Seasonal activity, reproductive cycles and growth of the pickerel frog *Lithobates palustris* (LeConte, 1825), from Pennsylvania

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ABSTRACT - Seasonal activity, reproduction and post-metamorphic growth were examined in the pickerel frog *Lithobates palustris* from Pennsylvania using 572 museum specimens collected during 1899-2009 from 66 of 67 counties. Frogs were active from March to November, with a peak in July. Testes were at their maximum dimensions in the fall, and females overwintered with ripened eggs. The greatest frequency of males with enlarged thumbs occurred during March to May, and females were gravid during April to May, indicating early spring breeding, which was typical for populations of the pickerel frog in the middle of its geographic range. Estimated clutch size co-varied positively with female body size and averaged 1,785 eggs. Both sexes reached sexual maturity approximately one year after transformation. Sexual dimorphism in body size was pronounced in this population, with males (mean = 54.9 mm SVL; range = 40.9-66.5) having matured and averaged significantly smaller than females (mean = 67.0 mm SVL; range = 54.1-87.4 mm). Findings from this study corroborate a latitudinal trend in breeding season that followed cool weather and also demonstrated an otherwise inflexible response in other life history traits despite a geographically broad distribution.

THE pickerel frog *Lithobates palustris* (LeConte, 1825) is an eastern North American ranid found in a wide range of aquatic habitats (Conant & Collins, 1998; Redmer, 2005). Its breeding season is well documented and has been summarised by Redmer (2005). Following a latitudinal cline in breeding season the pickerel frog breeds earliest and over the longest period in the south, and progressively shorter and later in the late spring to early summer in the north of its range (Redmer, 2005). In turn, eggs can hatch between 10-24 days and tadpoles can transform in two to three months (Johnson, 1984; Harding, 1997; Redmer, 2005). Reproductive biology of the pickerel frog, its gonadal cycles and clutch characteristics are less well known (Resetarits & Aldridge, 1988; Trauth et al., 1990). In Pennsylvania little is also known concerning its reproductive characteristics (Hulse et al., 2001). The goal of this study was to clarify aspects of the breeding phenology of the pickerel frog in Pennsylvania and relate our findings to the broader topic of geographic variation in its life history traits.

MATERIALS AND METHODS

We examined 575 pickerel frogs that were collected during 1899-2009 from 66 of the 67 Pennsylvania counties in the holdings of the Carnegie Museum of Natural History in Pittsburgh and the State Museum of Pennsylvania in Harrisburg. Using calipers, the snout-vent length (mm SVL) of all size classes and of tadpoles was measured.

Sexual maturity in males was determined by the presence of enlarged testes and enlarged thumbs. Length and width of the left testis as a percentage of body size was used to measure seasonal differences in testis dimension. Monthly frequencies of enlarged thumbs also served as a measure of seasonal patterns of fertility. Sexually mature females were associated with one of four ovarian stages. In the first stage, oviducts were thin and just beginning to coil, and the ovaries were somewhat opaque. In the second stage, the oviducts were larger and more coiled, and the ovaries contained some pigmented oocytes. In the third stage, oviducts were thick and heavily coiled, and the ovaries were in various stages of clutch development. In the fourth stage, oviducts were thick and heavily coiled, and the ovaries were full of polarized ova with few non-polarized ova, indicating a fully ripened clutch and gravid female (Meshaka, 2001).

Fat body development was scored as absent, intermediate in volume in the body cavity, to extensive development that reached cranially in the body cavity. The latter was used as an estimated monthly incidence of extensive fat relative to all females examined from each month.

A subset of females was examined for clutch characteristics. Ten clutches were removed, patted on a paper towel to remove excess moisture, a subset of ova weighed on an electronic scale, and that mass then extrapolated to estimate clutch size. From each clutch, the diameters of 10 ova were measured using a dissecting scope fitted with an ocular micrometer; the largest ovum was used in comparative relationships with clutch size and female body size.

Tadpole developmental status was scored using Gosner (1960) staging protocol. For practical purposes, tadpoles were assigned to categories of having poorly developed hind legs (less than Gosner stage 37) or well developed hind legs (Gosner stage of at least 37). Metamorphs were distinguished from tadpoles by the presence of forelimbs (Gosner stage 42) and distinguished from juveniles by the presence of a tail. Statistical analysis and graphs were conducted with the use of Microsoft Excel™ software. Two tailed t-tests were used to compare means between samples and significance was recognised at $P < 0.05$.

RESULTS

Seasonal Activity

Over a period of 100 years pickerel frogs have been collected during March-November

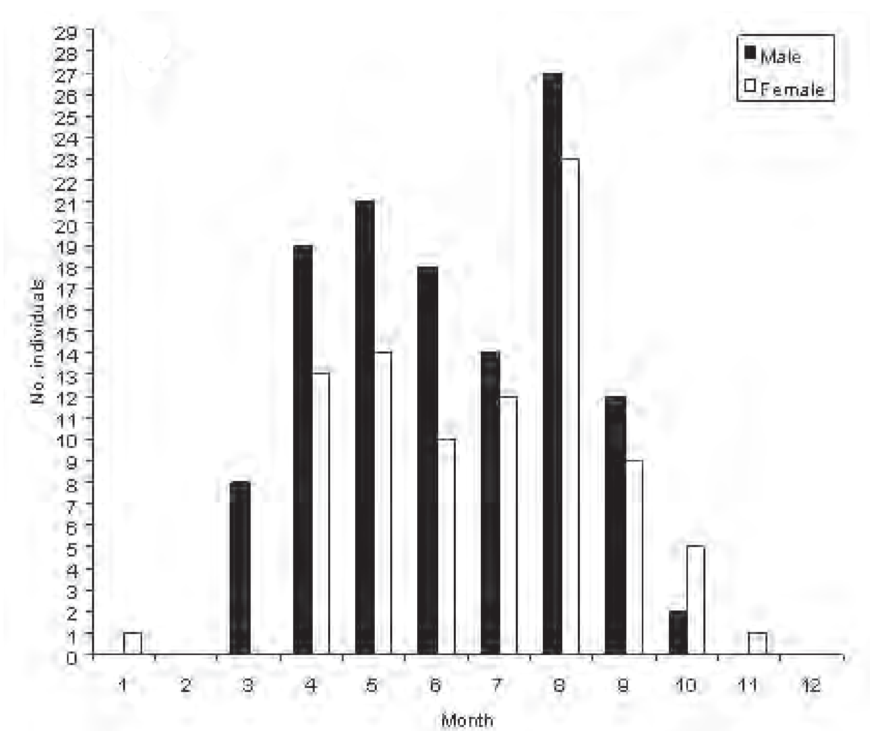

Figure 1. Seasonal incidence of capture of pickerel frogs *Lithobates palustris* from Pennsylvania. (males n = 121 and females n = 88).

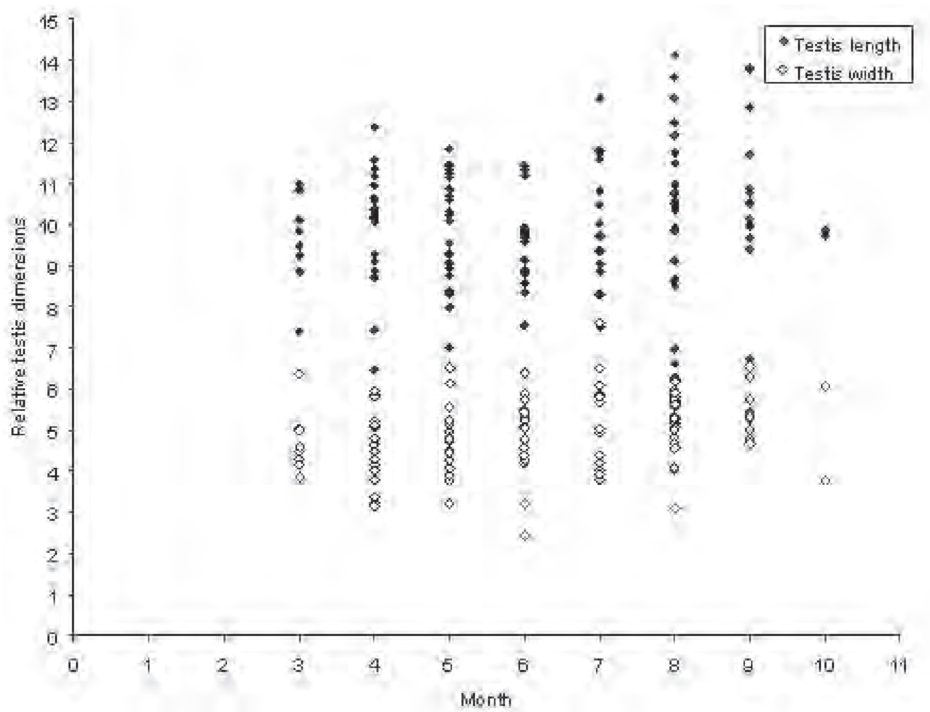

Figure 2. Monthly distribution of testis size of 121 pickerel frogs *Lithobates palustris* from Pennsylvania.

(Fig. 1). An exception to this was a single female individual found in January that was taken from a cave in Indiana County. Commensurate with breeding activities, males outnumbered females in collections during the early part of the year more so than at other times. Peak collections occurred in July, synchronous with the appearance of Metamorphs and perhaps with greater terrestrial activity after breeding. This suggests a need by adults to replenish fat stores before the end of autumn. Metamorphs were apparent from June to August, whereas juveniles were present from March to October. Both life stages were most apparent in August (23/48 and 101/315 individuals respectively).

Seasonal Changes in Testis Size

The monthly distribution of testis length as a percentage of snout-vent length was reduced from March through June and followed by an abrupt increase which peaked soon thereafter (Fig. 2). Seasonal changes in testis width were less apparent but likewise appeared to reach maximum dimensions by autumn. The seasonal distribution of testis dimensions corroborated spring breeding, as sperm was drained from maximally expanded testis by June and followed by recrudescence thereafter. In this way, enlarged testis toward the end of the year would accompany dormancy so that males would be fertile immediately upon spring emergence.

Male Thumbs

The highest monthly incidences (100%) of enlarged thumbs in males occurred during March to May and again in October. As was the case for the seasonal changes in testis size, enlarged thumbs (a measure of fertility), were highest in frequency at the beginning of dormancy. Enlarged male thumbs lasted through May and were, expectedly, associated with spring breeding. The lowest incidence of enlarged thumbs occurred in June (61.1%) and steadily increased thereafter.

Male Fat and Presence of Food

Extensive fat development was present in males

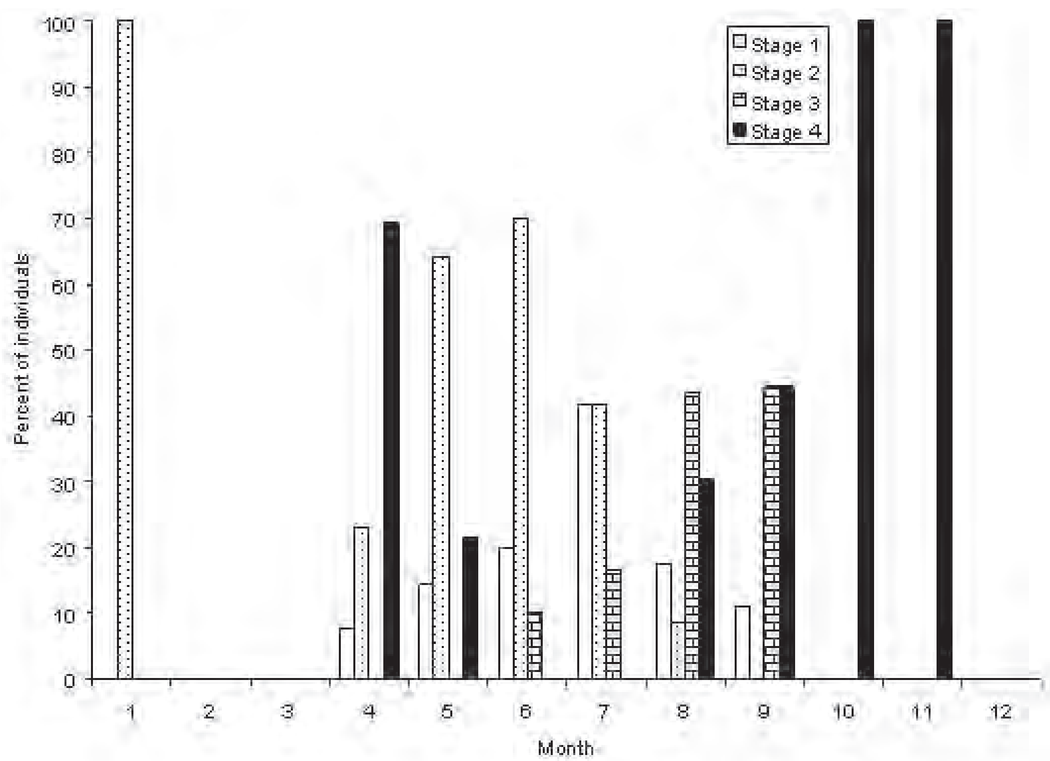

Figure 3. The annual ovarian cycle of 88 pickerel frogs *Lithobates palustris* from Pennsylvania.

throughout their active season. However, males emerged with depleted fat stores which, in turn, were at their lowest at the end of breeding in May. Fat development increased monthly, beginning in June until winter dormancy began. At this time all males of the October sample contained extensive fat. Only 25% of males contained food upon emergence in spring. The monthly incidence of males containing food increased rapidly in April (78.9%) while males were breeding. It then continued to increase over the remaining season and peaked in July, sufficient to sequester fat for the winter.

Ovarian Cycle

Having emerged shortly before breeding the majority of females in April were gravid (Fig. 3). The last gravid female of May was collected on 18 May. In June females began the production of clutches as evidenced by a steady decrease in frequencies of stage 1 and 2 females and the steady increase in frequency of stage 3 females during June to September. This trend resulted in the presence of gravid females by August and a full complement of gravid females ready to enter dormancy in preparation for breeding soon after spring emergence.

Clutch Characteristics

Ten females (mean = 74.2 ± 5.01 mm SVL; range = 66.1-79.9 mm) collected during the breeding season produced a mean clutch size of 1,785 eggs (SD = ± 531.4; range = 850-2450). A significant and positive relationship existed between clutch size and female SVL ($r^2 = 0.7199$, $P < 0.002$) but not between maximum ova size and female SVL (Fig. 4). The relationship between maximum ova diameter and clutch size was positive and significant ($r^2 = 0.5116$, $P < 0.02$).

Female Fat and Presence of Food

Eight months of collections had females available for analysis. The frequencies of females with extensive fat development were generally low (<

22.2%) but highest shortly after breeding during June (70.0%) and July (41.7%) and among the 52.9% of females whose clutch development was well underway (stage 3). Only 10.3% of gravid females contained extensive fat. Extensive fat development was also low in stage 1 (25.0%) and stage 2 (11.1%) females. The monthly frequencies of digestive tracts containing food indicated that females were foraging extensively throughout much of the active season. Only in April were frequencies of females having contained food less than 60% of the monthly sample. Between spring emergence and commencement of breeding (after April) food in digestive tracts increased rapidly and then decreased in October as females prepared to enter dormancy. Likewise, food in digestive tracts was present in all four ovarian stages with high frequencies (>80%) found among spent and yolking females while less than one half the frequency (39.7%) was found among gravid females.

Growth and Sexual Maturity

Across Pennsylvania, metamorphs were present during June to August (Fig. 5), indicating a two to three month larval period after April and May breeding. Body sizes of metamorphs ranged 18.6-31.7 mm SVL (mean = 25.6 ± 3.9 mm; n = 48). The monthly distribution of body sizes suggested that males reached a minimum of 40.9 mm SVL the following spring at 10 months of post-metamorphic age. Mean body size of sexually mature males was 54.9 mm SVL (SD = ± 5.7 mm; range = 40.9-66.5 mm; n = 121).

The monthly distribution of body sizes (Fig. 5) suggests that although some females could reach maturity of at least 54.1 mm SVL they reached sexual maturity the following summer at approximately 13 months post-metamorphic age. Females appeared to have bred for the first time when they were 22-23 months post-metamorphic age. Mean body size of sexually mature females was 67.0 mm SVL (SD = ± 8.6 mm; range = 54.1-87 mm; n = 88) (Fig. 5).

Among 88 sexually mature females the mean body size of gravid females (71.2 ± 7.6 mm SVL; range = 57.0-86.5 mm; n = 29) was significantly

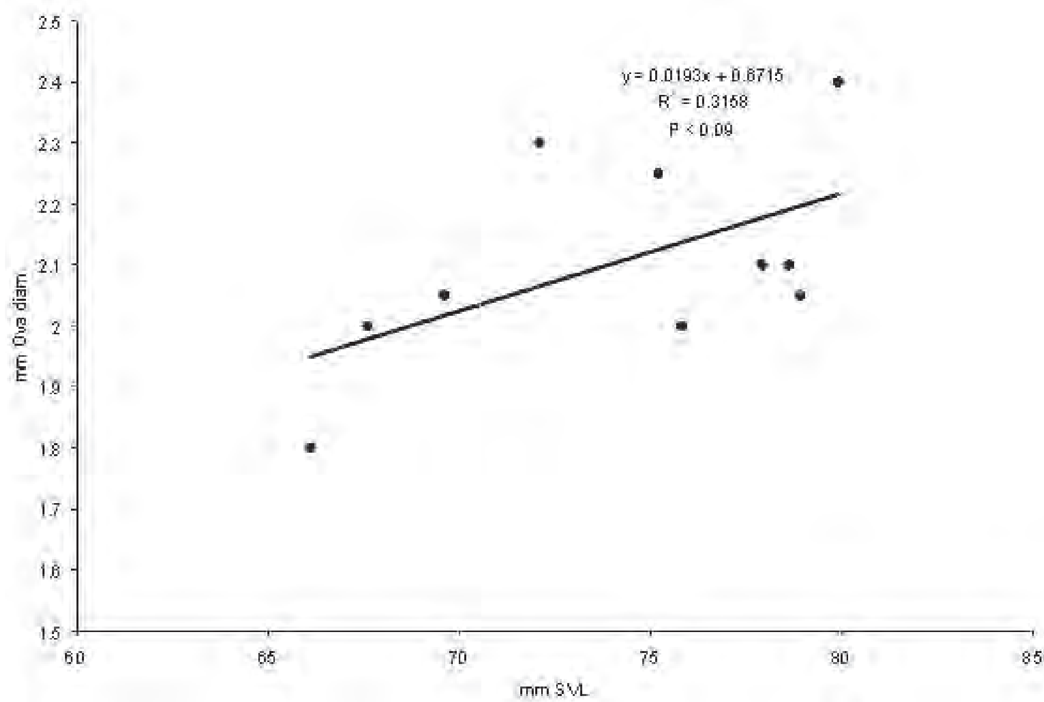

Figure 4. The relationship between largest ova diameter and body size in mm SVL of 10 female pickerel frogs *Lithobates palustris* from Pennsylvania.

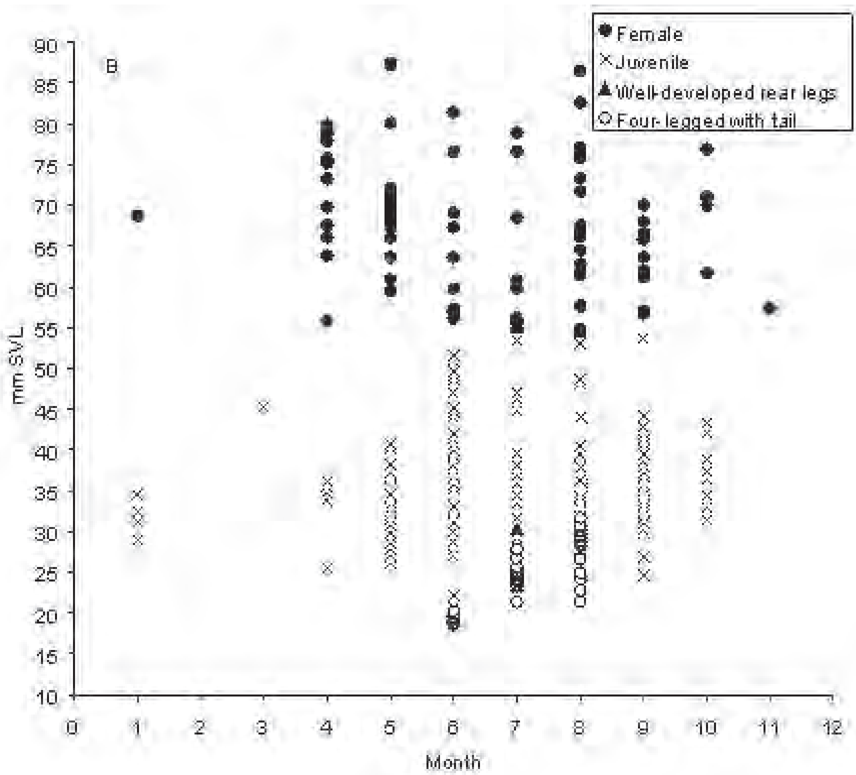

Figure 5. Monthly distribution of body sizes of females (n = 88), juveniles (n = 315), tadpoles with well-developed rear legs (n = 3) and metamorphs (n = 48) of the pickerel frog *Lithobates palustris* from Pennsylvania.

larger (t = -3.383, df = 86, P < 0.001) than that of non-gravid counterparts (65.0 ± 8.3 mm SVL; range = 54.1-87.4 mm; n = 59). Among all sexually mature adults, mean body size of females was significantly larger (t = -12.274, df = 207, P < 0.001) than that of males.

DISCUSSION

The geographic range of the pickerel frog extends southward from the Canadian Maritime Provinces of New Brunswick and southern Quebec to southern Mississippi (Conant & Collins, 1998), thereby placing Pennsylvania midway across the latitudinal range for the species. This large latitudinal distribution seems to correspond with a cline in the reproductive season of this species, whereby southern populations were typified by December to May breeding, central populations were typified by March to May breeding, and northern populations were typified by May to June breeding (Redmer, 2005). These are broad ranges that are useful within the context of large geographic areas. Within these seasonal ranges can exist shorter seasons but within a broad range. Therefore it is not surprising that within such a large central portion of the pickerel frog's geographic range that breeding seasons may have narrower subsets of the March to May range as in Missouri (Johnson, 1984). For example, in Missouri the breeding season was reported during March to May (Johnson, 1984: Redmer 2005) and that of Wisconsin was April to May (Vogt, 1981; Redmer 2005). The latter season is also true of findings by Hulse et al. (2001), Klemens (1993) for Connecticut and adjacent areas, Great Lakes region (Harding, 2000), and Ithaca, New York (Wright & Wright, 1949). Breeding occurred in April in Indiana (Minton, 2001) and in Pennsylvania Surface (1913) noted egg-laying in May. Our findings are best characterised as that expected for the central portion of the pickerel frog's geographic range having bred during April to May (Redmer, 2005).

The similarity in breeding season between

Pennsylvania and Missouri was reflected in similar gonadal cycles. For example, in Missouri testis mass was lowest in late winter and followed by an increase in mass during April to July (Resetarits & Aldridge, 1988). Similarly, in our study, testis size was smallest in fall and spring and increased in size during May to September.

Two studies, in Missouri (Resetarits & Aldridge, 1988) and our Pennsylvania contribution, show that full complements of eggs were present by the end of summer. Mean clutch size was remarkably stable in the few regions from which such data is available. A mean clutch size of 1,759 eggs was reported for populations in both Missouri (Resetarits & Aldridge, 1988) and adjacent Arkansas (Trauth et al., 1990), similar to the mean values of 1,785 eggs of our study. In all three studies clutch size was positively associated with female body size.

With one difference, the fat cycles of males and females from our study were similar to those from Missouri (Resetarits & Aldridge, 1988). For example, overwintering males initially stored greater amounts of fat than females in Missouri (Resetarits & Aldridge, 1988) and the frequency of males with extensive fat at that time was greater than that of females in Pennsylvania (this study). On the other hand, both sexes entered overwintering with maximum fat stores in Missouri (Resetarits & Aldridge, 1988), whereas in our Pennsylvania study this occurred in males only.

In Missouri, later depletion of fat in males (February) than females (January) was thought to be associated with mobilisation of fat stores by males for breeding activity and to initiate spermiogenesis (Resetarits & Aldridge, 1988). Likewise, fat made in the summer by females in Pennsylvania was possibly converted quickly into clutches for the following season, with less of a requirement for extensive fat storage through the winter than male counterparts. Males, in turn, may possibly have needed more fat through the winter to be used in the spring for calling activity.

Appearance of metamorphs ranged from June in Arkansas (Trauth et al., 1990) and Illinois (Smith, 1961) to June and July in Indiana (Minton, 2001) and Louisiana (Hardy & Raymond, 1991), June to August in Pennsylvania (this study), July to September in Connecticut (Klemens, 1993) and August and September in Pennsylvania (Surface, 1913). A comparison of these emergence dates with respective breeding dates suggests a larval duration of two to three months across populations, although, a four month larval duration may have occurred during one of the breeding seasons studied by Raymond & Hardy (1991). Larval periods of the pickerel frog were noted to be 60 to 80 days in Wisconsin (Vogt, 1981), three months in Missouri (Johnson, 1987), approximately 90 days in West Virginia (Green & Pauley, 1987), three and 1.5 months in Arkansas (Trauth et al., 1990), 60 to 90 days in the Great Lakes Region (Harding, 1997), and 70 to 80 days in the Carolinas and Virginia (Martof et al., 1980). In laboratory conditions, metamorphosis took place 75 to 90 days after hatching (Redmer, 2005). Our data suggest a two, and up to three month, larval period in Pennsylvania, the duration of which was not surprising in light of the frogs known breeding season.

Post-metamorphic growth to sexual maturity was relatively rapid in males (10 months) and females (13 months) from Pennsylvania and most males and all females would reproduce for the first time the breeding season thereafter. Estimated age at sexual maturity of both sexes in our study was earlier than ages noted by others across the region; second spring after transformation in Pennsylvania (Hulse et al., 2001); second spring after hatching in the Great Lakes region (Harding, 1997); three years of age in West Virginia (Green & Pauley, 1987).

The minimum and mean body sizes of adult males (SVL) ranged less so than those of females across the geographic range with no apparent latitudinal trend. Likewise, the degree of sexual size dimorphism was constant across locations whereby males averaged approximately 80% the size of females. The largest adults, however, ranged broadly in both sexes across the range of the species.

CONCLUSION

Pennsylvania populations of the pickerel frog conformed to the latitudinal gradient associated with the breeding season of the species (Redmer, 2005), with Pennsylvania falling midway between northerly and southerly extremes. Concomitant to geographic differences in breeding season, the months in which Pennsylvania pickerel frog metamorphs appeared also differed from other areas of its range, with those of Pennsylvania intermediate between the geographic extremes. Not enough data has yet been collected to determine growth rates beyond age at sexual maturity for the pickerel frog, and to date, the few estimations regarding age at sexual maturity ranged widely. This highlights the requirement for more data before meaningful trends can be proposed. However, despite this, adult body sizes were relatively fixed across its geographic range with a constant ratio of size dimorphism. Mean clutch sizes reported from three states were also similar. Although the larval period of the pickerel frog ranged from one and a half to three and possibly four months, most records noted above were within the two to three month range, which typified Pennsylvania. Thus, Pennsylvania populations of the pickerel frog appear to adhere to the latitudinal pattern associated with cool weather breeding but otherwise exhibit little variability with respect to adult body size and clutch characteristics.

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REFERENCES

Conant, R. & Collins, J.T. (1998). *Reptiles and Amphibians of Eastern/Central North America.* New York: Houghton Mifflin Co.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.

Green, N.B. & Pauley, T.K. (1987). *Amphibians and Reptiles in West Virginia.* Pennsylvania: University of Pittsburgh Press.

Harding, J.H. (1997). *Amphibians and Reptiles of the Great Lakes Region*. Michigan: University of Michigan Press.

Hardy, L.M. & Raymond, L.R. (1991). Observations on the activity of the pickerel frog, *Rana palustris* (Anura: Ranidae), in northern Louisiana. *J. Herpetol.* **25**, 220-222.

Hulse, A.C., McCoy, C.J. & Censky E.J. (2001). *Amphibians and Reptiles of Pennsylvania and the Northeast.* New York: Cornell Univ. Press.

Johnson, T.R. (1987). *The Amphibians and Reptiles of Missouri.* Jefferson City: Missouri Department of Conservation.

Klemens, M.W. (1993). Amphibians and Reptiles of Connecticut and Adjacent Regions. State Geol. and Nat. Hist. Survey, Bull. No. 112.

Martof, B.S., Palmer, W., Bailey, J. & Harrison J.R. (1980). *Amphibians and Reptiles of the Carolinas and Virginia.* North Carolina: University of North Carolina Press.

Meshaka, W.E., Jr. (2001). *The Cuban Tree Frog in Florida: Life History of a Successful Colonizing Species*. Gainesville: University Press Florida.

Minton, S.A., Jr. (2001). *Amphibians and Reptiles of Indiana.* Indianapolis: Indiana Academy of Science.

Redmer, M. (2005). *Rana palustris* LeConte, 1825; pickerel frog. In: *Status and Conservation of North American Amphibians.* M. Lannoo (Ed.). Pp. 568-570. California: Univ. California Press.

Resetarits, W.J., Jr. & Aldridge R.D. (1988). Reproductive biology of a cave-associated population of the frog *Rana palustris*. *Can. J. Zool.* **66**, 329-333.

Smith, P.W. (1961). The amphibians and reptiles of Illinois. *Ill. Nat. Hist. Surv. Bull.* **28**, 298.

Surface, H.A. (1913). First report of the economic features of the amphibians of Pennsylvania. *Penn. Zoo. Bull. Div. Zoo. Penn. Dept. Agri*. **3**, 68-152.

Trauth, S.E., Cox, R., Butterfield, B., Saugey, D. & Meshaka, W.E., Jr. (1990). Reproductive phenophases and clutch characteristics of selected Arkansas amphibians. *Proc. Ark. Acad. Sci.* **44**, 107-113.

Trauth, S. E., Plummer, M.J. & Robison H.K. (2004). *Amphibians and Reptiles of Arkansas.* Little Rock: Arkansas Fish and Game Commission.

Vogt, R.C. (1981). *Natural History of Amphibians and Reptiles of Wisconsin.* Milwaukee: Milwaukee Public Museum.

Wright. A. H. & A.A. Wright. (1949). Handbook of Frogs and Toads of the United States and Canada. Ithaca: Cornell University Press.