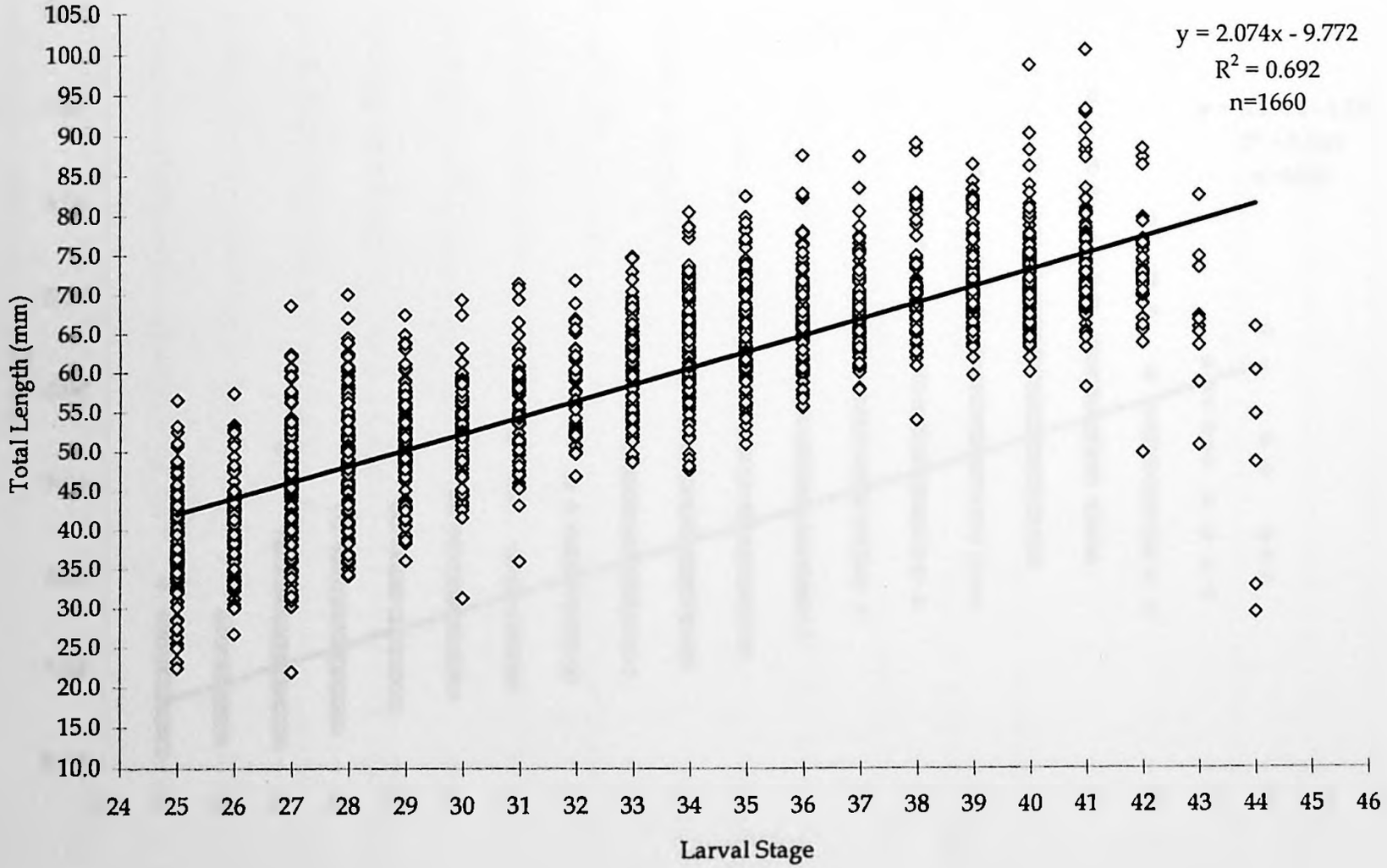
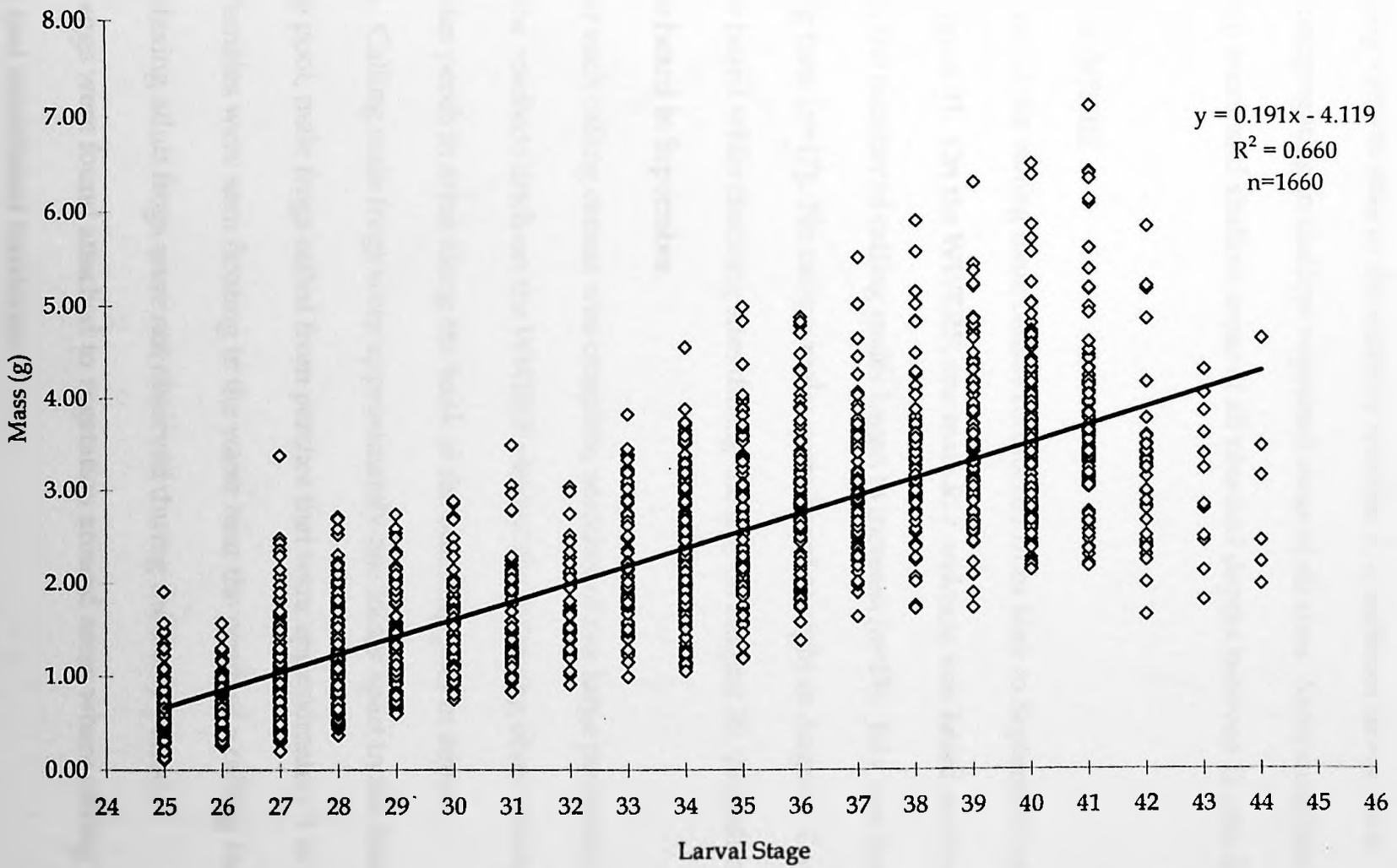


Figure 30: MNF Barton Knob and Westvaco WERF summer (May - August 1997) larval stage vs. mass linear regression graph



(Figure 13). Of the larvae marked on MNFBK study sites, 8 from the June group were recaptured throughout the remainder of the study period.

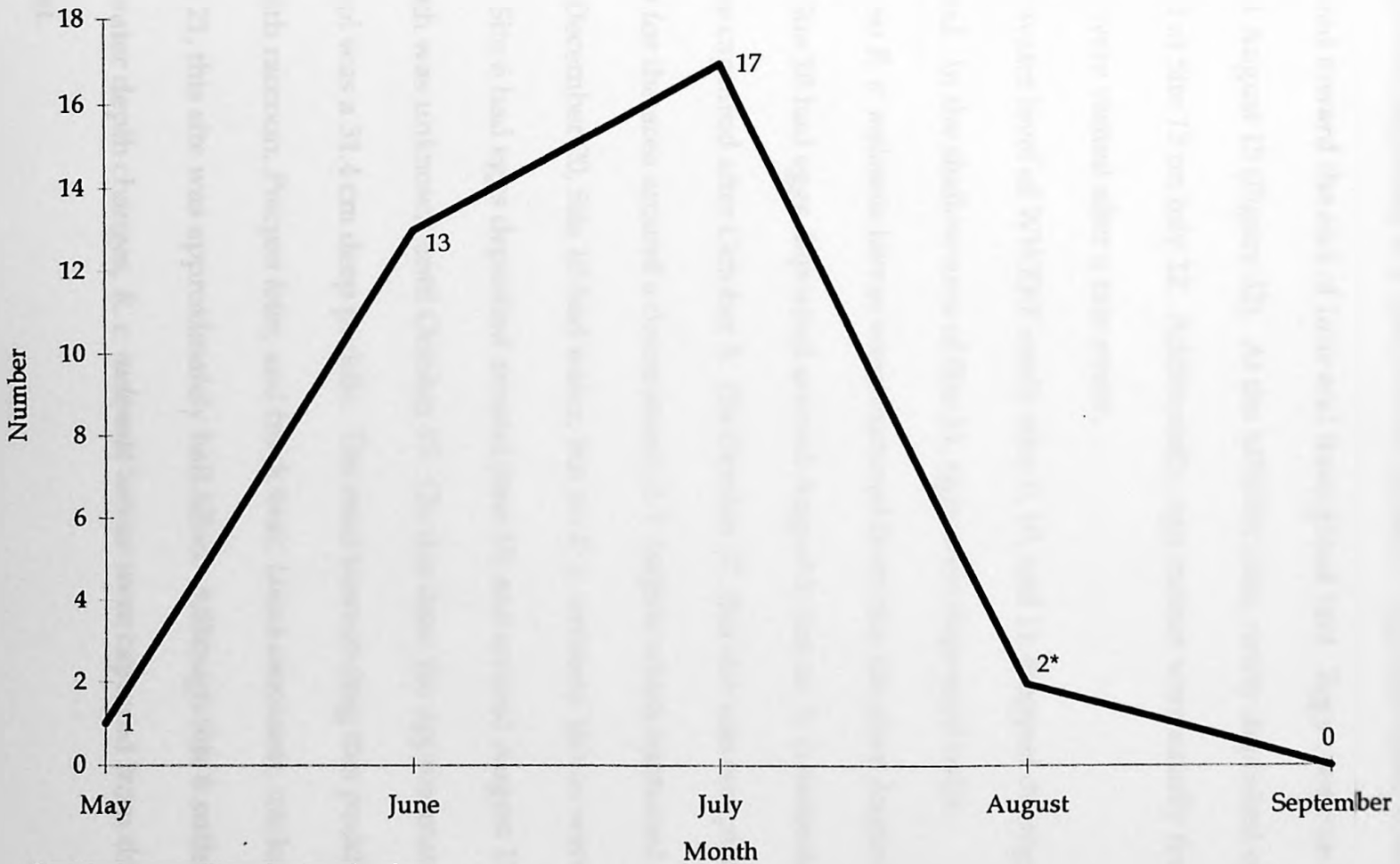
During visits to sites in the summer months, *R. c. melanota* larvae were often seen congregating in shallow vegetated areas of all sites. Most study sites were in open areas, and shallow areas of all sites had depths between 12 and 20 cm.

Breeding Activity

Results of the calling male census conducted from May to September are shown in Figure 31. On the WWERF, one male *R. c. melanota* was heard on May 19. In June, the number of calling males began to increase (n=13). July was the peak calling time (n=17). No calling males were heard at night in August. Two males were heard while checking sites during the day on August 30. No calling males were heard in September.

After each calling census was complete, searches of one large permanent pool and one roadside ditch on the WWERF yielded the following observations. Calling males perch in areas along the bank of the breeding pool in dense vegetation. Calling male frogs were approximately one meter apart in the ditch. In the large pool, male frogs called from perches that were approximately 1 to 3.5 m apart. Females were seen floating in the water near the perch of a calling male frog. Amplexing adult frogs were not observed during the study period. However, eggs were found attached to vegetation around areas where calling male frogs had established territories.

Figure 31: Line graph of *R. c. melanota* calling males at Westvaco WERF study sites from May to September 1997



* Male frogs calling during the day.

On the WWERF, *R. c. melanota* eggs were first discovered on June 19. These eggs were deposited in a shallow area of Site 6. Egg deposition at other sites occurred toward the end of June and throughout July. Eggs continued to be found until August 15 (Figure 32). At the MNFBK sites, newly deposited eggs were found at Site 13 on July 12. Additionally, egg masses were usually found when sites were visited after a rain event.

The water level of WWERF study sites 6, 10, and 11 dropped during the study period. In the shallow area of Site 11, eggs were deposited twice. However, no *R. c. melanota* larvae were captured from this site after August 16. Similarly, Site 10 had eggs deposited around August 1, but no *R. c. melanota* larvae were captured after October 3. On October 17, this site was completely dry except for the area around a dense stand of *T. latifolia* which contained soft mud. On December 20, Site 10 had water, but no *R. c. melanota* larvae were captured. Site 6 had eggs deposited around June 19, and around August 15. The pool's depth was unknown until October 17. On this date, the approximately 2.1 m deep pool was a 31.4 cm deep puddle. The mud surrounding this puddle was covered with raccoon, *Procyon lotor,* and black bear, *Ursus americana,* tracks. On December 21, this site was approximately half filled. Although Site 6 suffered dramatic water depth changes, *R. c. melanota* larvae were captured from this site at each visit.

Figure 32: Photograph of *R. c. melanota* eggs collected on August 15, 1997 at Site 6.

The two ditches, Site 4 and Site 5 had eggs deposited between August 1 and August 16. Eggs were never found at the oxbow site (Site 9). Furthermore, after August 1, few *R. c. melanota* larvae were captured from this site.

Figure 33 shows mean total length measurements of egg and early larval stages of *R. c. melanota* that were collected at various study sites on the WWERF. Newly deposited *R. c. melanota* eggs (stage class 2), had an average diameter (shown as total length) of 1.67 mm. Early stage class 25 larvae ranged from 9.77 to 16.00 mm.

Froglet dispersal, size, and diet

Rana c. melanota froglet dispersal from pitfall trap data collected from Site 6 is shown in Figure 34. Froglets inside the pool area were first found in pitfall traps in June (n=13). *Rana c. melanota* froglet dispersal increased in July (n=115), peaked in August (n=151), and sharply declined in September (n=3). October (n=6) was the final month *R. c. melanota* froglets were found in the pool. Twenty-two of the 310 froglets captured in pitfall traps were found outside the pool area.

Figures 35 through 37 are length frequency histograms for the froglet, juvenile, subadult, and adult morphological characters of SUL, CW, and RTL, respectively. Figure 35 shows the morphological character SUL. Most *R. c. melanota* froglets had a SUL between 26.0 and 26.9 mm. Figure 36 shows the morphological character CW. Most froglets had a CW between 9.0 and 9.9 mm. Figure 37 shows the morphological character RTL. Most froglets had a RTL between 14.0 and 14.9 mm. SUL and CW ranked median values were found to

Figure 33: Graph of lengths of early stage *R. c. melanota* larvae collected from Westvaco WERF study sites (June - August 1997).

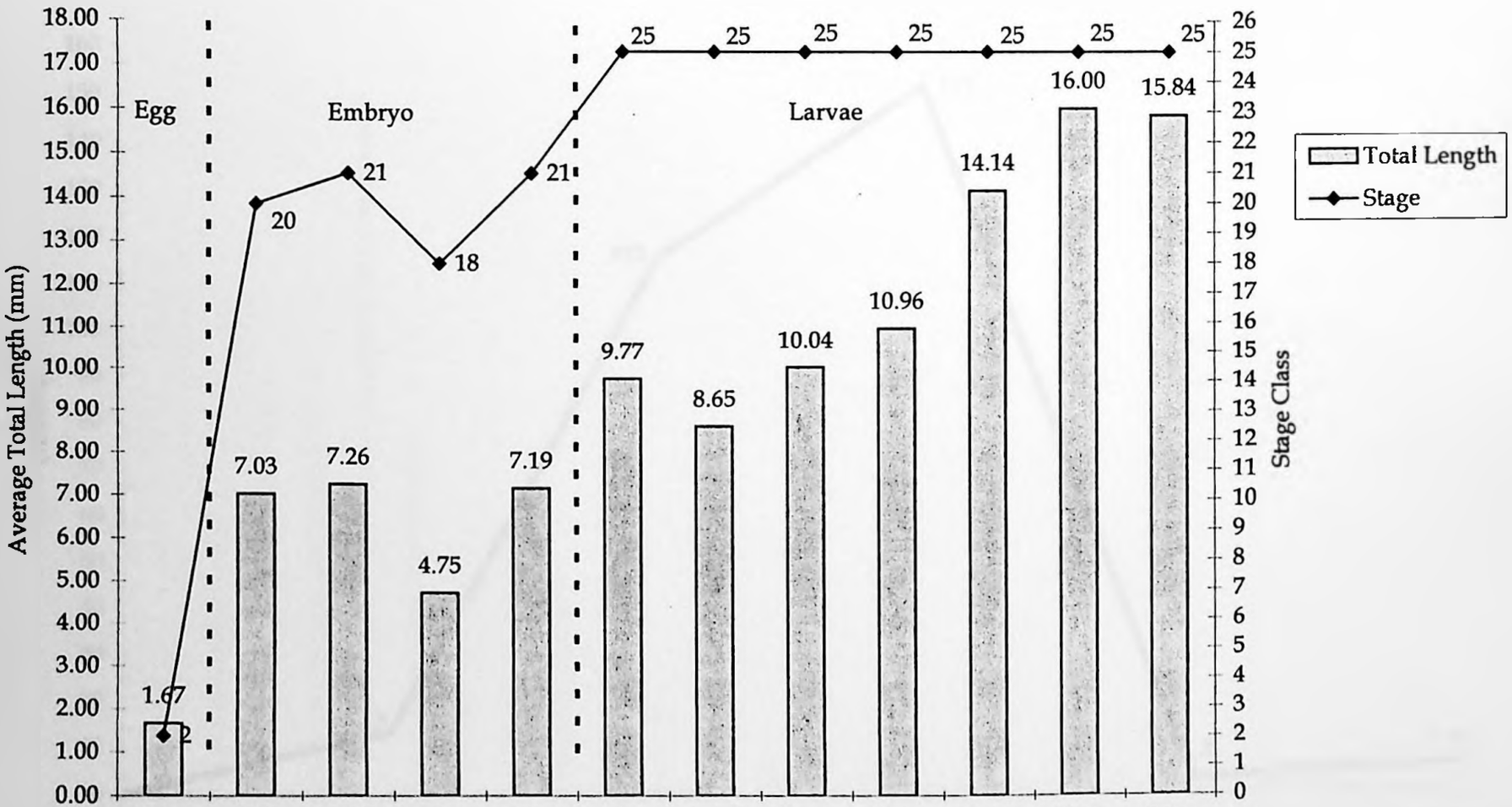


Figure 34: Line graph of *R. c. melanota* froglet dispersal from Westvaco WERF Site 6 (May - October 1997)

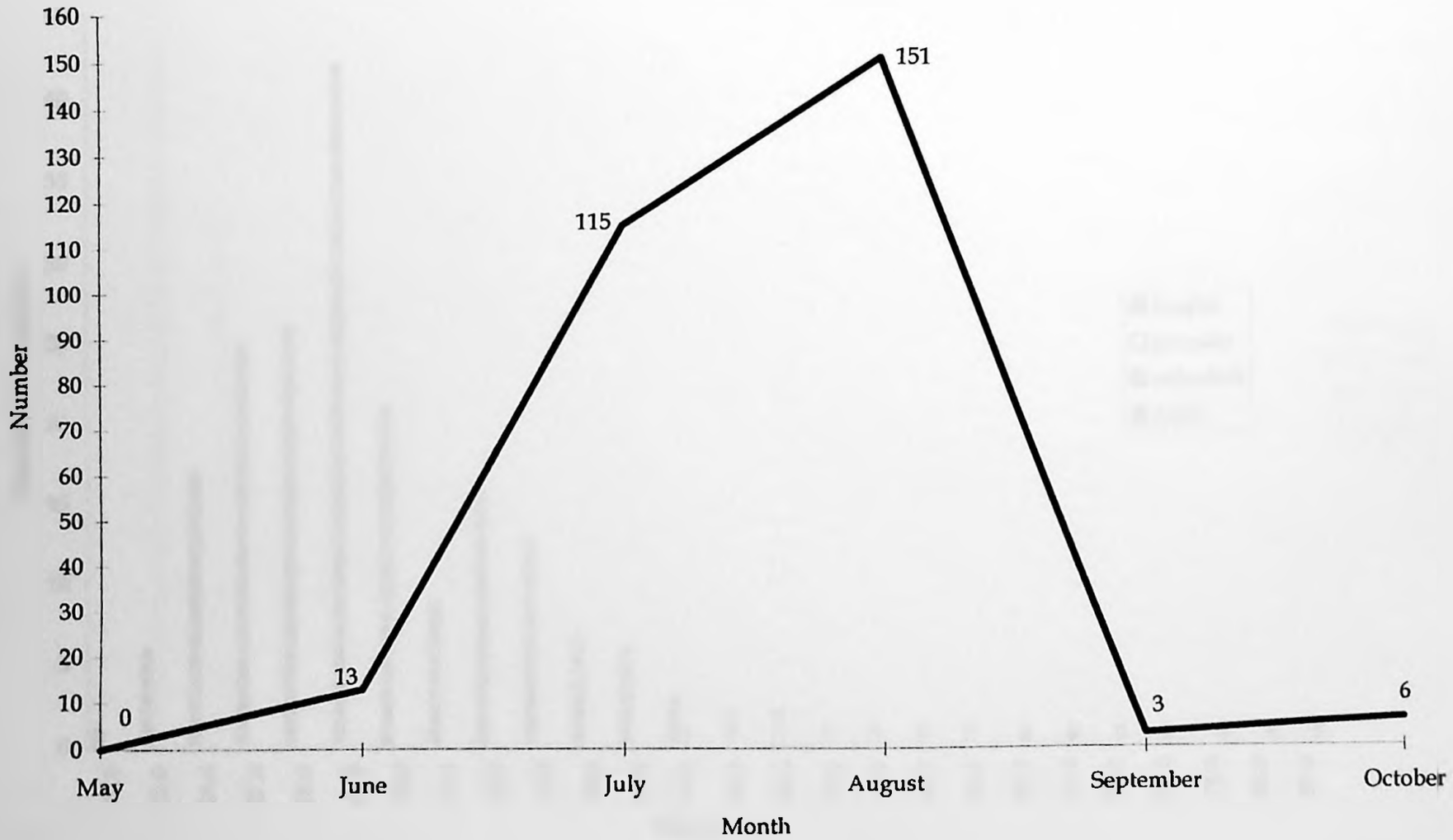


Figure 35: Length frequency histogram for *R. c. melanota* frog snout-to-urostyle length (SUL)

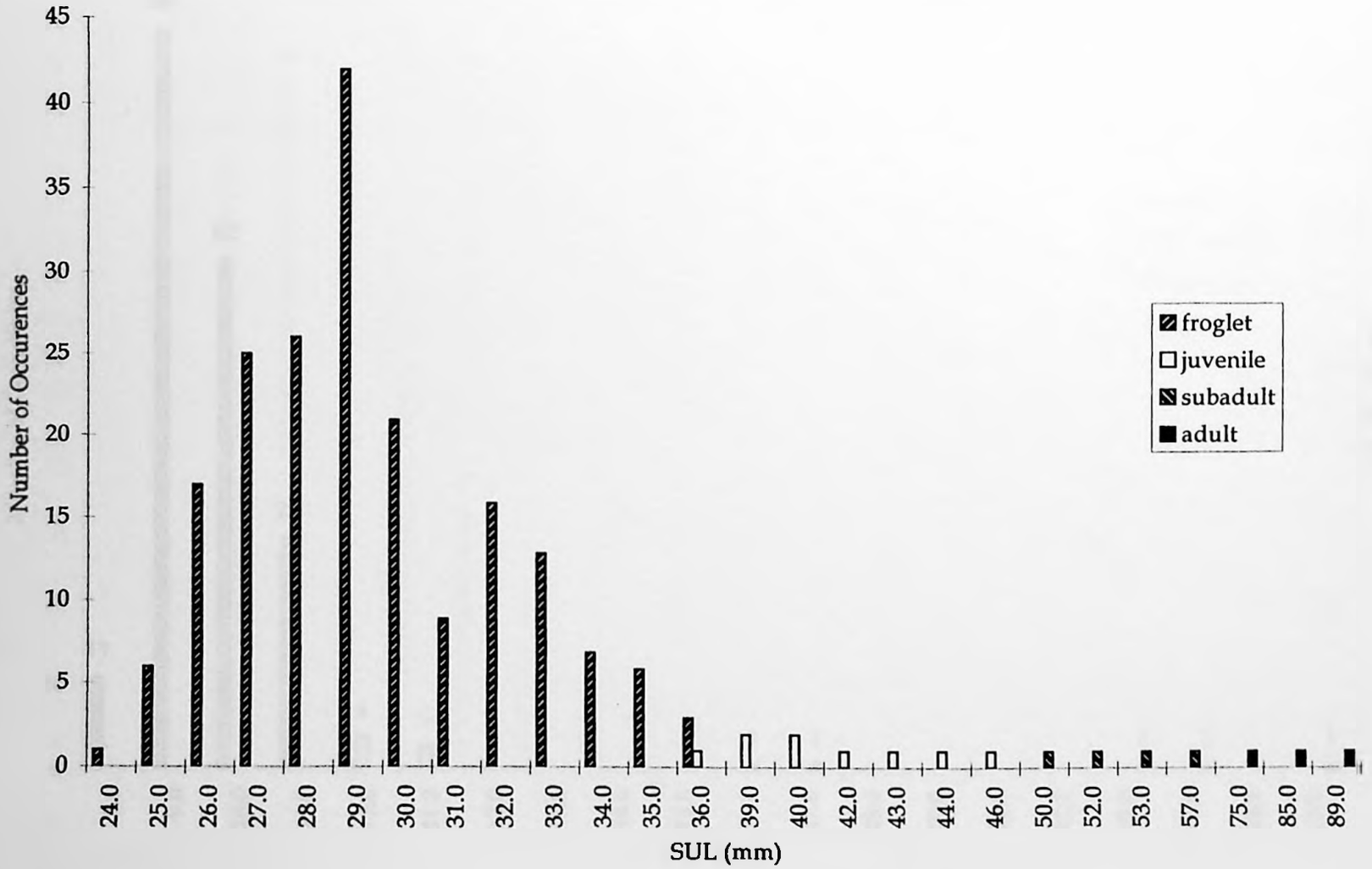


Figure 36: Length frequency histogram for *R. c. melanota* frog cranial width (CW)

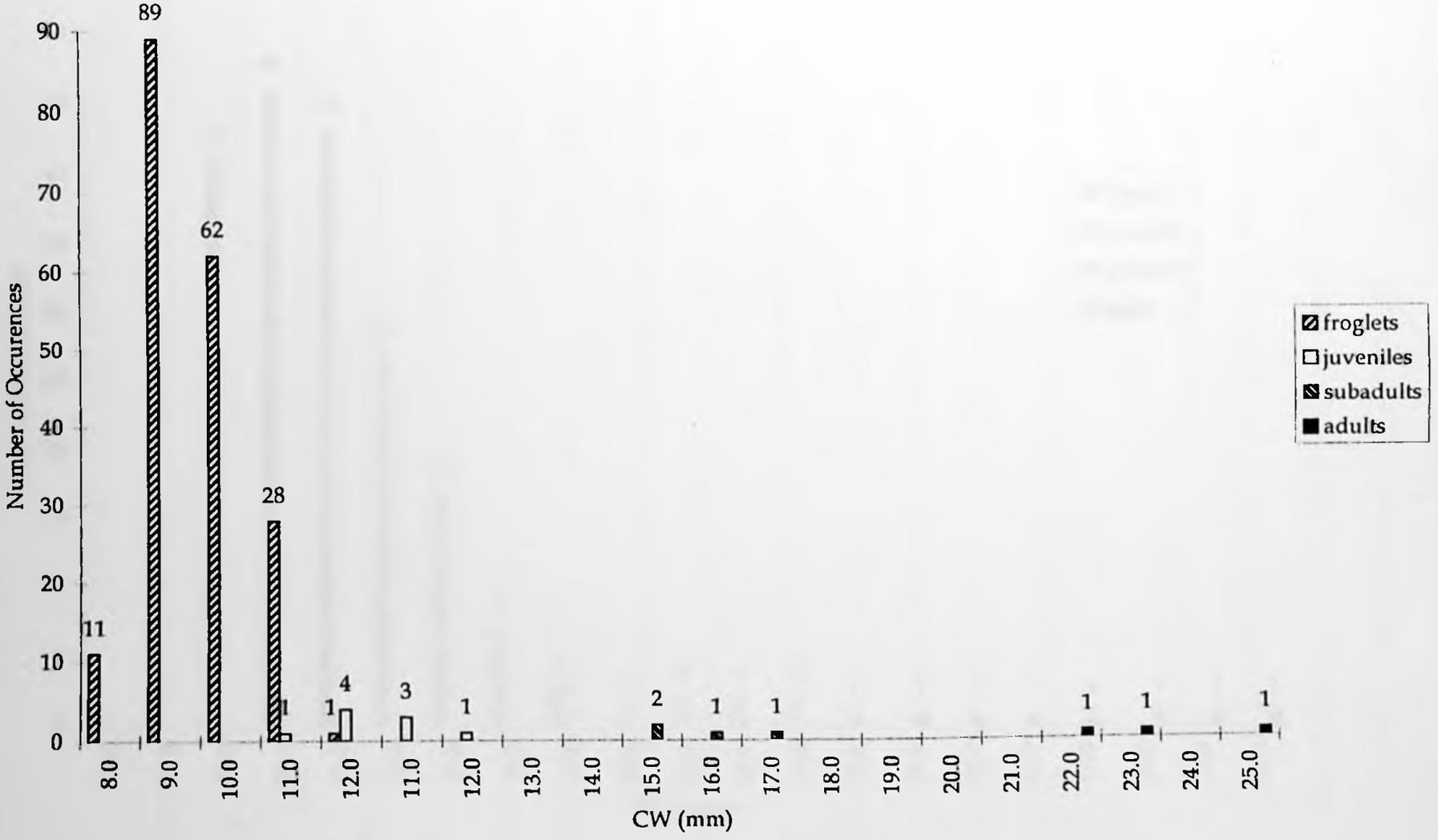
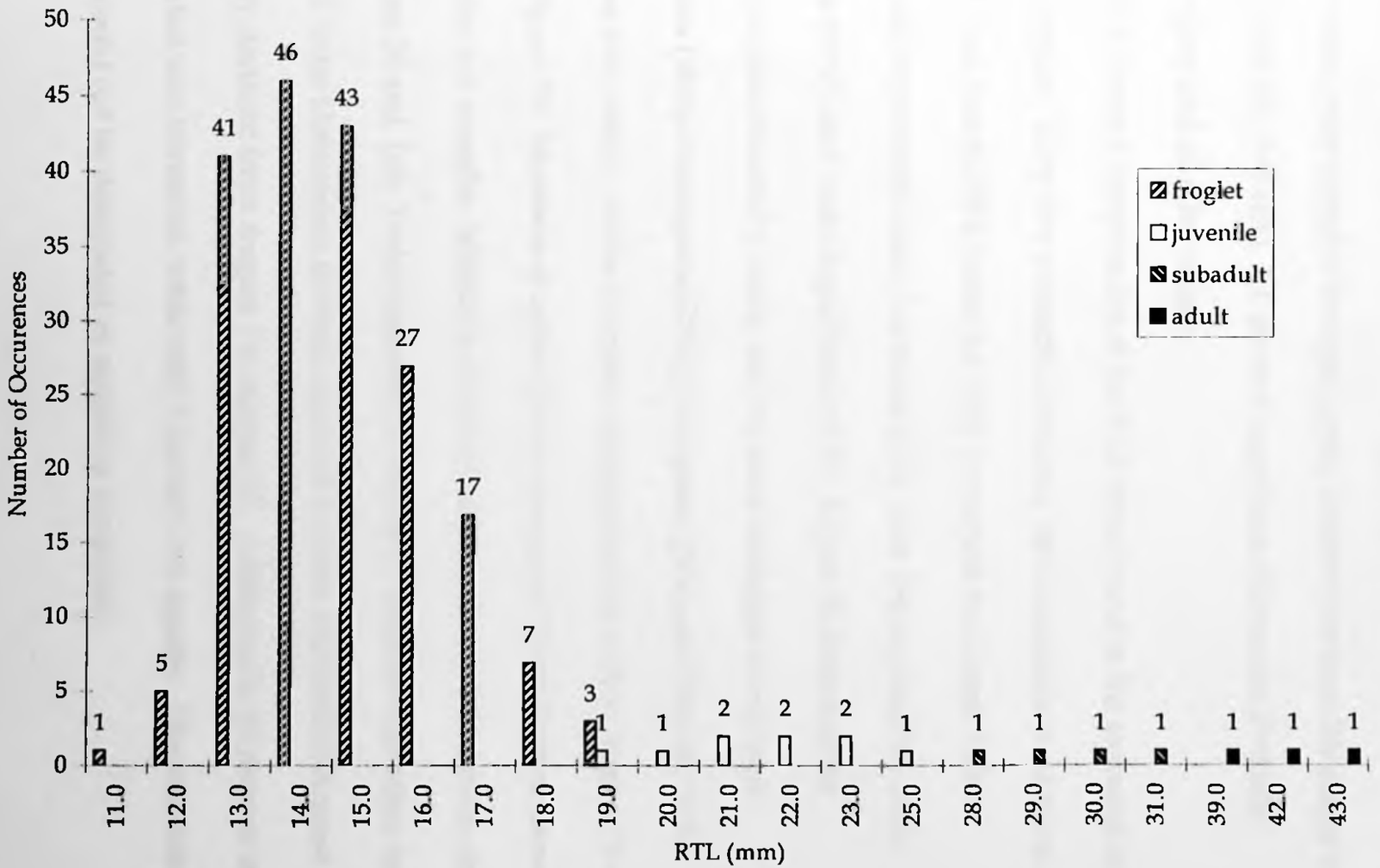


Figure 37: Length frequency histogram for *R. c. melanota* frog right tibia length (RTL)



be significantly different between life stages by K-WANOVA (SUL: H=44.621; 4 df; P<0.001; CW: H=44.632; 4 df; P<0.001). DM showed significant differences (P<0.05) between froglets and all other life stages for SUL and CW characters. RTL mean values were found to be significantly different between life stages by ANOVA (F=306.725; P<0.001). TT showed significant differences (P<0.001) between froglets and all other stages.

Table 9 shows a complete list of the food items found in the stomachs of *R. c. melanota* froglets. Sixty-five stomachs contained 341 identifiable food items. Animals of class Insecta (93%) were the most prominent food item. Other animals found represented class Arachnida (4%), class Diplopoda (2%), class Gastropoda (<1%), and class Oligochaeta (<1%). Figure 38 illustrates the breakdown of class Insecta by order, and the most abundant orders were Hymenoptera (28%), Homoptera (27%), Coleoptera (21%), and Diptera (13%). Within these four orders, stomach content changes from June 20 to August 15 are shown in Figure 39. Members of order Hymenoptera and Order Diptera showed distinct peaks and troughs. Members of order Coleoptera were taken more often between June 20 and July 3 when members of the order Diptera were taken less. Members of order Homoptera showed a gradual increase and peaked August 1, then sharply declined from August 1 to August 15. Additionally, 83 percent of the froglet diet was terrestrial, while only 4 percent was aquatic. The remaining 13 percent could not be designated as aquatic or terrestrial.

Table 9: Detailed Contents of *R. c. melanota* Froglet Stomachs (n=65)

Class	Order	Family	Number
Insecta	Hymenoptera	Formicidae	49
Insecta	Hymenoptera	*	40
Insecta	Homoptera	*	85
Insecta	Coleoptera	Curculionidae	10
Insecta	Coleoptera	Dytiscidae	9
Insecta	Coleoptera	Staphylinidae	6
Insecta	Coleoptera	Chrysomelidae	4
Insecta	Coleoptera	Carabidae	2
Insecta	Coleoptera	Cerambycidae	2
Insecta	Coleoptera	Noteridae	2
Insecta	Coleoptera	Nitidulidae	1
Insecta	Coleoptera	Scarabaeidae	1
Insecta	Coleoptera	*	30
Insecta	Diptera	Stratiomyidae	8
Insecta	Diptera	Tipulidae	1
Insecta	Diptera	*	32
Insecta	Lepidoptera	*	17
Insecta	Hemiptera	Miridae	4
Insecta	Hemiptera	Nabidae	2
Insecta	Hemiptera	*	4
Insecta	Orthroptera	*	4
Insecta	Collembola	*	2
Insecta	Odonata	Coenagrionidae	1
Arachnida	Araneae	*	15
Diplopoda	Polydesmida	*	5
Gastropoda	*	*	3
Oligochaeta	*	*	2

Figure 38: Percent of insect orders consumed by *R. c. melanota* froglets from 20 June to 15 August 1997 by Order

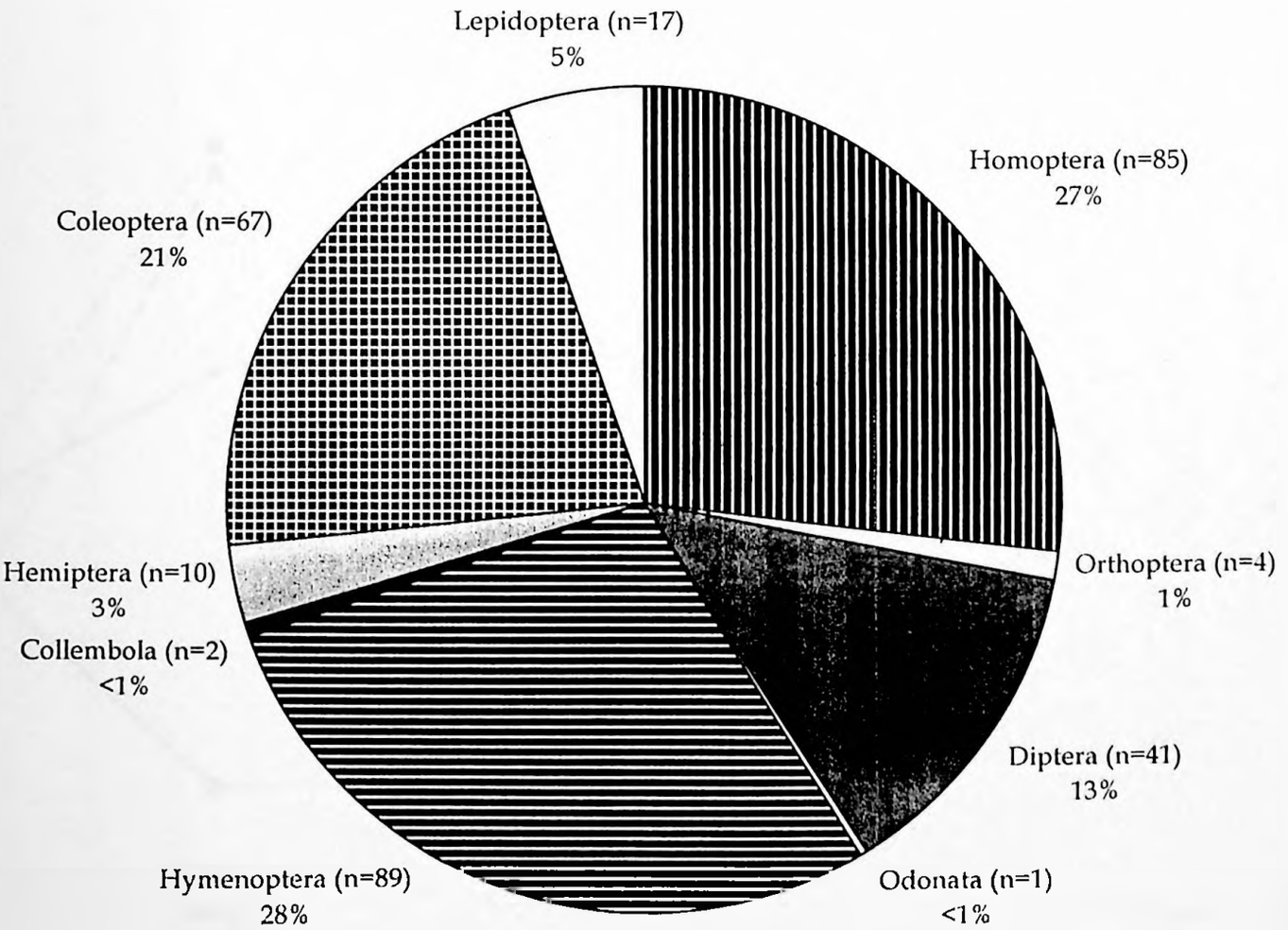
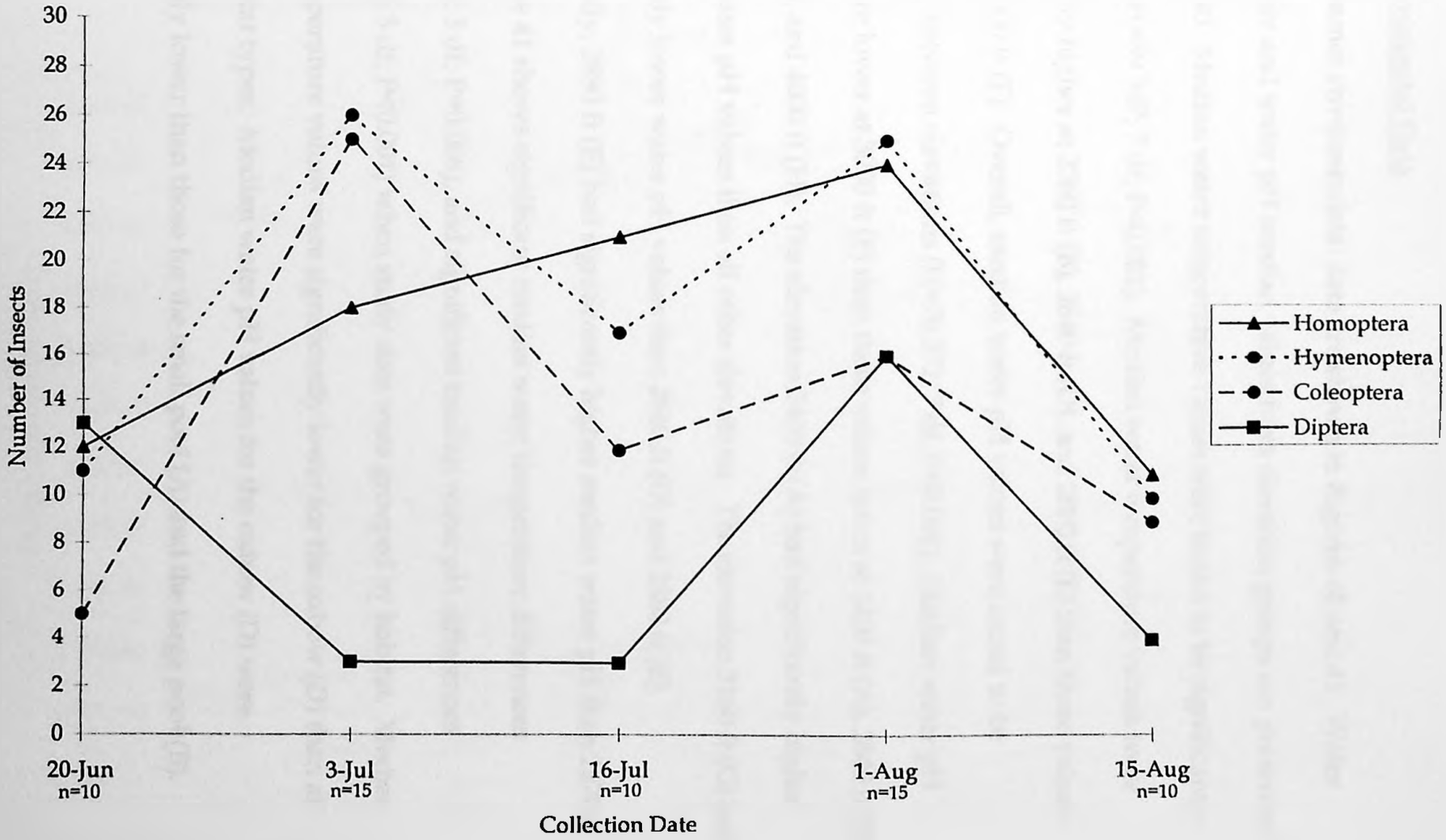


Figure 39: Line graph of *R. c. melanota* froglet stomach content changes from 20 June to 15 August 1997.



Environmental Data

Summer environmental data are shown in Figures 40 and 41. Water temperature and water pH median values from elevation groups are presented in Figure 40. Median water temperature values were found to be significantly different ($H=39.547$; 7 df; $P<0.001$). Median water temperature values were significantly higher at 2560 ft (B), 2840 ft (D), and 2880 ft (E) than those values taken at 3000 ft (F). Overall, median water pH values were found to be significant between elevations ($H=76.572$; 7 df; $P<0.001$). Median water pH values were lower at 3000 ft (F) than those values taken at 2400 ft (A), 2840 ft (D), 2880 ft (E), and 4000 ft (H). The elevation 2400 ft (A) had significantly higher median water pH values than all other elevations. The elevation 3160 ft (G) had significantly lower water pH values than 2840 ft (D) and 2880 ft (E). Additionally, 2880 ft (E) had significantly higher median water pH than 2570 ft (C). Figure 41 shows significant median water temperature differences ($H=21.330$; 3 df; $P=0.008$), and significant median water pH differences ($H=31.474$; 3 df; $P<0.001$) when study sites were grouped by habitat. Median water temperature values were significantly lower for the oxbow (D) than all other habitat types. Median water pH values for the oxbow (D) were significantly lower than those for the small pool (A) and the large pool (B).

Figure 40: MNF Barton Knob and Westvaco WERF summer (May - August 1997) environmental data (grouped by elevation)

Data labels with letters matching elevational group designations indicate significant differences in median values (K-WANOVA, P<0.05).

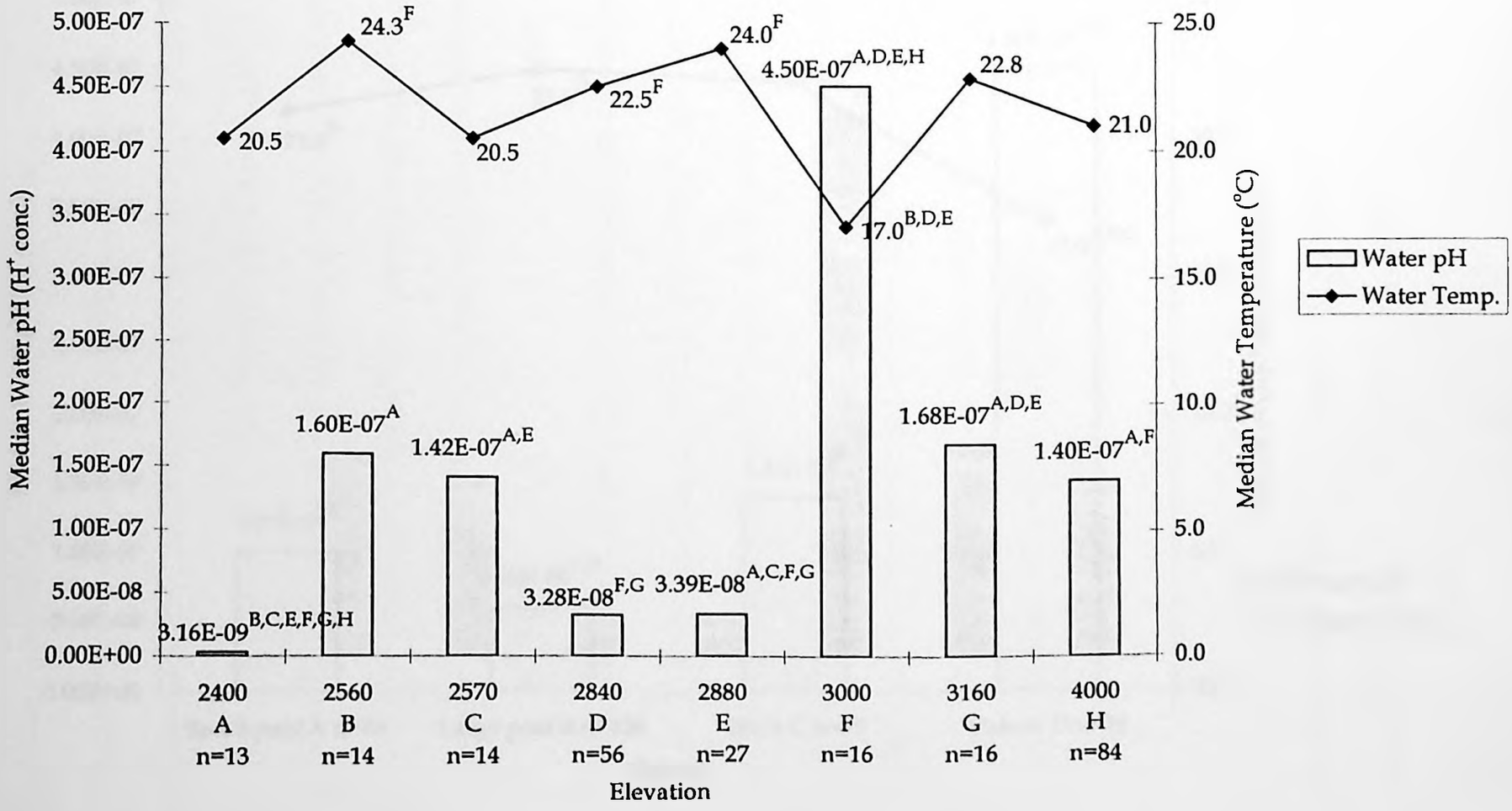
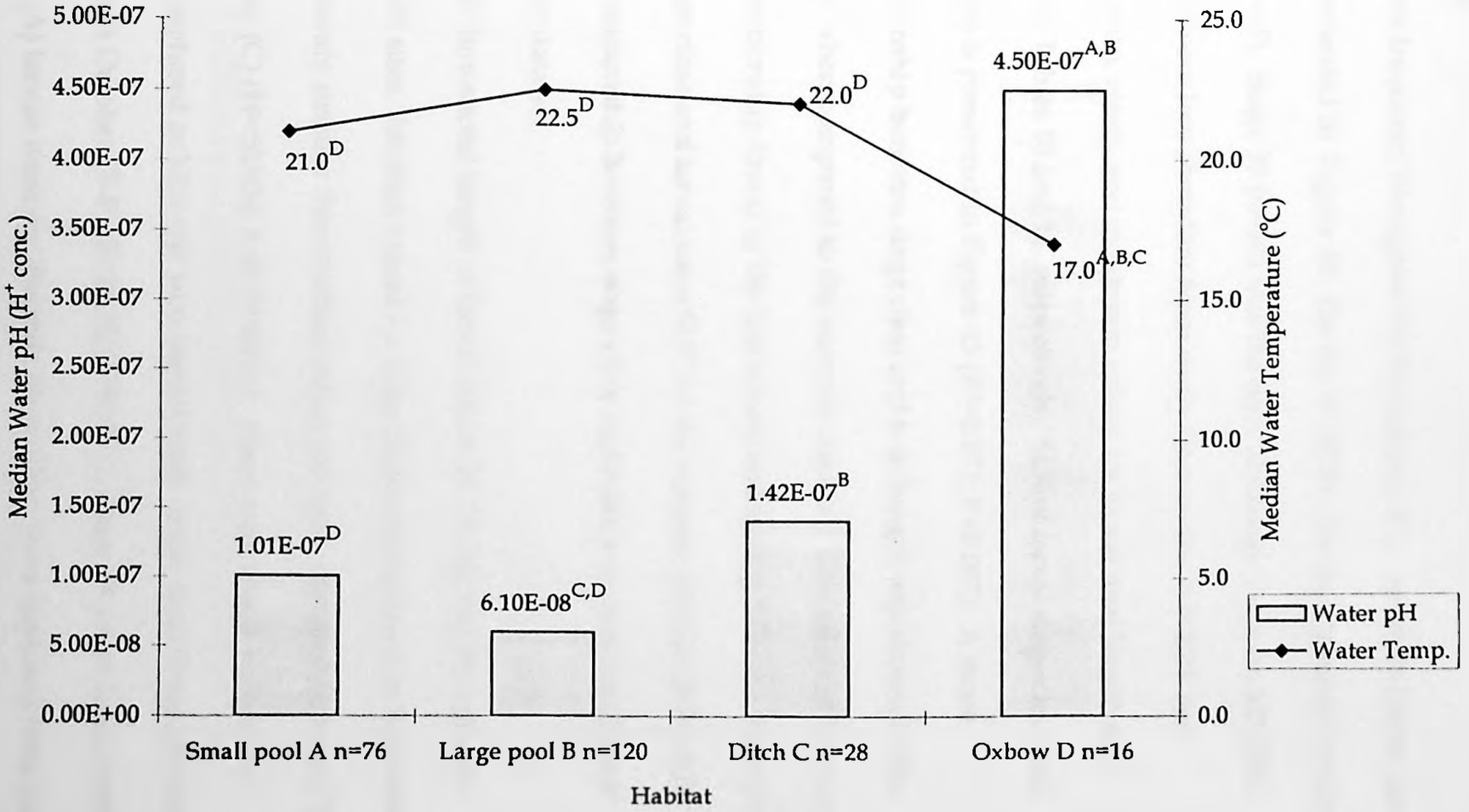


Figure 41: MNF Barton Knob and Westvaco WERF summer (May - August 1997) environmental data (grouped by habitat)

Data labels with letters matching habitat group designations indicate significant differences in median values (K-WANOVA, P<0.05).



Autumn and Early Winter (September 1997 - December 1997)

Larvae Data

Stage class frequency histogram for the autumn *R. c. melanota* larvae data set (n=296) is presented in Figure 42. On the WWERF, the most abundant stage class was 26 (n=43). Stage 35 (n=10) was the most abundant stage on MNFBK. Stages 40 and 41 were less abundant from study sites on the WWERF and MNFBK. Minimum, mean, and maximum values for larval total length and larval mass are in Tables 10 and 11, respectively. SLR of larval stage class and larval total length is presented in Figure 43 (R^2=0.871; P<0.001). A more significant relationship between stage class and total length was shown in the autumn data set when compared to the summer data set. This relationship was similar to the relationship found in the late winter and spring data set. Figure 44 is the larval stage class and larval mass SLR for the autumn data set (R^2=0.810; P<0.001). The relationship between stage class and mass was more significant than the summer data set.

Figure 45 shows total length of larval stages 26, 32, 36, and 38 captured from the WWERF sites. Median values for stage 26 larvae captured in September (E) were significantly smaller than median values for those captured in May (A), June (B), and July (C) (H=50.934; 5 df; P<0.001). Mean total length values for stage 32 larvae captured in May (A) were significantly larger than those captured from June through October (B-F) (F=12.312; P<0.001). Stage 36 mean total length values for May (A) larvae were significantly larger than those captured from July

Figure 42: MNF Barton Knob (BK) and Westvaco WERF (WV) autumn (September - October 1997) larval stage frequency histogram

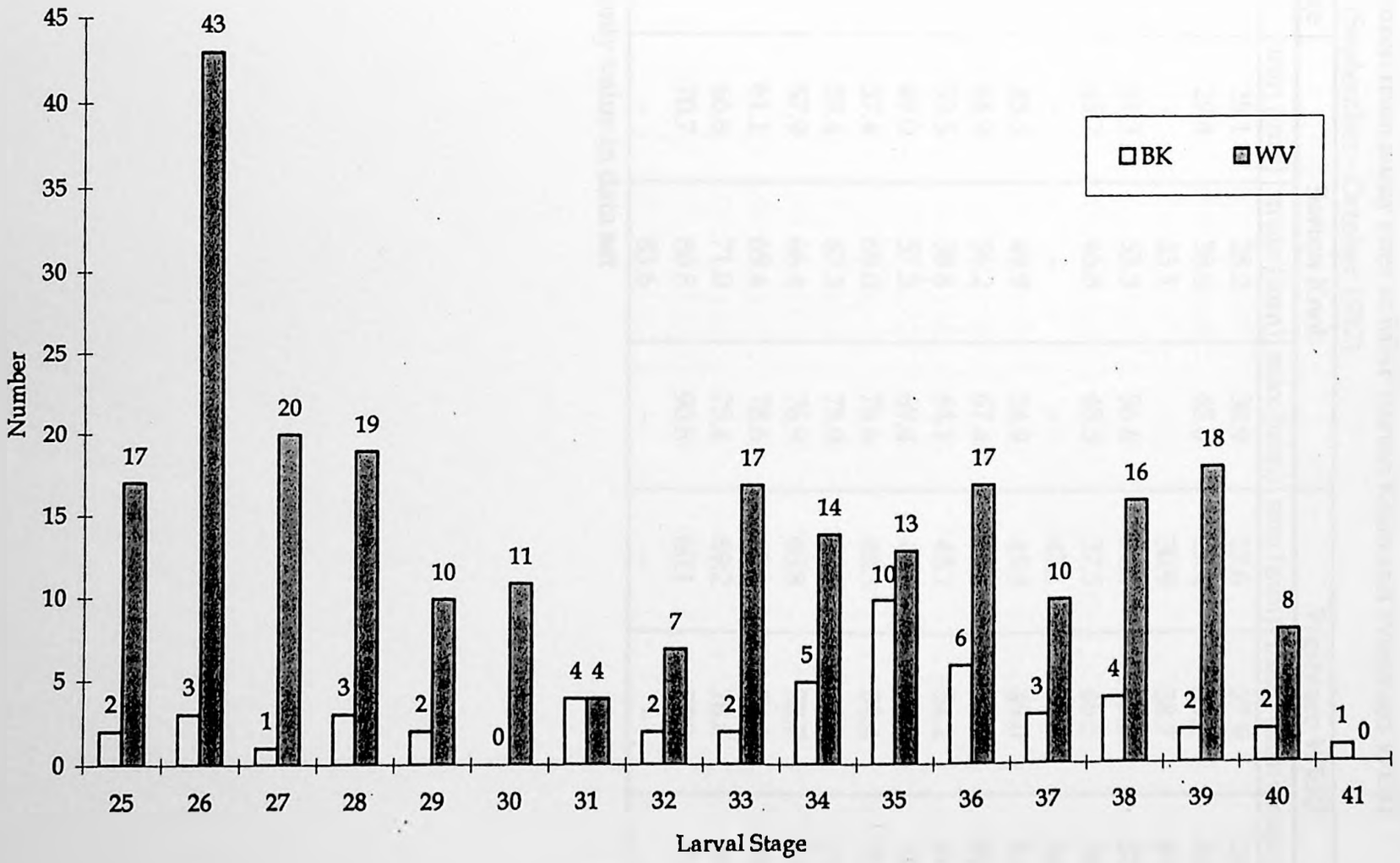


Table 10: Minimum, mean, and maximum total length values for *R. c. melanota* larvae captured from study sites at MNF Barton Knob and Westvaco WERF in autumn (September - October 1997).

Larval Stage	Barton Knob			Westvaco WERF		
	min (mm)	mean (mm)	max (mm)	min (mm)	mean (mm)	max (mm)
25	25.1	28.0	30.9	17.6	27.9	35.0
26	29.8	38.6	45.0	27.4	33.1	49.2
27	-	43.1	-	30.9	38.7	49.4
28	51.3	53.3	56.6	34.8	43.5	52.5
29	45.2	46.8	48.3	37.5	46.5	59.6
30	-	-	-	43.1	51.4	56.6
31	45.5	49.9	54.9	45.3	49.0	52.7
32	44.9	56.2	67.4	43.4	53.4	59.0
33	53.5	58.8	64.1	48.1	54.2	61.5
34	49.0	57.5	69.4	49.7	58.5	77.6
35	57.4	69.0	76.6	48.3	59.4	73.2
36	58.4	67.3	73.9	58.9	66.3	75.4
37	57.9	66.4	76.9	65.8	70.7	76.7
38	61.1	69.4	78.6	62.4	74.1	85.1
39	66.6	71.0	75.4	69.2	78.8	86.1
40	70.7	80.8	90.8	60.1	77.3	85.8
41	-	83.6	-	-	-	-

*indicates only value in data set

Table 11: Minimum, mean, and maximum mass values for *R. c. melanota* larvae captured from study sites at MNF Barton Knob and Westvaco WERF in autumn (September - October 1997).

Larval Stage	Barton Knob			Westvaco WERF		
	min (g)	mean (g)	max (g)	min (g)	mean (g)	max (g)
25	0.18	0.22	0.25	0.05	0.21	0.43
26	0.34	0.64	0.88	0.14	0.39	1.14
27	-	0.76*	-	0.22	0.54	1.01
28	1.33	1.51	1.61	0.49	0.82	1.23
29	0.89	0.97	1.04	0.63	0.92	1.28
30	-	-	-	0.74	1.16	1.42
31	0.87	1.07	1.50	0.72	1.01	1.28
32	0.69	1.57	2.45	0.89	1.41	1.80
33	1.52	1.76	1.99	0.94	1.51	3.78
34	1.05	1.77	2.74	0.83	1.61	2.14
35	1.63	2.47	3.45	0.94	1.77	2.95
36	1.98	2.62	3.56	1.64	2.47	3.23
37	1.49	2.39	3.50	2.39	2.93	3.81
38	1.85	2.97	4.07	2.00	3.45	5.04
39	2.55	3.21	3.86	3.17	4.04	4.93
40	2.84	3.92	5.00	3.68	4.24	5.26
41	-	5.41*	-	-	-	-

*indicates only value in data set

Figure 43: MNF Barton Knob and Westvaco WERF autumn (September - October 1997) larval stage vs. total length linear regression graph

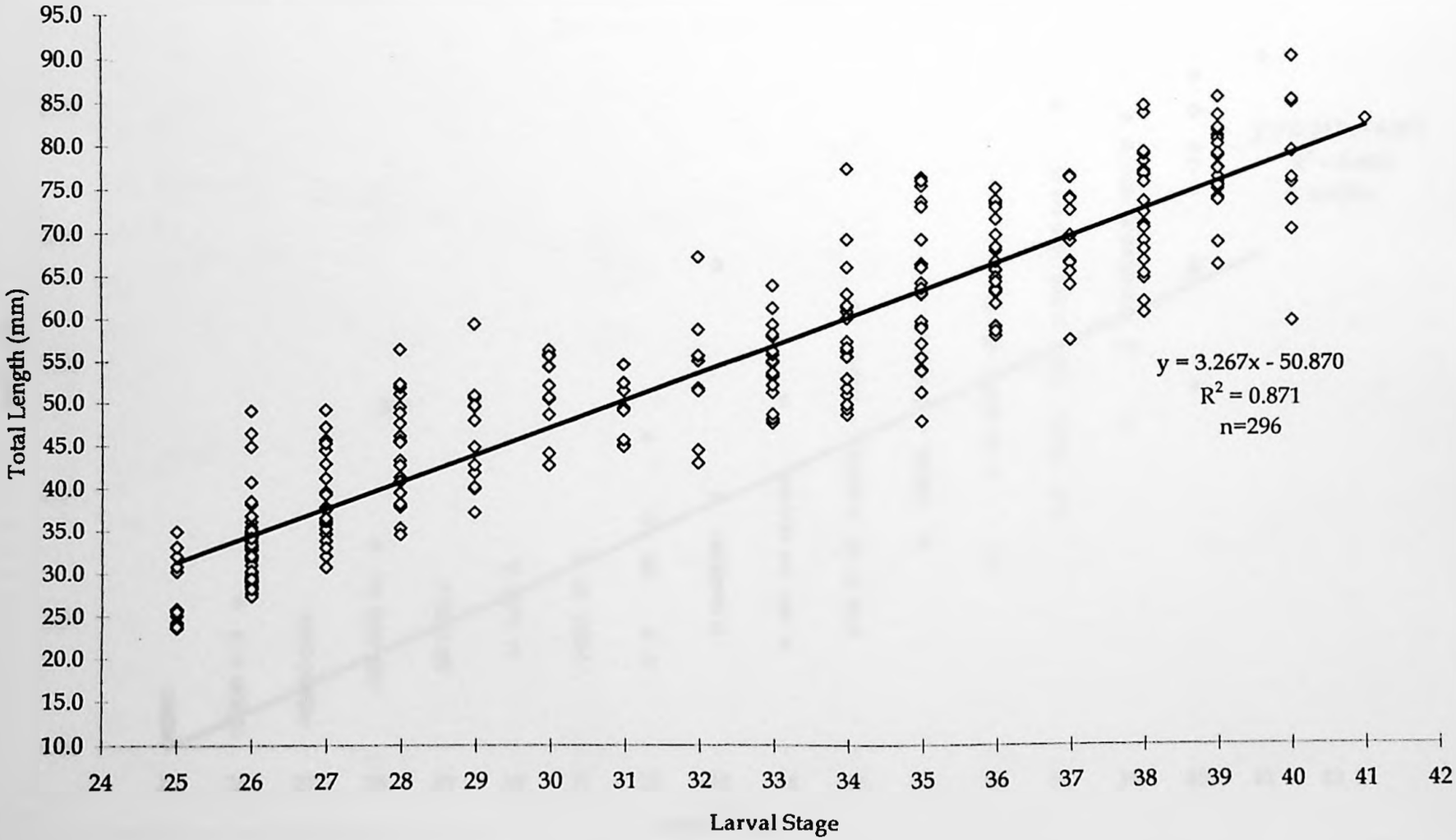


Figure 44: MNF Barton Knob and Westvaco WERF autumn (September - October 1997) larval stage vs. mass linear regression graph

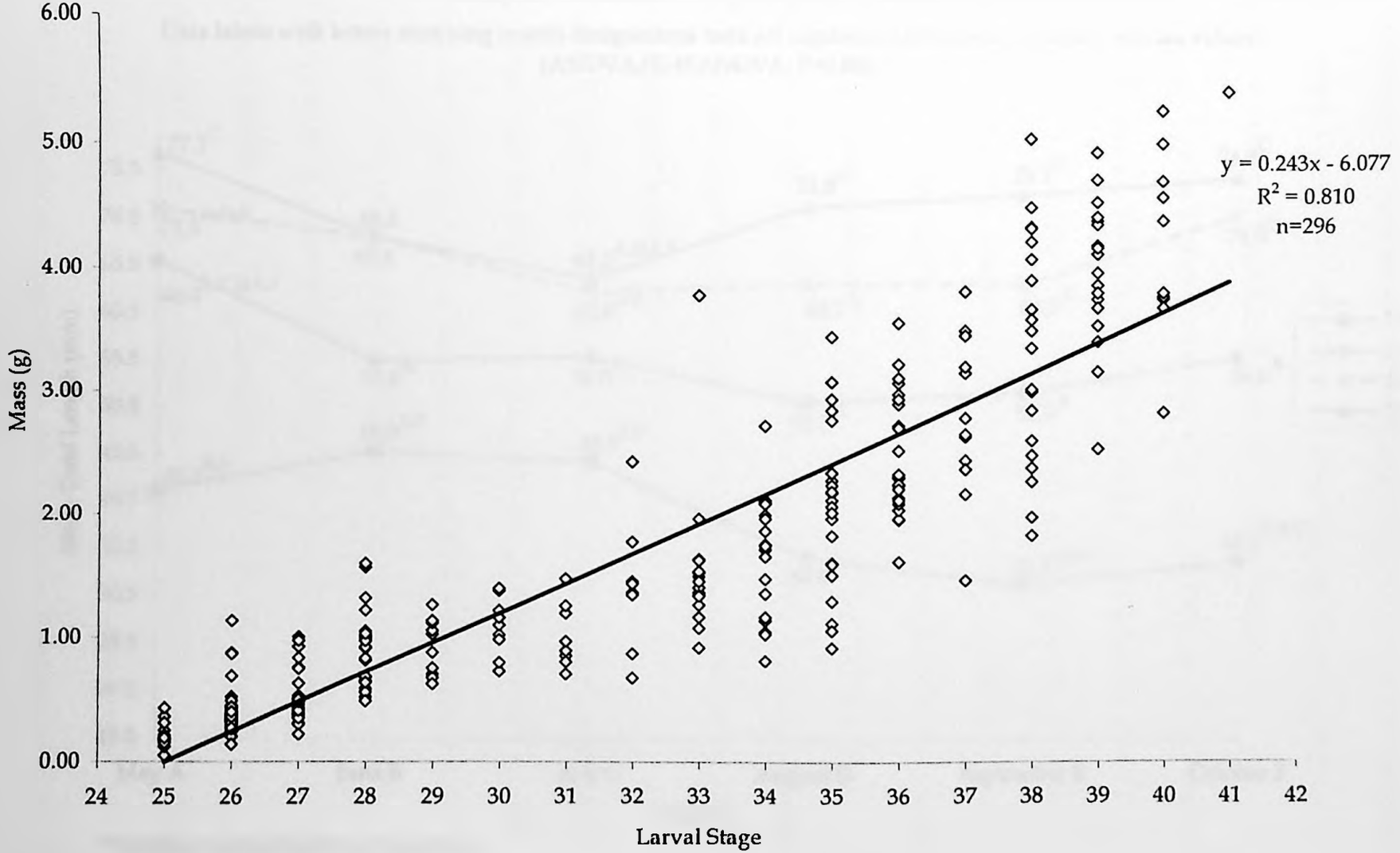
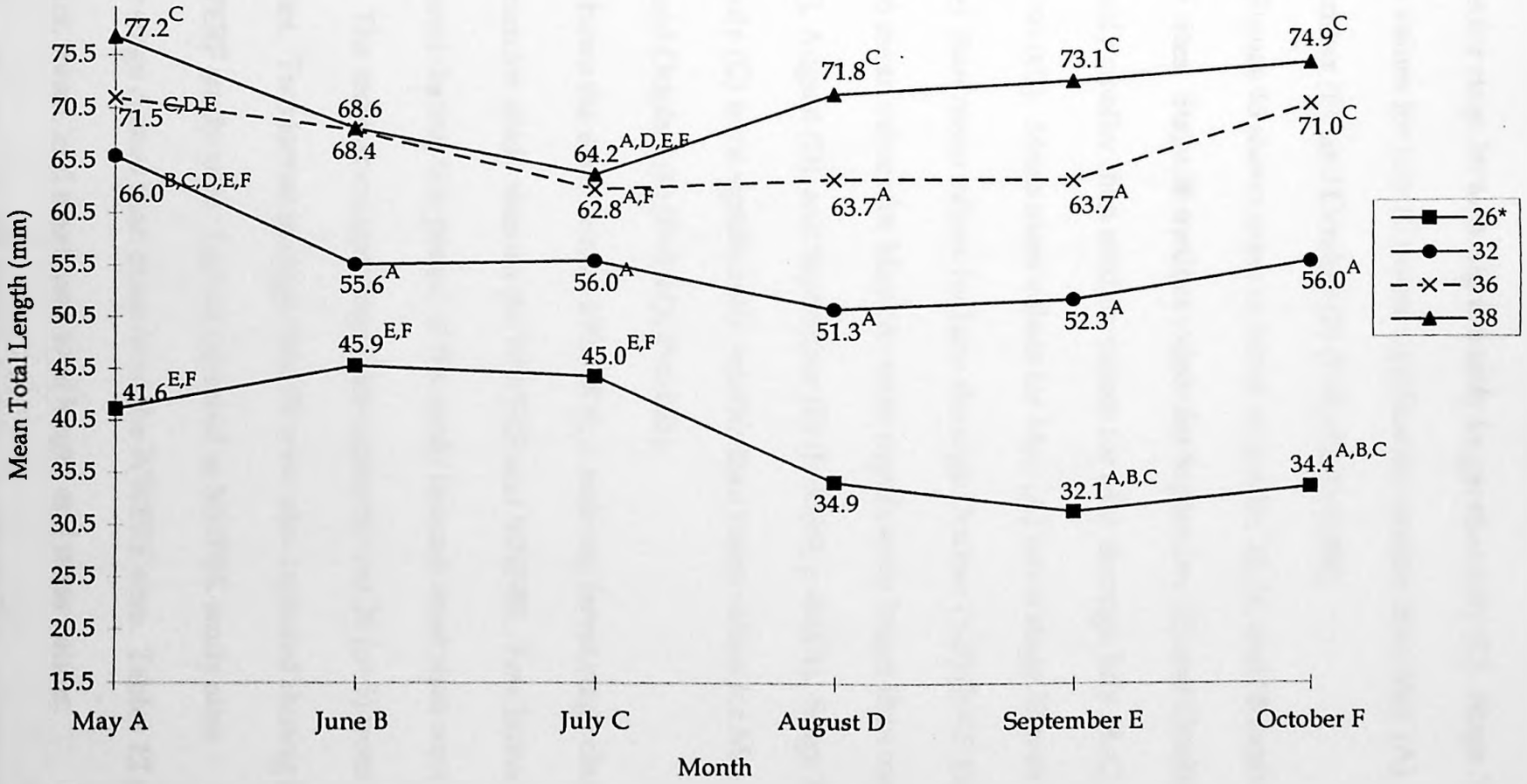


Figure 45: Larval stage total length of *R. c. melanota* collected from Westvaco WERF study sites May to October 1997 (grouped by month)

Data labels with letters matching month designations indicate significant differences in mean/median values (ANOVA/K-WANOVA, P<0.05).



* Median values shown for this stage.

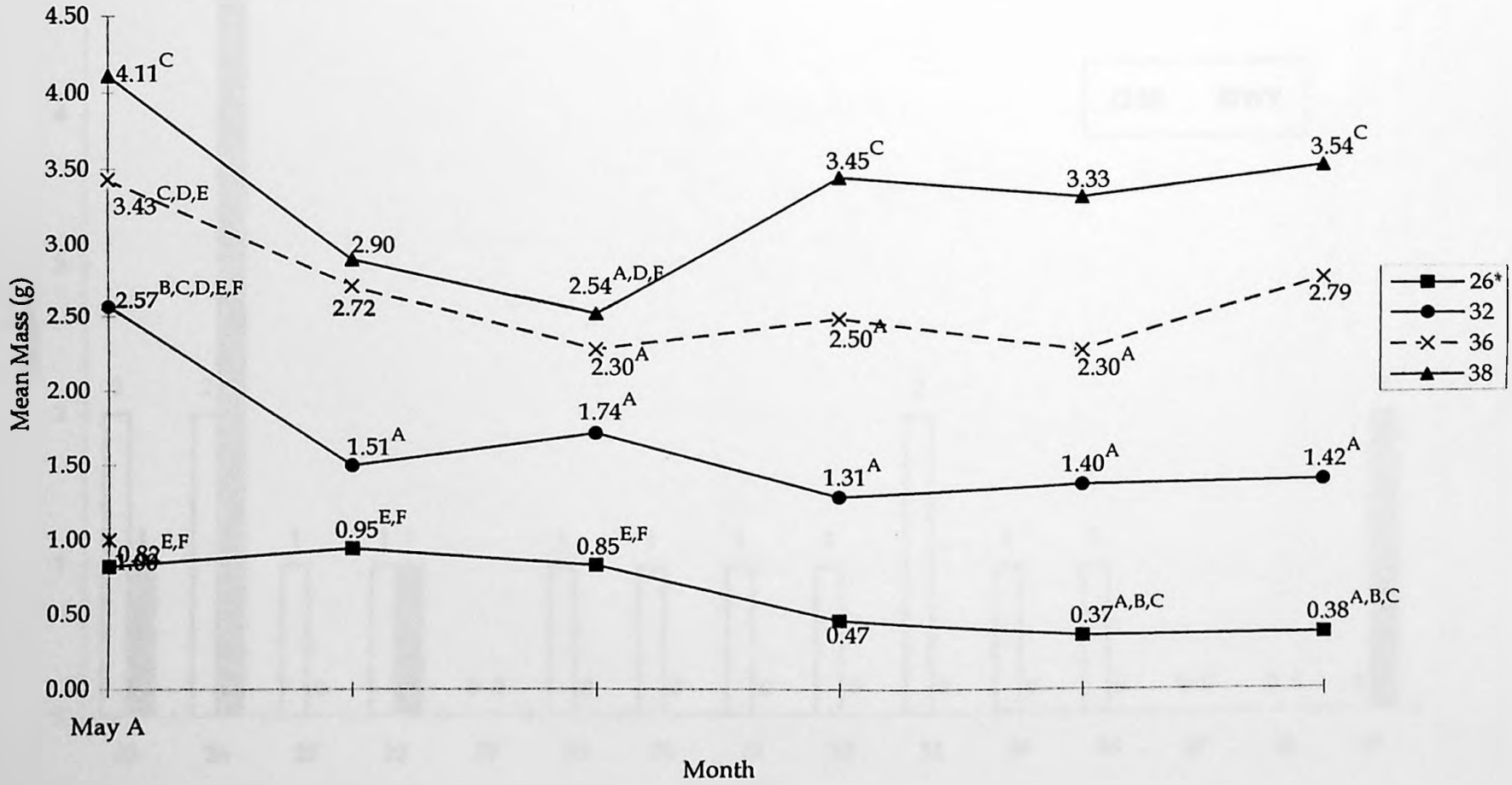
through September (C-E) ($F=8.204$; $P<0.001$). Additionally, October (F) mean total length values for stage 36 were significantly larger than July (C). Stage 38 mean total length values for July (C) were significantly smaller than May (A), August (D), September (E), and October (F) ($F=8.452$; $P<0.001$).

Similarly, Figure 46 shows mass of larval stages 26, 32, 36, and 38 captured from the WWERF sites. Stage 26 median values for September (E) and October (F) were significantly smaller than median values for May through July (A-C) ($H=52.700$; 5 df; $P<0.001$). Mean mass values for May (A) larval stage 32 were significantly larger than mean values for June through October (B-F) ($F=15.151$; $P<0.001$). Stage 36 mean values for May (A) were significantly larger than mean values for July (C), August (D), and September (E) ($F=7.689$; $p<0.001$). Stage 38 mean values for July (C) were significantly smaller than mean values for May (A), August (D), and October (F) ($F=5.972$; $P<0.001$).

Figure 47 shows the early winter 1997-98 *R. c. melanota* larval stage class frequency histogram for study sites on the WWERF and MNFBK. Few larvae ($n=26$) were captured during this period of the study because most sites were covered with ice. The most abundant stage class captured was 26 ($n=5$) from the WWERF study sites. Two larvae in stage class 39 were also captured during this period at the WWERF study sites. Larvae captured at MNFBK study sites represented more stage classes than those from the WWERF sites. Tables 12 and 13 report minimum, mean, and maximum total length and mass values, respectively. Figure 48 shows the SLR for larval stage and total length ($R^2=0.885$;

Figure 46: Larval stage mass of *R. c. melanota* collected from Westvaco WERF study sites May to October 1997 (grouped by month)

Data labels with letters matching month designations indicate significant differences in mean/median values (ANOVA/K-WANOVA, P<0.05).



*Median values shown for these stages.

Figure 47: MNF Barton Knob (BK) and Westvaco WERF (WV) early winter larval stage frequency histogram

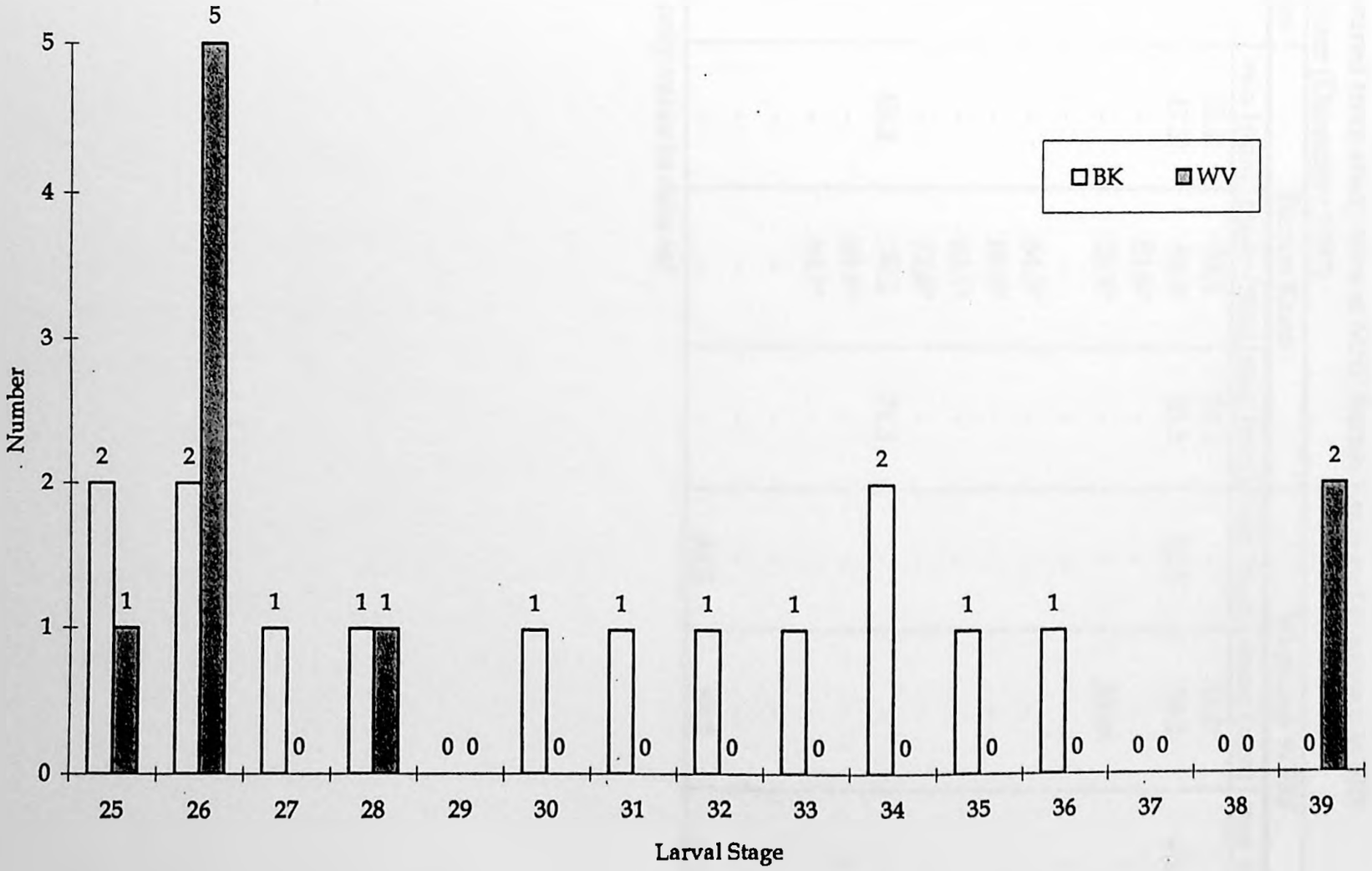


Table 12: Minimum, mean, and maximum total length values for *R. c. melanota* larvae captured from study sites at MNF Barton Knob and Westvaco WERF in early winter (December 1997).

Larval Stage	Barton Knob			Westvaco WERF		
	min (mm)	mean (mm)	max (mm)	min (mm)	mean (mm)	max (mm)
25	32.2	33.2	34.1	-	33.5*	-
26	47.2	50.3	53.3	32.5	38.2	43.7
27	-	51.8*	-	-	-	-
28	-	55.1*	-	-	39.8*	-
29	-	-	-	-	-	-
30	-	64.3*	-	-	-	-
31	-	60.0*	-	-	-	-
32	-	63.1*	-	-	-	-
33	-	72.0*	-	-	-	-
34	69.2	70.2	71.1	-	-	-
35	-	69.9*	-	-	-	-
36	-	69.1*	-	-	-	-
37	-	-	-	-	-	-
38	-	-	-	-	-	-
39	-	-	-	84.0	88.0	92.0

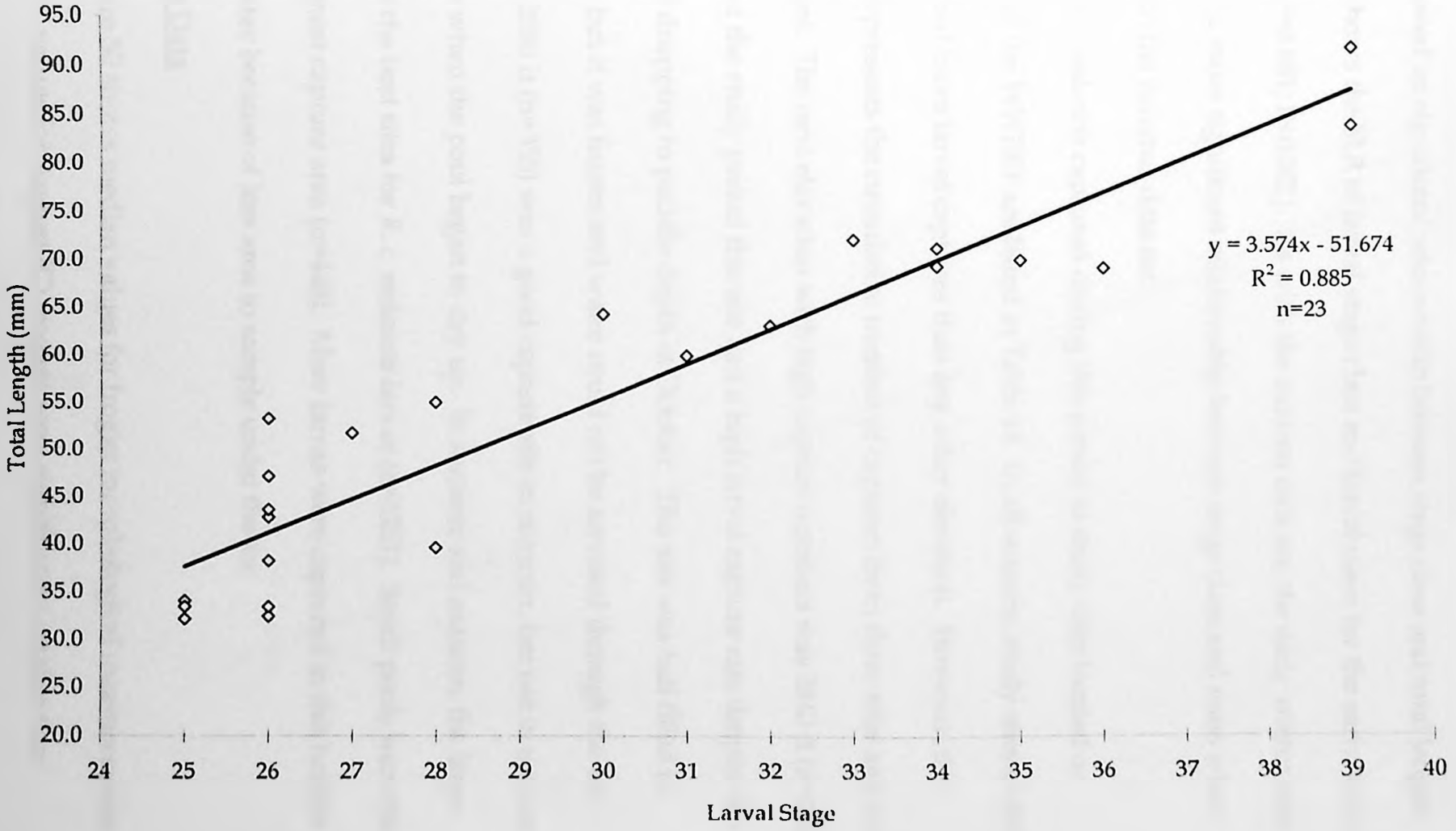
*indicates only value in data set

Table 13: Minimum, mean, and maximum mass values for *R. c. melanota* larvae captured from study sites at MNF Barton Knob and Westvaco WERF in early winter (December 1997).

Larval Stage	Barton Knob			Westvaco WERF		
	min (g)	mean (g)	max (g)	min (g)	mean (g)	max (g)
25	0.48	0.49	0.50	-	0.45*	-
26	1.22	1.31	1.40	0.51	0.68	0.93
27	-	1.37*	-	-	-	-
28	-	1.57*	-	-	0.98*	-
29	-	-	-	-	-	-
30	-	2.32*	-	-	-	-
31	-	1.63*	-	-	-	-
32	-	2.23*	-	-	-	-
33	-	2.72*	-	-	-	-
34	2.86	2.87	2.88	-	-	-
35	-	2.56*	-	-	-	-
36	-	2.59*	-	-	-	-
37	-	-	-	-	-	-
38	-	-	-	-	-	-
39	-	-	-	5.09	5.39	5.69

*indicates only value in data set

Figure 48: MNF Barton Knob and Westvaco WERF early winter (December 1997) larval stage vs. total length linear regression graph



P<0.001). As with the autumn data set, *R. c. melanota* larvae collected in early winter showed an significant relationship between stage class and total length. Figure 49 shows the SLR of larval stage class and larval mass for the early winter data set (R^2=0.881; P<0.001). As with the autumn data set, the early winter data set showed a more significant relationship between stage class and mass when compared to the summer data set.

Rana c. melanota captured during this period at study sites located on MNFBK and the WWERF are listed in Table 14. In all seasons, study sites at 4000 ft (n=627) had more larval captures than any other elevation. However, this elevation represents the cumulative number of captures from three sites and two habitat types. The next elevation with high capture numbers was 2840 ft (n=515). Throughout the study period this site had a high larval capture rate despite the water level dropping to puddle depth in October. This site was half filled in December, but it was frozen and water could not be accessed through the ice. The site at 2880 ft (n=355) was a good capture site in summer, but not in autumn and winter when the pool began to dry up. In summer and autumn, the large pools were the best sites for *R. c. melanota* larvae (n=1251). Small pools were the second highest capture area (n=448). More larvae were captured in this habitat type in winter because of less area to sample under the ice.

Froglet Data

Figure 50 shows median values for froglet morphological measurements. Median SUL values for August (C) froglets were significantly smaller than

Figure 49: MNF Barton Knob and Westvaco WERF early winter larval stage vs. mass linear regression graph

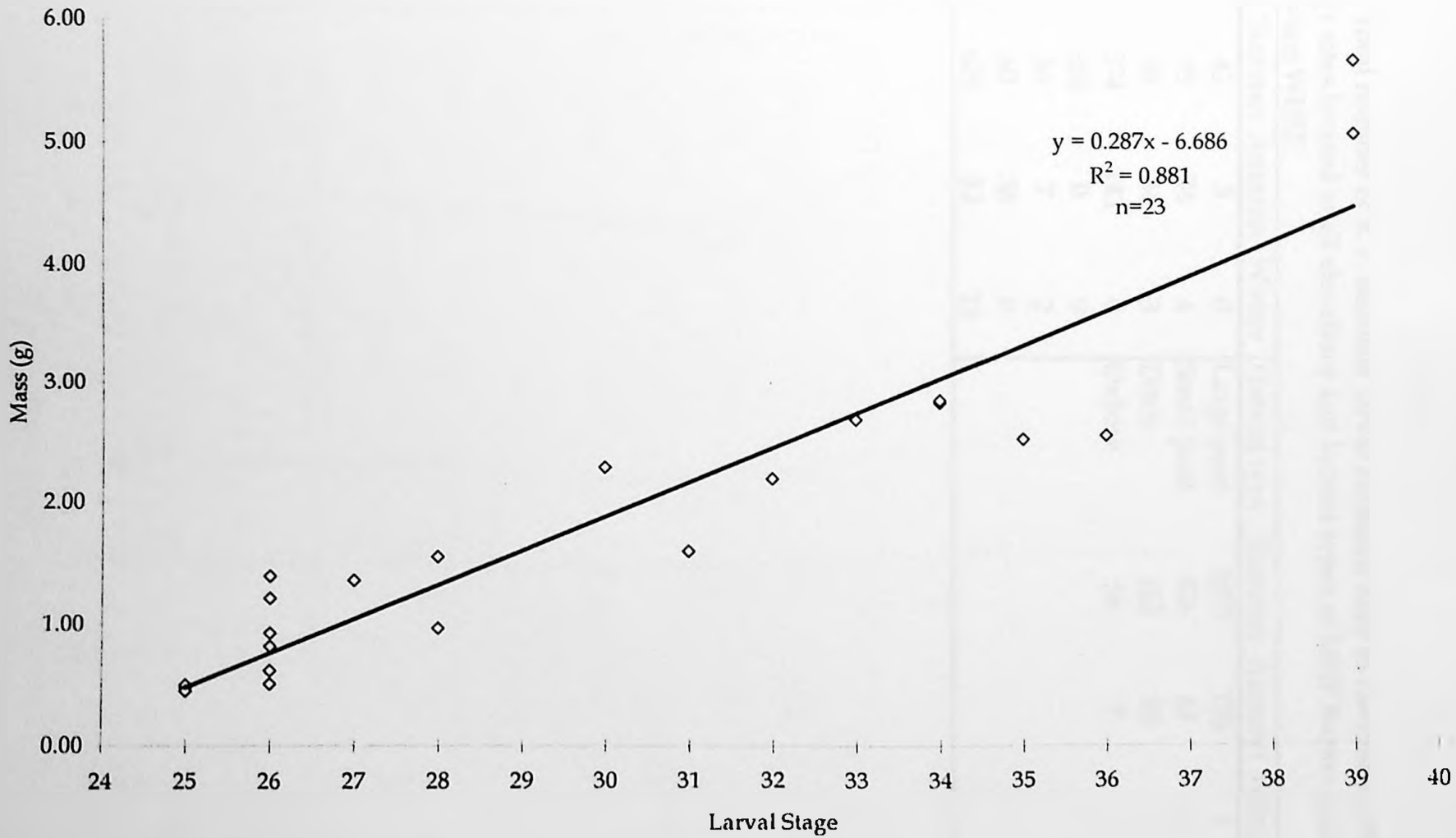
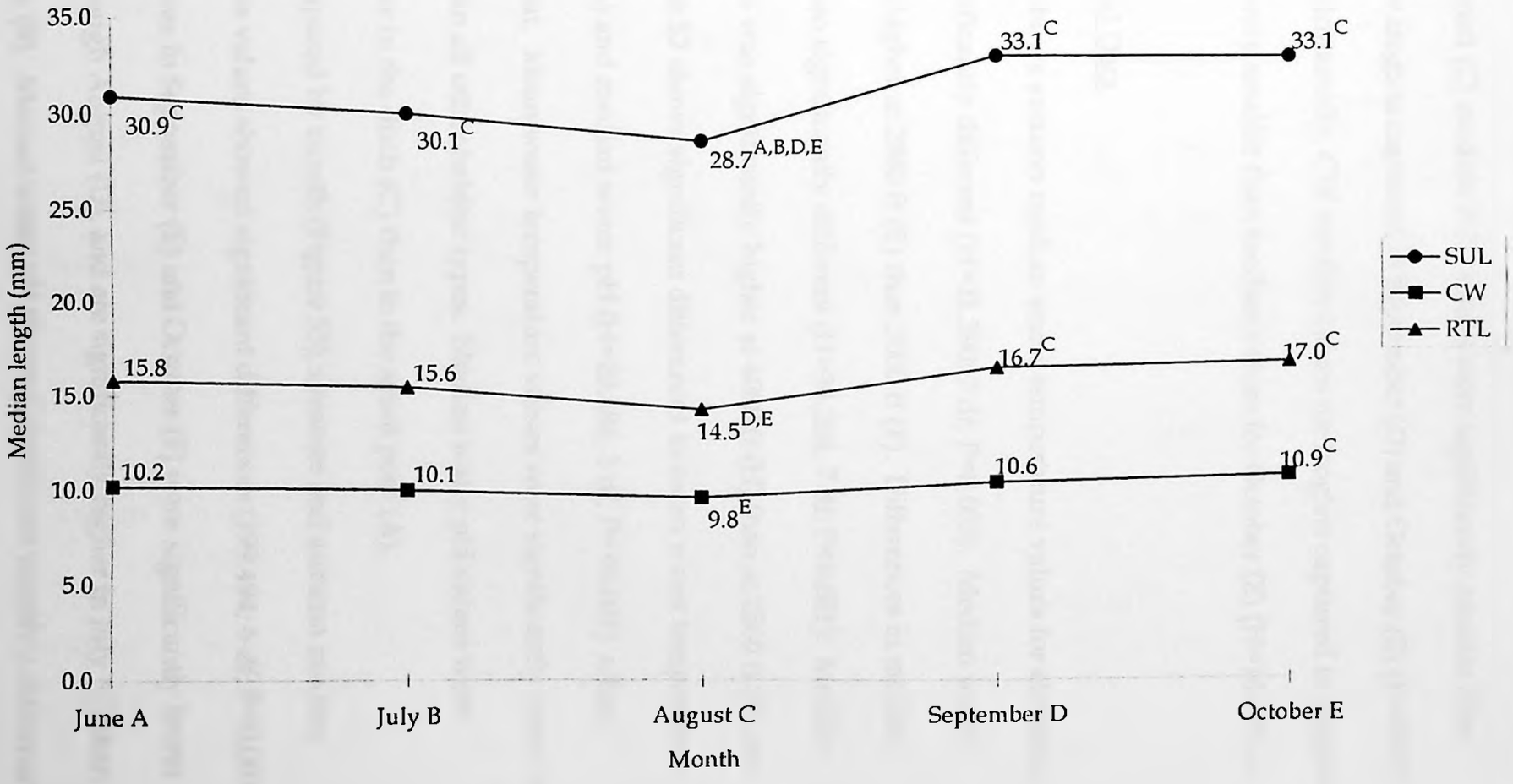


Table 14: Total number of *R. c. melanota* larvae captured May to December 1997 from study sites located at all elevations and habitat types at MNF Barton Knob and Westvaco WERF.

Elevation	Summer	Autumn	Winter	Habitat type	Summer	Autumn	Winter
2400	42	3	0	Large pool	1075	173	3
2560	95	31	4	Small pool	376	61	11
2570	58	34	3	Ditch	153	55	7
2840	374	141	0	Oxbow	56	7	2
2880	355	0	0				
3000	56	7	2				
3160	60	38	0				
4000	620	52	15				

Figure 50: *R. c. melanota* Froglet Morphological Measurements collected at Westvaco WERF Site 6 from June to October 1997 (grouped by month)

Data labels with letters matching month designations indicate significant differences in median values (K-WANOVA, P<0.05).



median values for June (A), July (B), September (D), and October (E) (H=35.612; 4 df; P<0.001). August (C) median RTL values were significantly smaller than median values for froglets captured in September (D) and October (E) (H=29.357; 4 df; P<0.001). Additionally, CW median values for froglets captured in August (C) were significantly smaller than median values for October (E) (H=16.337; 4 df; P=0.003).

Environmental Data

Figure 51 shows autumn median water temperature values for elevation groups were significantly different (H=21.390; 7 df; P=0.003). Median water temperature was higher at 2880 ft (E) than 3000 ft (F). Differences in median water pH were also significantly different (H=34.264; 7 df; P<0.001). Median autumn water pH was significantly higher at 4000 ft (H) than at 2560 ft (B) and 2880 ft (E). Figure 52 shows significant differences in mean water temperature (F=6.711; P<0.001) and median water pH (H=20.484; 3 df; P=<0.001) when grouped by habitat. Mean water temperature values were significantly lower in the oxbow (D) than all other habitat types. Median water pH values were significantly lower in the ditch (C) than in the small pool (A).

When compared by month (Figure 53), summer and autumn median water temperature values showed significant differences (199.494; 6 df; P<0.001). Water temperatures in September (E) and October (F) were significantly lower than May (A) through August (D), and are significantly higher in July (C) than May (A) and June (B). Median water pH showed significant monthly differences

Figure 51: MNF Barton Knob and Westvaco WERF autumn (September - October 1997) environmental data (grouped by elevation)

Data labels with letters matching elevational group designations indicate significant differences in median values (K-WANOVA, P<0.05).

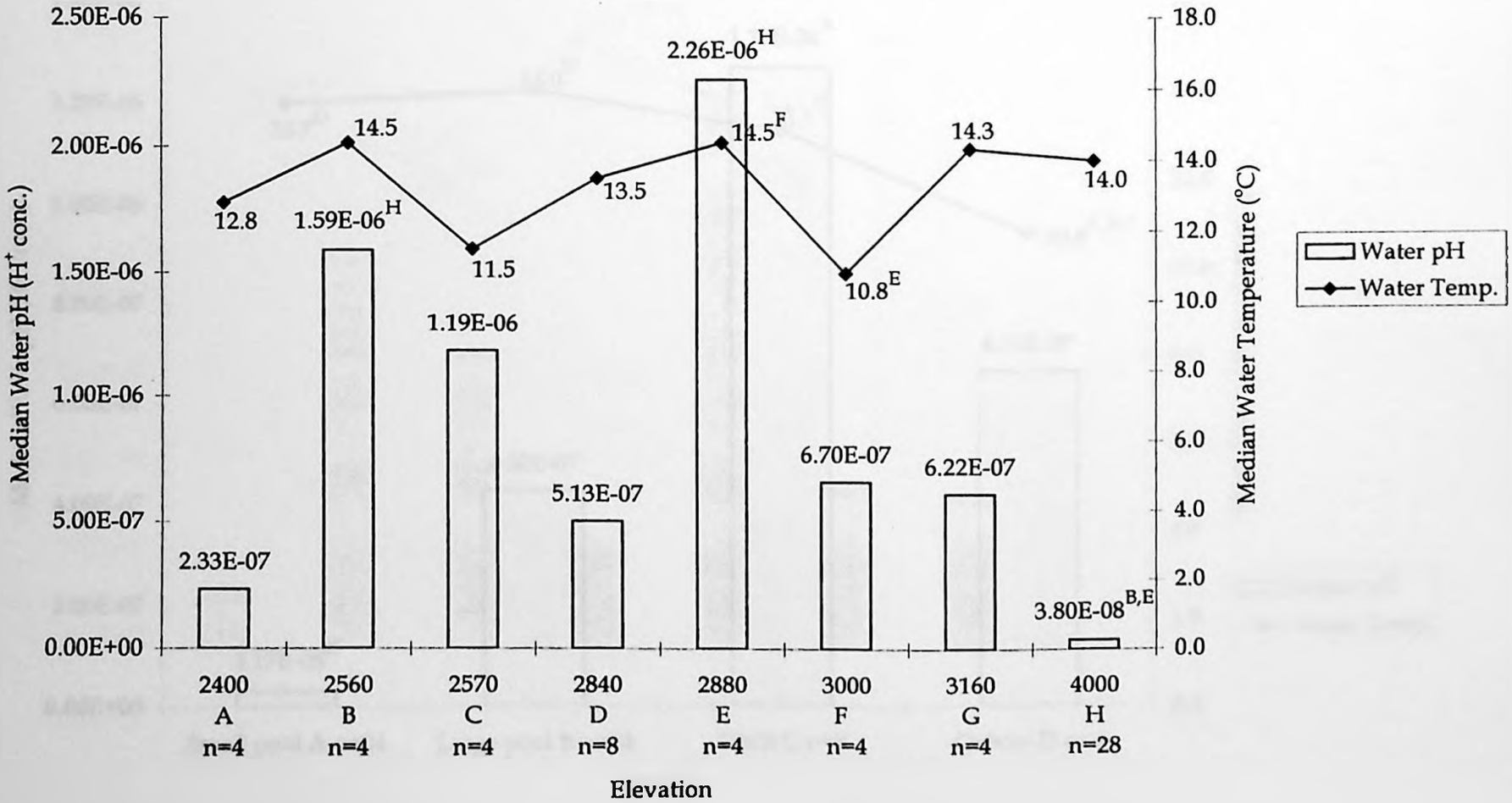


Figure 52: MNF Barton Knob and Westvaco WERF autumn (September - October 1997) environmental data (grouped by habitat)

Data labels with letters matching habitat group designations indicate significant differences in median/mean values (K-WANOVA/ANOVA, P<0.05).

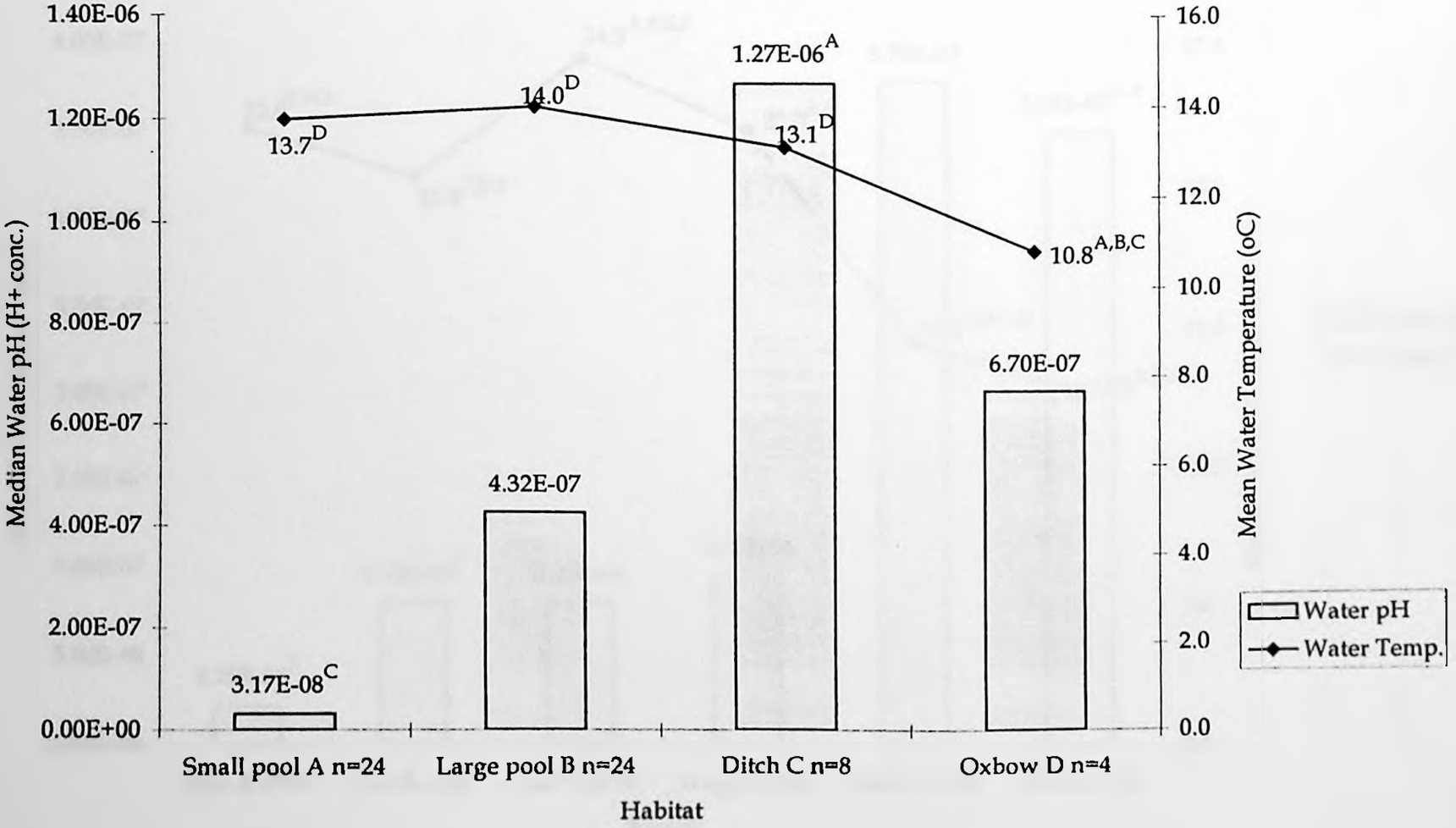
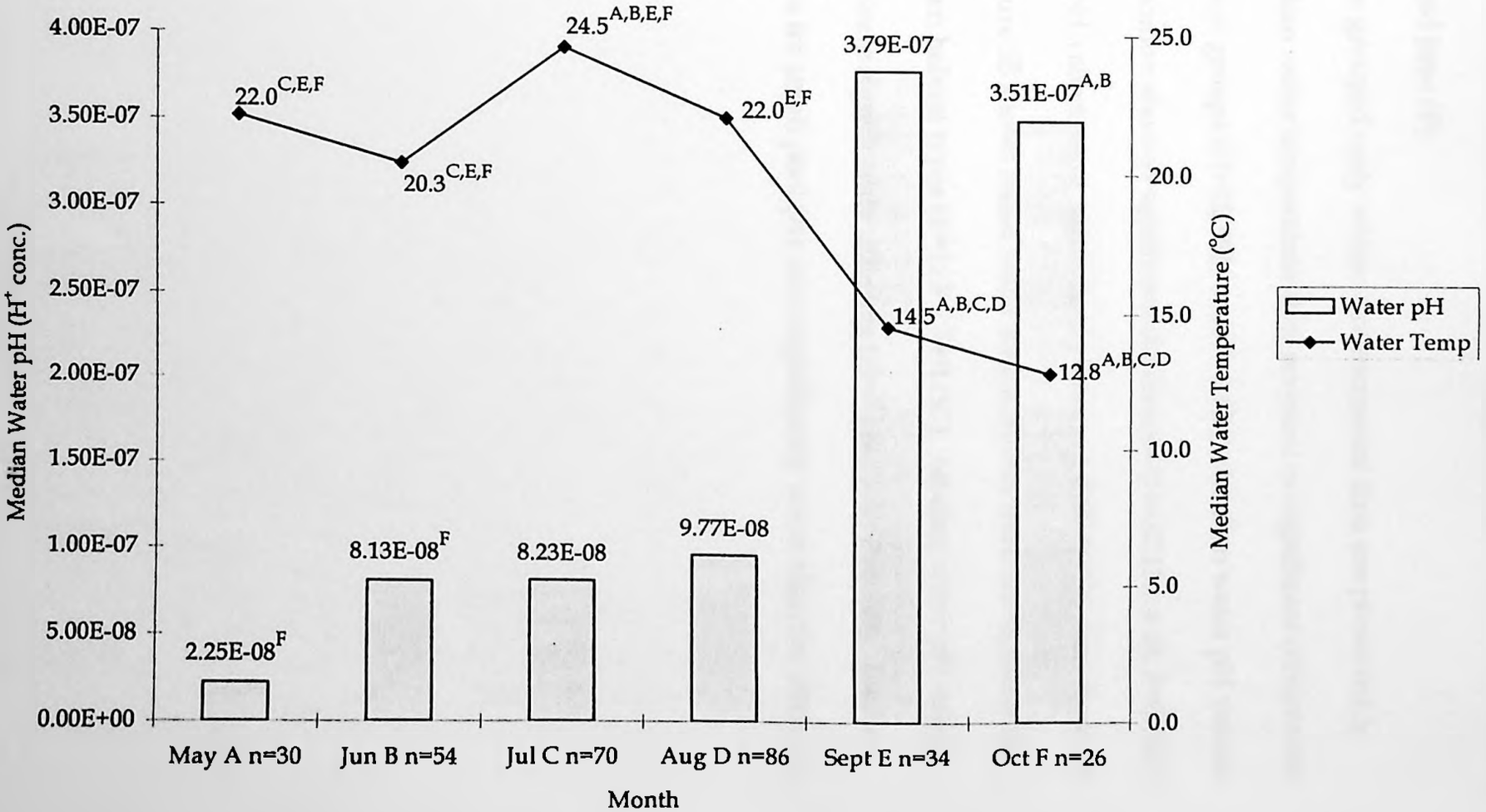


Figure 53: MNF Barton Knob and Westvaco WERF environmental data from May to October 1997 (grouped by month)

Data labels with letters matching month designations indicate significant differences in median values (K-WANOVA, P<0.05).



(H=40.890; 6 df; P<0.001). Median water pH is significantly lower in October (F) than May (A) and June (B).

Elevation grouped early winter environmental data are presented in Figure 54. Median water temperature data revealed no significant differences between elevation groups (H=12.395; 6 df; P=0.054). Median water pH values grouped by elevation showed significant differences (H=22.130; 6 df; P=0.001). Median water pH values were significantly lower at 4000 ft (G), than those at 2880 ft (D). Figure 55 shows mean water temperatures were not significantly different between habitat types (F=1.696, P=0.197). Median water pH within habitat groups were significantly different (H=7.886; 3 df; P=0.048). Median water pH values for small pool (A) were significantly lower than the ditch (C).

Figure 54: MNF Barton Knob and Westvaco WERF early winter (December 1997) environmental data (grouped by elevation)

Data labels with letters matching elevational group designations indicate significant differences in median values (K-WANOVA, P<0.05).

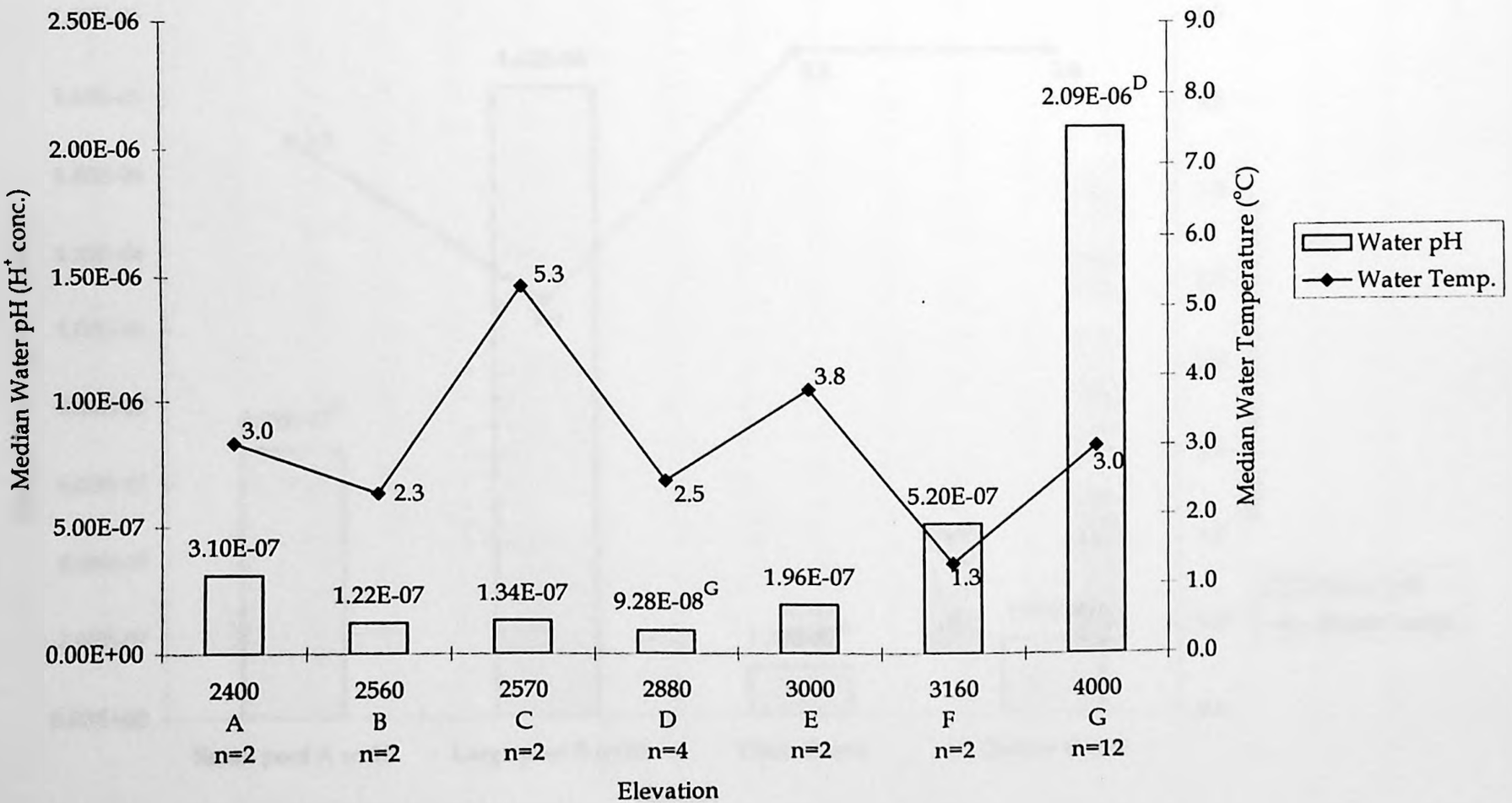
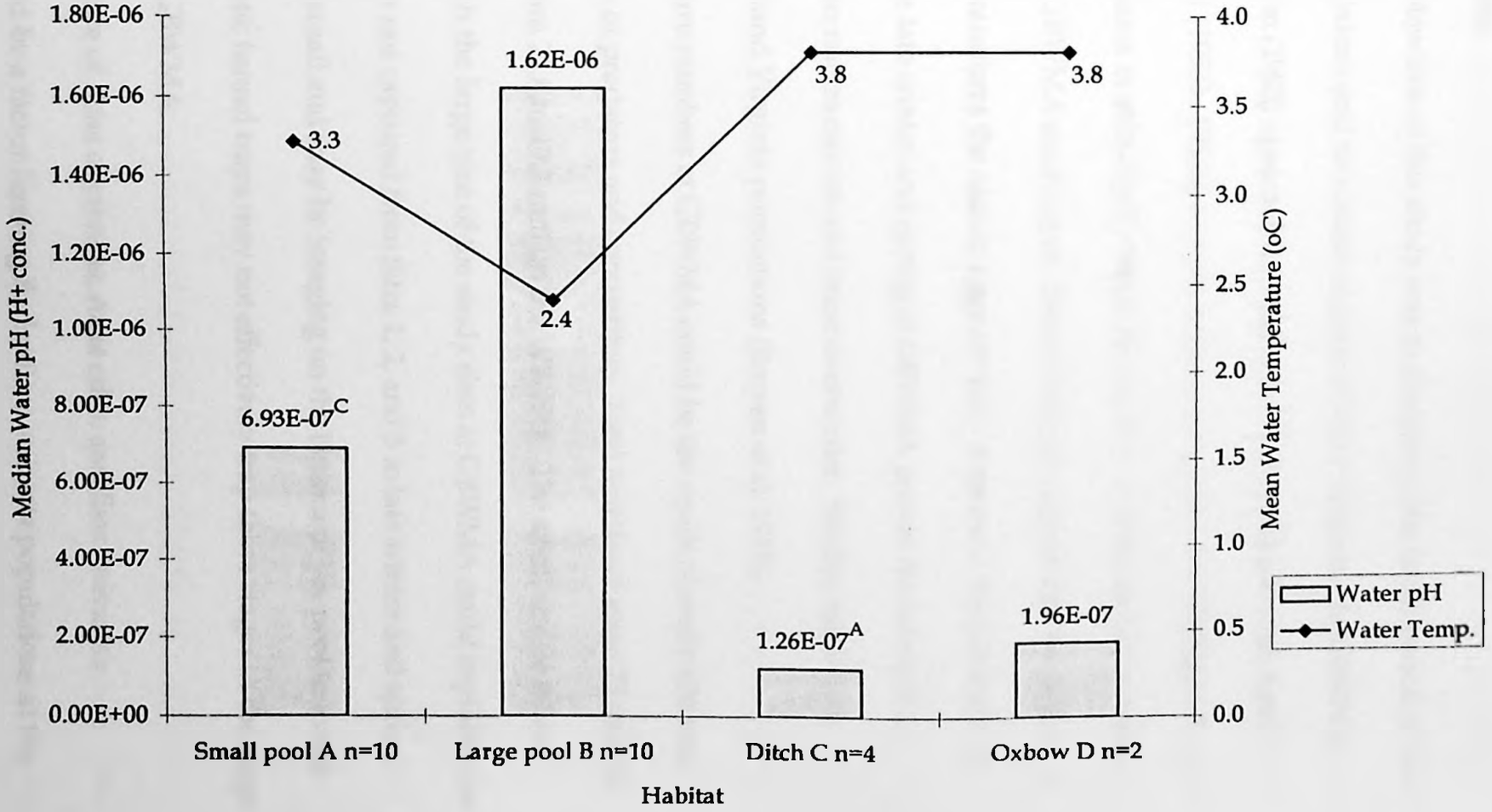


Figure 55: MNF Barton Knob and Westvaco WERF early winter (December 1997) environmental data (grouped by habitat)

Data labels with letters matching habitat group designations indicate significant differences in median/mean values (K-WANOVA/ANOVA, P<0.05).



Discussion

Biological Data

The first objective of this study was to determine the larval period of *R. c. melanota* in the plateau and mountain regions of West Virginia. At GBWMA, Pauley and Barron (1995) reported *R. c. melanota* calling males in mid-April. Similarly, Martof (1953b, 1956b) reports initial calling dates for Michigan *R. c. melanota* populations in mid-April. Stage 25 and 26 *R. c. melanota* larvae were absent from the GBWMA study sites. These findings suggest that some *R. c. melanota* larvae transform the season eggs are laid. However, the presence of larvae during the late winter and spring at GBWMA proves that some *R. c. melanota* larvae do not transform and must overwinter. Similar results are reported for lowland Virginia populations (Breven et al. 1979).

Low capture numbers at GBWMA could be the result of study site size, and the presence of predators and competitors. Total lengths of stage 25 and 26 larvae ranged from 21.8 to 49.7 mm on the WWERF. The small size of these stages paired with the large size of the study sites at GBWMA could explain why these stages were not captured from Sites 1, 2, and 3 in late winter and spring. These stages are small and may be foraging on the bottom of the pool to avoid predators. Aquatic funnel traps may not effectively trap these stages in the large pool habitats at GBWMA.

The presence of *Rana catesbeiana, Amia calva,* and *Esox americana vermiculatus* could be a factor limiting *R. clamitans melanota* populations at the

GBWMA sites. *Rana catesbeiana* is a known predator of *R. clamitans melanota* larvae and adults (Stewart and Sandison 1972; McAlpine and Dilworth 1989; Werner et al. 1995). *Amia calva* is a carnivorous fish species (Trautman 1981), and an *E. a. vermiculatus*, another carnivorous fish species, was captured in one funnel trap with a *R. c. melanota* larvae tail sticking out of its mouth. It is also important to note that three other species of ranid frogs with similar natural histories occur with *R. c. melanota* at GBWMA. *Rana catesbeiana*, *R. pipiens*, and *R. palustris* occur at GBWMA. The latter two species breed earlier than *R. clamitans melanota*, and utilize different breeding habitats (McAlpine and Dilworth 1989; Pauley and Barron 1995). However, *R. c. melanota* competes with *R. catesbeiana* for food, and breeding sites (McAlpine and Dilworth 1989; Werner 1994; Werner et al. 1995; Hecnar and M' Closkey 1997). It has been suggested by Hecnar and M'Closkey (1997) that in the absence of *R. catesbeiana*, *R. clamitans melanota* will be the dominant anuran species in a given habitat. However, the absence of the mentioned predators and other ranid frog species at the WWERF study sites could be a contributing factor to the success of *R. c. melanota* on the WWERF. Further study of the interspecific relationships between *R. c. melanota* and other frog species present at GBWMA is needed to determine any population limitations.

Stage specific development tables are useful to predict a point of larval development for many different anuran species regardless of size (Gosner 1960). Total length and mass measurements of larvae in a particular stage can be used

to predict larval stage for a specific species (Gosner 1960). Simple linear regression analysis yielded a significant relationship between total length and larval stage in all seasons sampled. A similar relationship was also shown between mass and larval stage in all seasons sampled. These results show that the use of total length and mass measurements to determine stage specific body size is a useful tool for West Virginia *R. c. melanota* populations. Minimum, mean, and maximum measurements for these measurements provide additional data for future research on *R. c. melanota* larvae in West Virginia.

In summer, the relationship of total length and larval stage of *R. c. melanota* was less significant than other seasons. The same trend occurred with the relationship between mass and larval stage in the summer period. A decrease in total length and mass values occurs with the appearance of stages 40 through 44. It is important to note that these stages are not collected during the late winter, spring, and early winter periods of this study. The appearance of stage 40 in *R. c. melanota* larval populations is important because this stage marks the beginning of drastic body changes in anuran larvae. These changes include tail absorption, development of forelimb buds, and the breakdown of larval mouth parts (Gosner 1960). Absorption of the tail obviously causes a decrease in total length. Furthermore, *R. c. melanota* larvae at this stage of development do not feed; hence, the decrease in mass of the tadpole (Jenssen 1967).

Stage 25 represents the first free-living stage of anuran larvae, and larvae can spend a considerable amount of time in this stage (Gosner 1960). Total

lengths of this stage are variable. Hand collected samples of newly hatched larvae show total lengths ranging from 9.77 to 16.0 mm. Stage 25 larvae captured by dip net sweeps and aquatic funnel traps had total lengths between 17.6 and 56.6 mm.

Larval stage frequency histograms for the summer period show distinct shifts in the peak stages from the May/June period and the July/August period. In May and June, the most abundant stages are 27, 28, and 29 which comprise the first peak. The second peak shows the most abundant stages to be 34, 35, 36, and 39. In July and August, the two peaks in the May and June period are reduced, and the most abundant stages are now stages 25 and 39. The peak calling male activity was in July. Likewise, peak egg deposition time was in July and August, which explains the abundance of stages 25 larvae during the July/August period. Similarly, the abundance of stage 39 larvae during the July/August period correlates with the peak of stages 40 through 44 larvae captures in July. It also correlates with peak froglet dispersal from Site 6 on the WWERF in July and August.

If cell differentiation in *R. c. melanota* ceases at water temperatures of 10 C, then larvae that overwinter will continue to grow, but will not undergo further development (Smith-Gill and Breven 1979). Hence, stage specific body measurements of larvae captured in May will be larger than body measurements of the same stage captured in September and October. Monthly total length and mass values for stages 26, 32, 36, and 38 follow this trend. Similarly, *R. catesbeiana*

larvae overwinter and have been shown to vary in body size depending upon how many winters are passed as larvae (Collins 1979).

The second objective was to document the breeding activity of *R. c. melanota*. The focus of this part of the study was in the mountain region of West Virginia. Wright (1914), Martof (1953b, 1956b), Breven et al. (1979), and Smith-Gill and Breven (1979) discussed the importance of water temperature in the breeding and development of *R. c. melanota*. The beginning of the *R. c. melanota* breeding season is temperature dependent with threshold water temperatures of around 25 C (Wright 1914, Breven et al. 1979). Water temperatures of 25 C were recorded at the WWERF study sites around May 19, which is when the first calling male was heard. Adult *R. c. melanota* were active around June 12 at MNFBK sites, but calling males were not recorded until July 1. Water temperatures at these sites were above 25 C around June 26. Smith-Gill and Breven (1979) reported threshold water temperatures for the cessation of *R. c. melanota* cell differentiation to be 10 C. These water temperatures were recorded at the WWERF sites around October 3, and at the MNFBK sites around August 24.

The results of breeding activity, and egg deposition data from the WWERF and MNFBK study sites show that *R. c. melanota* in the mountain region of West Virginia exhibit a shorter breeding season than Martof (1953b, 1956b) describes for Michigan populations. Threshold water temperatures necessary to initiate breeding activity in this species are a month behind Michigan

populations. Additionally, Martof (1956a) suggests that *R. c. melanota* larvae from eggs laid before June 25 will develop and transform in the season laid. Larvae from eggs laid after this time will not develop until the following summer. Eggs were first deposited at sites on the WWERF on June 19, and were first deposited at MNFBK sites on July 12. Martof's findings suggest that most *R. c. melanota* larvae on the WWERF, and all larvae on MNFBK will not transform in the season laid. Breven et al. (1979) reports populations of *R. c. melanota* in mountain regions of Virginia must overwinter once and sometimes twice before transformation.

Lack of rainfall during the study period caused extreme water level fluctuations at some sites. Lab reared *R. c. melanota* larvae will develop in 92 days (Ting 1951). Field observations of *R. c. melanota* larval development suggest that during periods of pool drying, larval growth rates will increase (Richmond 1964). On the WWERF, sites 6, 10, and 11 suffered water level changes during the summer. In the fall, Site 6 became a small puddle with many *R. c. melanota* larvae of various stages. Some larvae were eaten by *Procyon lotor* and *Ursus americanus*, while others took refuge in the mud and leaves at the bottom of the puddle. Site 10 contained stages 25 through 27 larvae until it completely dried up in October. No larvae were captured when the pool filled again, but the ice layer over the pool in December made sampling the pool area with a D-frame net difficult. The shallow area of Site 11 dried two weeks after egg deposition in July and August. The deep area of the pool was sampled with a D-frame net, but no

larvae were found. It is possible for *R. c. melanota* larvae to survive periods of pool drying by burrowing in the mud (Pauley pers. comm.). Water temperature data collected from sites 10 and 11, and *R. c. melanota* larval growth rates suggest that the larvae at these sites did not transform before the pools dried. It is possible that the small stage 25, 26, and 27 larvae could have burrowed in the mud, and were missed during the sampling periods in the fall and winter.

Larval stage frequency, larvae size, water temperature data, and breeding activity data support the hypothesis that *R. c. melanota* larvae overwinter at least one year in plateau and mountain regions of West Virginia. Evidence of an extended two year larval period in the mountain regions of the state lies in mark and recapture results. In October, stage 34 and 39 larvae marked in May and June were recaptured at Site 6 on the WWERF. This finding is corroborated by observations of a two year larval period in the mountain regions of Virginia (Breven et al. 1979).

The third objective of the study was to determine size, dispersal, and basic dietary habits of *R. c. melanota* froglets. Mecham (1954) used morphological characters to distinguish between the subspecies of *R. clamitans*. Furthermore, studies of *R. sylvatica*, *R. pipiens*, *P. triseriata*, and *H. versicolor* have shown RTL measurements were the most useful morphological character in distinguishing species and subspecies (Mecham 1954). This study shows that morphological measurements can be used to distinguish between the post larval stages of *R. c.*

melanota. ANOVA results show the strongest morphological character to be RTL, and the weakest morphological character to be CW.

An increase in the larval development period due to low water temperatures, increases body size at metamorphosis (Ryan 1941). This trend is illustrated in the monthly differences in the morphological measurements of *R. c. melanota* froglets. Froglets captured in August have smaller SUL, RTL, and CW measurements than those captured in June and July. Froglets captured in June and July have spent more time in the larval stage than those captured in August. The nine froglets captured in September and October were also larger than those captured in August. However, these froglets were the last to transform that season, and were not exposed to the dispersal pressures of froglets which transformed in July and August.

The diet composition of *R. c. melanota* froglets captured on the WWERF differs from other reports by Hamilton (1948), Whitaker (1961), Stewart and Sandison (1972), and Werner et al. (1995). The insect orders in this study have been reported, but dietary percentages differ from previous studies. These findings are expected since the diet of *R. c. melanota* is largely dependent upon prey availability at a given location (Stewart and Sandison 1972).

Stomach contents revealed that *R. c. melanota* froglet diet largely consisted of terrestrial insects with a small amount of aquatic insects. Several trends occurred within the four most abundant insect orders found in froglet stomach contents over a two month period in the summer. The peaks and troughs

associated with order Diptera suggest that members of this order with bivoltine life cycles were taken by *R. c. melanota* froglets. Dipterans in families Stratiomyidae and Tipulidae have bivoltine life cycles, and were found in froglet stomachs (Merritt and Cummins 1996).

The peaks and troughs associated with orders Hymenoptera and Coleoptera cannot be determined within the scope of this study. Most hymenopterans were in family Formicidae, but identification beyond the ordinal level for other hymenopterans found in froglet stomachs was not possible. Most coleopterans could be identified to the family level; however, these results show that most coleopterans taken by froglets were terrestrial. This trend could indicate the froglets spend more time on land than in the water; however, defense mechanisms associated with aquatic coleopteran families such as Dytisidae make them unpalatable (Borror et al. 1989).

The trend of order Homoptera showed a steady rise and fall in the occurrence of this group of insects in froglet stomachs over the two month period. Most homopterans have univoltine life cycles which may explain this trend (Borror et al. 1989).

Measurements of prey eaten by *R. c. melanota* froglets in this study were not collected. McAlpine and Dilworth (1989) use prey size to determine dietary niche partitioning between *R. catesbeiana, R. c. melanota,* and *R. pipiens*. Since *R. c. melanota* was the dominant anuran species at most study sites on the WWERF and CMBK, it was not necessary to determine dietary overlap with other species.

Future studies of *R. c. melanota* at GBWMA should involve dietary overlap studies since *R. c. melanota* is not the dominant anuran species.

Environmental Data

The final objective was to determine if there were differences in water pH and water temperature of study sites at different elevations and of different habitat types. Aquatic habitat degradation has been the focus of many amphibian studies in the past fifteen years. Low water pH can be lethal to *Ambystoma* and *Bufo* larvae (Freda and Dunson 1986; Beebee et al. 1990; Sadinski and Dunson 1992). *Rana c. melanota* is tolerant of low water pH, and is found in many different aquatic habitats (Walker 1946; Wright and Wright 1949; Martof 1953a, 1953b, 1956; Barbour 1971; McDonald et al. 1984; Dale et al. 1985; Green and Pauley 1987; Freda and Taylor 1992; Pearman 1993). Although water pH of all fourteen sites was never below 6.0 standard units some general trends were observed. Sites at elevations of 3000 ft, 3160 ft, 3440 ft, and 4000 ft had lower water pH values during late winter, spring, and summer periods of the study. In autumn, these differences were not found because all sites had low water levels due to lack of rainfall in the summer months. In early winter, water pH values at 4000 ft were significantly lower than all other sites. Water pH was consistently lower in the temporary pool, ditch, and oxbow habitats except for the early winter sampling period. Rainfall increased pool water levels before early winter sampling, and may have influenced the results.

Studies of habitat degradation implicate acidic precipitation in the form of rain, snow, or fog (McDonald et al. 1984; Dale et al. 1985; Freda and Dunson 1986; Beebee et al. 1990; Sadinski and Dunson 1992). Temporary pools and small pools are more susceptible to acid deposition because of limited buffering capacity (Beebee et al. 1990). The results of elevation and habitat water pH analysis of the fourteen sites in this study suggest that acidic rainfall could influence water pH even on larger pools that have suffered periods of severe drying. Higher elevations of West Virginia are more susceptible to acidic deposition because they receive more rainfall (Pauley 1993). Smaller sites with fluctuating water levels are more susceptible because of limited buffer capacity (Freda and Dunson 1986; Beebee et al. 1990). However, caution must be used in interpretation of these results because of the lack of cation data. Cation measurements must accompany water pH results because of the interactions between H+ and reactions with the habitat substrate (Beebee et al. 1990). Since this study only addressed two environmental factors, water pH and water temperature, it should serve as a baseline for further studies. Future studies of these aquatic habitats must include cation analysis in association with water pH data.

Water temperature data between elevation groups generally show that sites at 3000 ft and 3160 ft are cooler than sites at all other elevations. Habitat groups generally show that water temperatures were usually lower for the oxbow. These results reflect that these sites were shaded by the forest canopy.

The other study sites were in open areas and had higher water temperature values.

Monthly water temperature differences support breeding activity and larval transformation results discussed in the biological data section of the manuscript. May and June were warmer than April, and mark the beginning of the *R. c. melanota* breeding activity. July was the warmest month, and was the peak of the *R. c. melanota* breeding season. Temperatures began to significantly drop in September and October, and these two months mark the end of larval development and transformation when threshold temperatures below 10 C were reached.

Of all habitat types sampled, large pools were the most successful for *R. c. melanota* breeding adults and larval development and transformation. Eggs were found more often in large pools, and more larvae were captured from large pools. These findings contradict Pearman's (1993) results of decreased survival of *R. c. melanota* larvae in large deep pools. The large deep pool in his study was an artificial pool in a laboratory setting, and was no more than 40 cm deep. All sites in this study were in the field, and most were larger and deeper than the artificial pool used by Pearman (1993). Differences between this study and Pearman (1993) reinforce the need to corroborate laboratory studies with field research.

Summary

Results of stage frequency histograms, SLR and ANOVA analysis of stage specific total length and mass, ANOVA analysis of froglet SUL, RTL, and CW measurements, breeding activity observations, and water temperatures indicate that some *R. c. melanota* larvae can spend one winter as larvae in plateau and mountain regions of West Virginia. Mark and recapture results of larvae captured at one site on the WWERF suggest that some *R. c. melanota* larvae spend two winters as larvae in the mountain regions.

Morphological characters SUL, RTL, and CW can be used to distinguish between post larval life stages of *R. c. melanota*. RTL is the strongest morphological character, and CW is the weakest morphological character. Percent composition of *R. c. melanota* froglet diet differs from previous reports, but the prey items are mostly terrestrial insects. The presence of the most abundant insect orders changed over the two month trapping period. These changes are in part due to insect life cycles which affect the availability of a given group to the froglet.

Generally, water pH was lower at higher elevation study sites, and at temporary pool, oxbow, and ditch habitats. Acidic precipitation could temporarily cause acidic conditions in these study sites. Further study of water pH of these aquatic habitats is needed to fully determine habitat differences. These studies need to focus on cation analysis for a better understanding of the water chemistry of these habitats.

Overall, water temperatures showed significant monthly differences. Lower water temperatures were found at two sites, but these differences are the result of forest canopy cover. Large pool habitats were the most successful habitats for adult *R. c. melanota* during the breeding season. *R. c. melanota* larvae were more abundant and more successful in large pool habitats.

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