Project 3: Assessment of population size, age structure and growth rates for cave inhabiting crayfish in Alabama

Final Report – December 2008

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Summary

A three-year mark-recapture study was conducted on three species of crayfish, *Cambarus tenebrosus*, *Cambarus hamulatus,* and *Orconectes australis,* found in four caves in northeastern Alabama. A total of 2,879 crayfish were marked. Due to low recapture numbers, growth models could not be developed for *C. tenebrosus* or *C. hamulatus*. However, relatively robust growth models were produced for three populations of *O. australis*. These models produced size-at-age relationships indicating that longevity and time-to-maturity to range from 19 to 50 and 4 to 28 years, respectively. These estimates varied among caves, indicating life history attributes are controlled to some degree by cave specific environmental characteristics. Our estimates of longevity and time-to-maturity are substantially lower than those provided for *O. australis* by Cooper (1975) and are more similar the upper range of longevity estimates available for other species of cave and surface crayfish. This study provides insight into the life history characteristics of *O. australis* from multiple populations and illustrates why conservation personnel should consider cave-specific variation when developing management strategies for cave species in Alabama.

Introduction

Six species of crayfish are obligate inhabitants of subterranean streams and pools (i.e., stygobionts) in Alabama. Four are endemic to the Tennessee River Basin in Alabama—*Cambarus jonesi*, *C. veitchorum*, *Orconectes sheltae*, *Procambarus pecki* (soon to be reclassified as *Cambarus pecki,* J. Cooper, personal communication)— the remaining species, *Cambarus hamulatus* and *Orconectes australis*, occur in northern Alabama and Tennessee. Until very recently, populations of *Orconectes australis* in Alabama were recognized as the subspecies *O. australis australis* (Buhay & Crandall 2008). None of these six crayfish species are listed as Federally Endangered or Threatened. Within Alabama, however, *P. pecki*, *O. sheltae* and *C. veitchorum* have been assigned a conservation status of Priority 1/Highest Conservation Concern, *C. hamulatus* and *C. jonesi* have been classified as Priority 2/High Conservation Concern, and *O. australis* (as *O. a. australis*) is considered Priority 3/Moderate Conservation Concern (ACWCS 2008). Most recently, Buhay & Crandall (2008) report that, compared with other closely related species, *O. australis* is of minor conservation concern due to its comparatively large geographic distribution (caves throughout NE Alabama and central Tennessee) and its occurrence in numerous and abundant populations with high levels of genetic diversity.

Analyses of population size, age structure, and longevity are critical for determining the viability of wildlife populations. Considering the relatively high level of imperilment of many of the stygobitic crayfish species of Alabama, as indicated by their conservation rankings, it is striking that so little is known about the attributes of single populations, not to mention the complete lack of knowledge about how these attributes vary among populations of different cave systems. Knowledge of population size, age structure, and longevity is critical information required for the assessment of population viability and for monitoring potential population imperilment.

In this report we summarize the results of research focused primarily on developing methods for the efficient assessment of population size, age structure, and individual growth rates and longevity for the widespread cave crayfish, Orconectes australis *and* Cambarus hamulatus. This was the primary objective of our research supported by Alabama SWG T-03-02. Our rationale for this research was to find an effective approach to assessing recruitment and overall population viability for cave crayfish using a common species as a model. The relatively widespread *O. australis* will be used as a surrogate for developing a protocol for the assessment of population attributes of other, more imperiled species of stygobitic crayfish.

It is important to note that this final report contains data that are part of a continuing study supported by a second Alabama SWG. Although they should be considered the definitive record of research conducted under T-03-02, they will be integrated into the data contained in the final report that will be supported by continuing Alabama SWG funding (final report to be provided in 2011).

Methods

Cave selection. Only caves with abundant populations of crayfish were selected for study. Caves surveyed were selected based on the recommendation of members of the National Speleological Society (Huntsville Grotto). This study would have not been possible without the close cooperation of the caving community. During the original cave-selection process, an attempt was made to include hydrologically-isolated caves (as can best be determined) to provide isolated populations of crayfish.

Habitat information. Temperature and water depth gauges were installed in aquatic habitats in each study cave. These were programmed to record measurements at 5 minute intervals. Temperature (^{o}C) and water depth (dm) were reduced to daily means. To enhance interpretation of seasonal patterns of changing water depth among caves, mean daily water depth was converted to relative depth by normalizing the data to a common average depth among caves. The length of each study reach was also measured and the percent pool and riffle was calculated.

Capture of crayfish. Crayfish were sampled using hand nets. A standardized system of collection was followed. This consisted of a pair of individuals moving in an upstream direction collecting crayfish as they are encountered. As the first individual moved up the reach collecting crayfish, the second waited for a ten-minute period before following. This method has proven to be very effective and was adopted in lieu of baited traps. The length of the stream reach sampled varied from cave to cave, and was determined by the physical characteristics of each cave (e.g., amount of accessible stream available, waterfalls, deep pools).

Size and reproductive status of crayfish. Standard length (tip of rostrum to posterior margin of carapace) and ocular length (posterior margin of ocular cavity to posterior-center margin of carapace) was measured for each crayfish to the nearest 0.1 mm with dial calipers. For males, the reproductive form (Form I or II) was recorded. For females the presence of active cement glands and presence of ova or young on pleopods were also recorded. Voucher specimens of mature individuals were taken from each population, preserved in ethanol, and deposited in the University of Alabama Decapod Collection.

Marking and recapture. Crayfish were marked using both internal tags (Visible Implant Alpha Tags-VIAT, Northwest Marine Technology) and Visible Implant Elastomer-VIE (Northwest Marine Technology). VIAT are small (1.0 X 2.5 mm), fluorescent, numbered tags that are placed internally using an injection apparatus. When placed beneath the cuticle against the ventral musculature of the tail of crayfish, they are readily visible and readable. Crayfish were also be marked by injecting VIE into soft tissues near the cuticle on the dorsum of the abdomen, where it cured into a soft rubber mark. Double marking allowed the assessment of tag loss, which was found to be low. Once processed, crayfish were released near the point of capture.

Individual longevity and growth rates. A bootstrap technique (Effron & Tibshirirani, 1993) was used to construct a minimum longevity model (Huryn et al. 1994; Whitmore & Huryn 1999) for *O. australis* in Hering, Tony Sinks, and Limrock Blowing caves. Measurements of annual gain in carapace length (AGCL) obtained by recapturing crayfish *marked for at least 350 days* provided the raw data required by the model. The AGCL of crayfish recaptured that were marked for longer than one year were transformed by dividing the gain in carapace length by the number of days elapsed since marking and multiplying the resulting value by 365. To account for variation in growth observed among crayfish, a bootstrap procedure was performed on AGCL by randomly resampling the data with replacement 1000 times. For each data set a regression equation of AGCL against initial carapace length (CL_i) was estimated. To reduce variability due to potential errors in carapace measurement, the highest and lowest AGCL rates were removed from each data set. In addition, the bootstrap model was constrained so that negative growth could not occur (i.e., individuals becoming smaller from year to year). To be conservative the longevity model was started with a CL assumed to represent one full-year's growth after hatching (based on observation on newly hatched crayfish maintained in the laboratory at temperatures equivalent to those found in the study caves). This seed CL was placed into the vector of 1000 equations and was used to generate estimates of predicted AGCL for the second year ($AGCL_{predicted}$). The size at the end of the second year's growth was calculated as:

$$CL_{\,year\ 2} \;=\; \mathrm{CL}_{\,\mathrm{seed}} + AGCL_{predicted} \qquad (1)$$

$CL_{year\ 2}$ was then be used as the seed for the third year of growth and so on. In this manner, the estimated CL for each year of growth was calculated as:

$$CL_{\,year\ \ i+1} \;= CL_{\,year\ i} + \;AGCL_{predicted} \qquad (2)$$

Confidence intervals for the expected mean CL of each year class were estimated using the frequency distribution of values estimated by the equation vectors. In this way, an estimate of age on the basis of CL was made with an explicit margin of error (e.g. 95% confidence intervals).

Sampling schedule. When cave conditions allowed, crayfish were collected and marked at approximately 2-month intervals from autumn 2005 through winter 2007, and at monthly intervals thereafter. This report includes data collected through October 2008. Depending upon the cave, crayfish were collected and marked on a minimum of 15 occasions and a maximum of 19 occasions.

Results

Description of study locations. Twenty seven Alabama caves were surveyed during this study. Abundant crayfish populations were located in four: Tony Sinks Cave System (E-1), Hering Cave, Limrock Blowing Cave and Bluff River Cave. All four caves contained streams providing riffle-pool habitat. *Orconectes australis* occurs in Tony Sinks, Hering Cave and, Limrock Blowing Cave. The populations of *O. australis* in Hering Cave and Limrock Blowing Cave were also used in the phylogenetic analyses of Buhay & Crandall (2005, 2008). *Cambarus hamulatus* was found only in Bluff River Cave. *Cambarus tenbrosus* also occurs in all four cave systems.

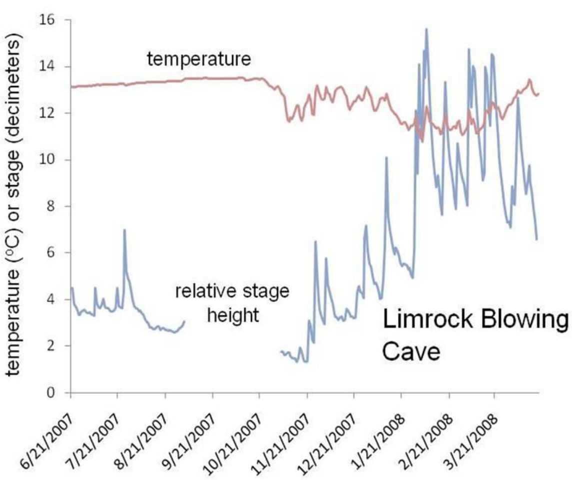

Fig. 2. Mean daily water temperature (^oC) and relative stage height (water level, dm) for Limrock Blowing Cave (21 June 2007 - 18 April 2008).

Length of sampling reach varied among the four caves. The reaches in Tony Sinks, Bluff River, Limrock Blowing, and Hering caves were 324, 690, 959, and 1203 m, respectively. Tony Sinks sampling reach consisted entirely of pools. The stream reaches sampled in Bluff River, Limrock Blowing and Hering caves consisted of 32%, 25%, and 15% riffle habitat, respectively.

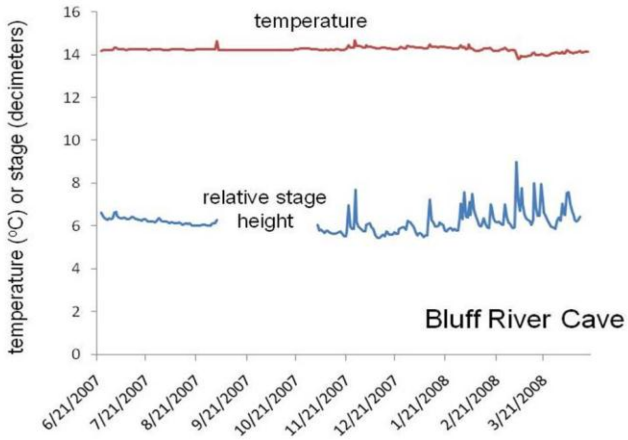

Fig. 1 Mean daily water temperature (^{o}C) and relative stage height (water level, dm) for Bluff River Cave (21 June 2007 - 18 April 2008).

Mean daily stream-water temperature, measured in each cave from 21 June 2007 to 18 April 2008 (Figs. 1-4), ranged from a minimum of 12.7 ^{o}C in Limrock Blowing Cave (Fig. 2) to a maximum of 14.1 ^{o}C in Bluff River Cave (Fig. 1). Water temperature was relatively constant in each of the caves, with mean daily temperatures, measured from 21 June 2007 to 18 April 2008, ranging from a minimum of 0.9 ^{o}C in Bluff River Cave (Fig. 1) to a maximum of 2.7 ^{o}C in Limrock Blowing Cave (Fig. 2). The temperature of the stream in Bluff River Cave (Fig. 1) showed little seasonality while the water temperature of Limrock Blowing Cave (Fig. 2) and Hering Cave (Fig. 3) showed relatively marked decreases during winter. In contrast with the previous caves, Tony Sinks showed an increase in water temperature during winter (Fig. 4). Seasonal shifts in water temperature appeared to be closely related to increases in groundwater flux. Mean daily air temperatures measured at a location in Stevenson, AL, during this period was 14.6 ^{o}C and ranged from -3.2 to 33.0 ^{o}C (compared to a maximum range of 2.7^{o}C measured for the cave streams).

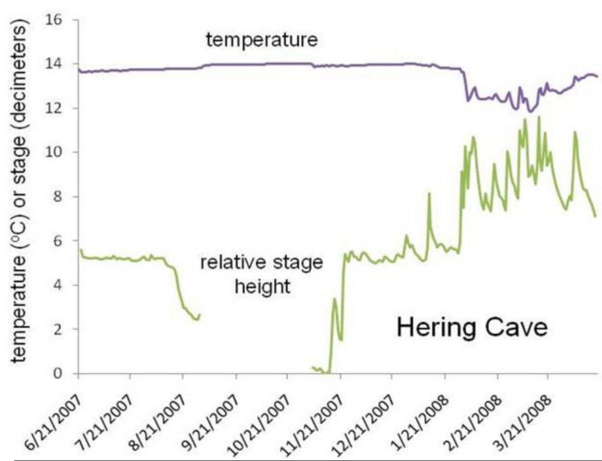

Fig. 3. Mean daily water temperature (^{o}C) and relative stage height (water level, dm) for Hering Cave (21 June 2007 - 18 April 2008).

Relative stage height, or water level, provides information about the variability of flow of the stream habitat provided by each cave. Changes in relative water level for the streams in Tony Sinks and Bluff River, Limrock Blowing and Hering Caves were measured from 21 June 2007 to 18 April 2008. As observed for the range of temperature fluctuation, the water level of Bluff River Cave showed the smallest range of variation (35 cm, Fig. 1) and Limrock Blowing Cave showed the greatest (143 cm, Fig. 2). Hering, and Tony

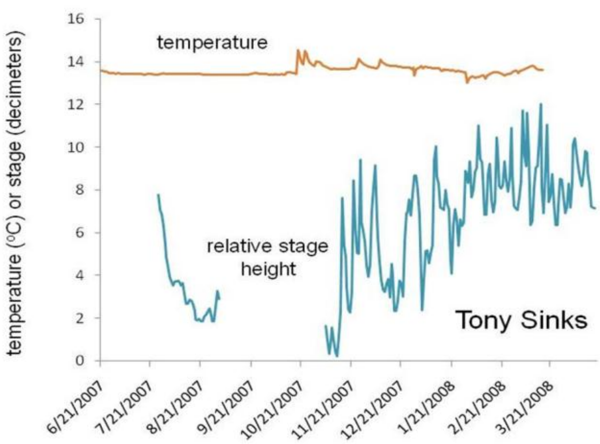

Fig. 4. Mean daily water temperature (^{o}C) and relative stage height (water level, dm) for Tony Sinks (21 June 2007 - 18 April 2008).

Sinks also showed changes in water level exceeding 100 cm (Figs. 3-4).

Of the four caves used in this study, Bluff River Cave was clearly exceptional due to its relatively stable water temperature and discharge; Bluff River Cave is also the only cave studied that contained *Cambarus hamulatus*. Limrock Blowing and Hering caves were most similar, showing distinct seasonal decreases in water temperature that corresponded to rapid increases with relative water stage during winter. Although Tony Sinks also showed a seasonal pattern in water stage that was similar to those observed for Limrock Blowing and Hering, it differed by showing an increase in water temperature as water levels rose in early winter. Several conclusions about these cave systems as habitat for crayfish can be drawn from these data. First, the temperature regime of each of the study caves was extremely stable compared with surface systems, as expected. Second, although thermal regimes were similar, there is a large range in the relative hydrological stability of the different caves, with Bluff River Cave providing a very stable physical habitat, while the other caves showed large fluctuations

Table 1. Size and reproductive status for specimens of *Orconectes australis* captured in Tony Sinks, and Hering and Limrock Blowing caves in Alabama (November 2006 – October 2008). Total ♂♂= total males observed. TCL(SE) = mean total carapace length in mm (standard error). M1 ♂♂= number of Form I (reproductively active) males. Total ♀♀=total females observed. ♀♀$_{cement}$= numbers of females observed with active cement glands, ♀♀$_{ovig}$=number of females observed with attached eggs or young.

Males

Cave	Total ♂♂	TCL (SE)	M1 ♂♂	TCL (SE)
Bluff River Cave	---	---	---	---
Tony Sinks	378	19.2 (0.2)	66	22.2 (0.4)
Hering Cave	210	23.6 (0.4)	36	28.6 (0.7)
Limrock Blowing Cave	276	20.6 (0.3)	30	24.4 (0.9)

Females

Cave	Total ♀♀	TCL (SE)	♀♀$_{cement}$	TCL (SE)	♀♀$_{ovig}$	TCL(SE)
Bluff River Cave	---	---	---	---	---	---
Tony Sinks	431	19.3 (0.2)	21	25.2 (0.6)	1	23.0 (--)
Hering Cave	275	23.6 (0.4)	8	30.9 (1.2)	2	30.6 (--)
Limrock Blowing Cave	322	21.1 (0.3)	2	32.3 (2.5)	1	29.7 (--)

in water volume. Third, the differences in thermal regimes and patterns of water depth indicated that, at least Bluff River Cave and Tony Sinks are apparently not hydrologically connected with Limrock Blowing and Hering caves.

Numbers of crayfish captured and tagged.

Crayfish have been captured and tagged from: Bluff Cave at monthly intervals from 8 May 2007 to 25 October 2008; Tony Sinks at seasonal intervals from 27 July 2006 to 27 January 2007, and at monthly intervals from 24 June 2007 to 19 October 2008; Hering Cave at seasonal intervals from 18 November 2005 to 17 September 2006, and at monthly intervals from 6 April 2007 to 18 October 2008; Limrock Blowing Cave at seasonal intervals from 14 January 2006 to 17 September 2006, and at monthly intervals from 9 May 2007 to 18 October 2008. As of October 2008, a total of 2,542 crayfish have been marked with VIAT tags and 2,879 crayfish have been marked with VIE implants in these four caves. All tagged crayfish were also marked with VIE, while crayfish too small to safely receive tags were marked with VIE only—thus explaining the discrepancy between numbers of crayfish marked by the different methods. Of these crayfish, 704, 405 and 541 specimens of *Orconectes australis* were tagged with VIAT in Tony Sinks, Hering Cave and Limrock Blowing Cave, respectively. Two hundred and eight, 197, 24 and 106 *Cambarus tenebrosus* were tagged with VIAT in Tony Sinks, Hering Cave, Limrock Blowing Cave and Bluff River Cave, respectively. Three hundred and fifty-seven specimens of *Cambarus hamulatus* were tagged with VIAT in Bluff River Cave.

Table 2. Size and reproductive status for specimens of *Cambarus hamulatus* captured in Bluff River Cave in Alabama (November 2006 – October 2008). Total ♂♂= total males observed. TCL(SE) = mean total carapace length in mm (standard error). M1 ♂♂= number of Form I (reproductively active) males. Total ♀♀=total females observed. ♀♀$_{cement}$= numbers of females observed with active cement glands, ♀♀$_{ovig}$=number of females observed with attached eggs or young.

Males

Cave	Total ♂♂	TCL (SE)	M1 ♂♂	TCL (SE)
Bluff River Cave	237	15.1 (0.1)	45	16.1 (0.2)

Females

Cave	Total ♀♀	TCL (SE)	♀♀$_{cement}$	TCL (SE)	♀♀$_{ovig}$	TCL(SE)
Bluff River Cave	239	15.0 (0.1)	20	16.2 (0.3)	---	---

Size and reproductive status of crayfish.

<u>*Orconectes australis.*</u> The mean total carapace lengths of reproductively active females (obvious cement glands) ranged from 25.2 mm in Tony Sinks to 32.3 mm in Limrock Blowing Cave (Table 1). The proportion of reproductively active females, compared with the total number captured, was low, ranging from <1% in Limrock Blowing Cave

Table 3. Size and reproductive status for specimens of *Cambarus tenebrosus* captured in Tony Sinks, and Hering, Limrock Blowing and Bluff River caves in Alabama (November 2006 – October 2008). Total ♂♂= total males observed. TCL(SE) = mean total carapace length in mm (standard error). M1 ♂♂= number of Form I (reproductively active) males. Total ♀♀=total females observed. ♀♀$_{cement}$= numbers of females observed with active cement glands, ♀♀$_{ovig}$=number of females observed with attached eggs or young.

Males

Cave	Total ♂♂	TCL (SE)	M1 ♂♂	TCL (SE)
Bluff River Cave	67	26.0 (1.1)	8	38.8 (1.1)
Tony Sinks	143	30.7 (0.8)	28	39.7 (0.7)
Hering Cave	108	39.4 (1.3)	45	46.3 (0.6)
Limrock Blowing Cave	12	38.6 (2.8)	1	48.2 (--)

Females

Cave	Total ♀♀	TCL (SE)	♀♀$_{cement}$	TCL (SE)	♀♀$_{ovig}$	TCL(SE)
Bluff River Cave	52	22.1 (0.8)	1	31.5 (--)	---	---
Tony Sinks	90	27.1 (0.7)	---	---	---	---
Hering Cave	102	28.9 (1.5)	23	42.0 (0.7)	1	44.1 (--)
Limrock Blowing Cave	11	37.7 (2.3)	---	---	---	---

to ~5% in Tony Sinks. The development of active cement glands showed no apparent pattern of seasonality for any population. Four females that were either ovigerous or carrying attached young were collected: 1 in Tony Sinks (8 January 2008), 2 in Hering Cave (4 November 2007, 8 January 2008), and 1 in Limrock Blowing Cave (2 December 2007). Although numbers of ovigerous females observed is low, it is apparent that the laying of eggs and hatching of young occurs in autumn and early winter.

The mean total carapace-length of reproductively active (Form I) males of *Orconectes australis* ranged from 22.2 mm in Tony Sinks to 28.6 mm in Hering Cave (Table 1). The proportion of reproductively active males, compared with the total number captured, was relatively high compared with females, ranging from <11% in Limrock Blowing Cave to ~17% in Tony Sinks and Hering Cave. The occurrence of Form I males showed no apparent seasonal pattern for any population. Sex ratios (females:male) for the total crayfish captured from the different populations were slightly biased toward females and ranged from 1.1 (Tony Sinks) to 1.3 (Hering Cave, Table 1). The largest female specimens had TCL of 32.4, 42.0 and 35.4 mm in Tony Sinks, Hering Cave and Limrock Blowing Cave, respectively, and the largest males had TCL of 29.5, 38.9 and 32.8 mm.

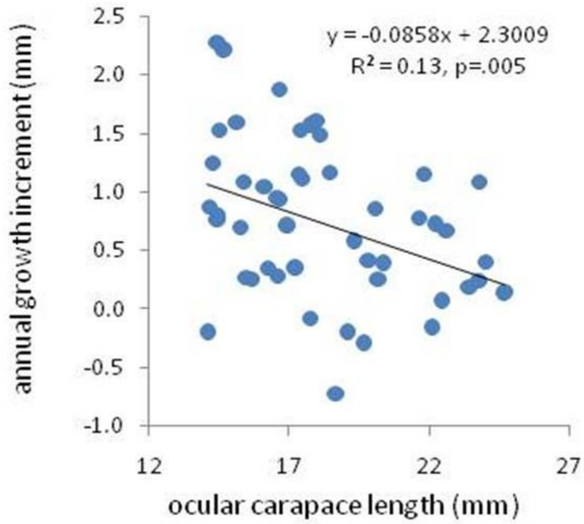

Fig. 5. Relationship between annual growth increment (mm ocular carapace length) and mean ocular carapace length for *Orconectes australis* in Limrock Blowing Cave. All specimens were marked for ≥ 350 d.

<u>*Cambarus hamulatus*</u> *Cambarus hamulatus* occurred only in Bluff River Cave. The mean total carapace lengths of reproductively active females (obvious cement glands) were 15.0 mm (Table 2). The proportion of reproductively active females, compared with the total number captured was ~8%. The development of active cement glands showed no apparent pattern of seasonality for any population. No ovigerous or young-bearing females were observed.

The mean total carapace-length of reproductively active (Form I) males of *C. hamulatus* was almost identical to the size of females (15.1 mm, Table 2). The proportion of reproductively active males, compared with the total number captured, was relatively high compared with females (~19%, Table 2). The occurrence of Form I males showed no apparent seasonal pattern.

Sex ratios (females:male) for the total crayfish captured was essentially 1:1 (Table 2). The largest female specimen observed had a TCL of 20.5 mm, the largest male had a TCL of 19.3 mm.

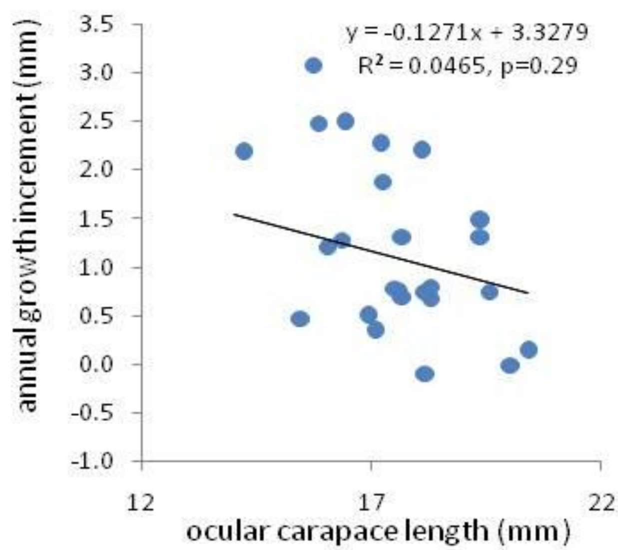

Fig. 6. Relationship between annual growth increment (mm ocular carapace length) and mean ocular carapace length for *Orconectes australis* in Tony Sinks. All specimens were marked for ≥ 350 d.

<u>*Cambarus tenebrosus*.</u> *Cambarus tenebrosus* occurred in all four study caves. The mean total carapace lengths of reproductively active females (obvious cement glands) ranged from 31.5 mm in Bluff River Cave to 42.0 mm in Hering Cave (Table 3). The proportion of

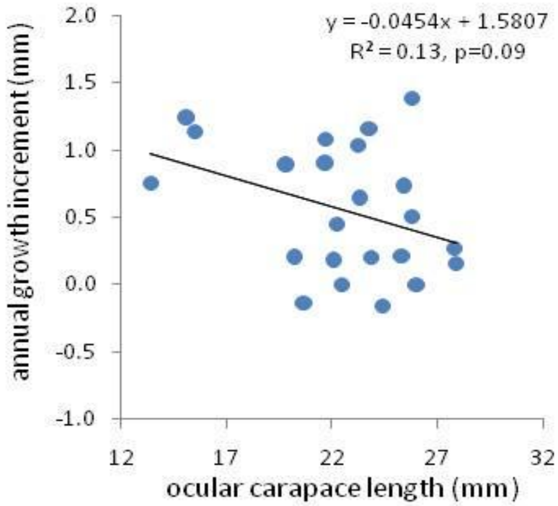

Fig. 7. Relationship between annual growth increment (mm ocular carapace length) and mean ocular carapace length for *Orconectes australis* in Hering Cave. All specimens were marked for ≥ 350 d.

reproductively active females, compared with the total number captured was low in most caves, ranging from 0% in Tony Sinks and Limrock Blowing Cave to ~0.01 % in Bluff River Cave. The proportion of reproductively active females in Hering Cave, however, was high (23%). The development of active cement glands showed no apparent pattern of seasonality for any population. One female bearing young was observed in Hering Cave (18 November 2005).

The mean total carapace-length of reproductively active (Form I) males of *Cambarus tenebrosus* ranged from 38.8 mm in Bluff River Cave to 48.2 mm in Limrock Blowing Cave (Table 3). The proportion of reproductively active males (compared with the total number captured) was relatively high, ranging from 8% in Limrock Blowing Cave to 42% in Hering Cave. The occurrence of Form I males showed no apparent seasonal pattern for any population. Sex ratios (females:male) for the total crayfish captured from the different populations were usually slightly biased toward males and ranged from 0.9 in Hering and Limrock Blowing caves, to 1.3 in Bluff River Cave (Table 3). The largest female specimens had TCL of 41.6, 53.4, 47.0 and 44.1 mm in Tony Sinks, Hering Cave, Limrock Blowing Cave, and Bluff River Cave, respectively, and the largest males had TCL of 47.2, 57.0, 51.0 and 44.0 mm.

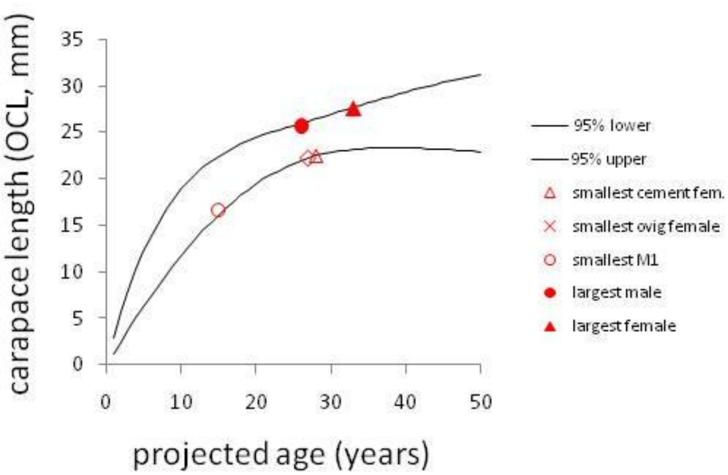

Fig. 8. Estimate of minimum projected age for *Orconectes australis* in Limrock Blowing Cave. Lines indicate the 95% confidence envelope based on a bootstrap model. "Smallest cement fem" indicates the smallest female observed with active cement glands; "smallest ovig female" indicates the smallest ovigerous or young-bearing female; "smallest M1" indicates the smallest reproductively active (Form I) male.

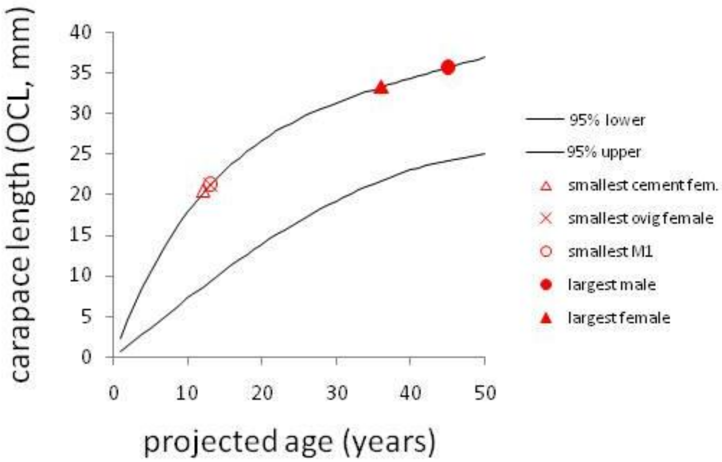

Fig. 9. Estimate of minimum projected age for *Orconectes australis* in Hering Cave. See **Fig. 8** for further explanation.

***Growth rates of* Orconectes australis *and* Cambarus hamulatus.**

<u>*Orconectes australis*.</u> As of October 2008, marked specimens of *Orconectes australis* have been recaptured for periods exceeding 1000 days after marking in Limrock Blowing Cave. To date, a total of 52 specimens of *Orconectes australis* were marked and recaptured after being marked for $\geq$ 350 d in this cave. The mean annual increase in carapace length (OCL) ranged from 1.1 mm/yr for 14.1 mm OCL individuals to 0.2 mm/yr for 24.7 mm OCL individuals. There was a negative, significant relationship between OCL and growth rate (Fig. 5). In Tony Sinks, marked individuals of *O. australis* have been recaptured after being marked for as long as 632 days. A total of 28 individuals were marked and recaptured for periods > 350 d. The mean annual increase in carapace length (OCL) for these individuals ranged from 1.7 mm/yr for 14.0 mm OCL individuals to 0.8 mm/yr for 20.4 mm OCL individuals. Note, however, that this relationship is not significant (i.e., p=0.05, Fig. 6). Specimens of *O. australis* were marked and recaptured over intervals as long as 883 days in Hering Cave. A total of 25 crayfish were marked and recaptured for periods exceeding 350 days. The mean annual increase in carapace length (OCL) for these individuals ranged from 1.1 mm/yr for 13.4 mm OCL crayfish to 0.3 mm/yr for crayfish with OCL of 27.9 mm. As observed for the population at Tony Sinks, however, this relationship was not statistically significant (Fig. 7).

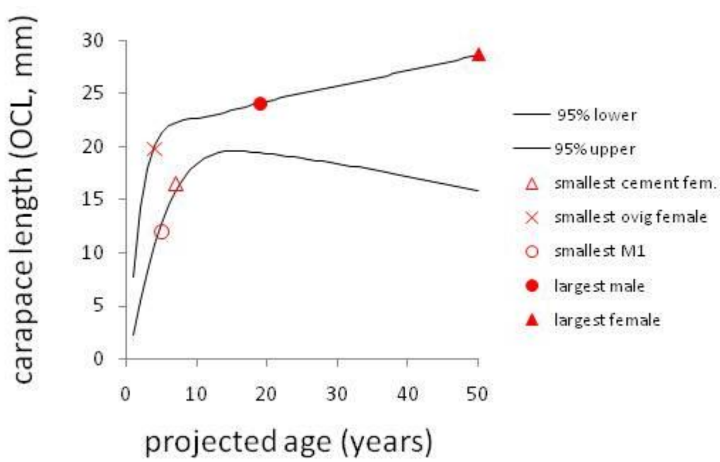

Fig. 10. Estimate of minimum projected age for *Orconectes australis* in Hering Cave. See **Fig. 8** for further explanation.

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As of October 2008, only four individuals of *Cambarus hamulatus* that were marked for a period exceeding 350 days have been recovered. Consequently, no estimates of growth-size relationships were attempted for the Bluff River Cave population.

***Estimates of longevity for* Orconectes australis.**

Bootstrap estimates of crayfish longevity were most statistically robust for the population of *O. australis* at Limrock Blowing Cave, due to the relatively high recapture rate of individuals marked for one year or more. The 95% confidence envelope (Fig. 8) indicated that the largest male and female crayfish observed in the cave had apparent minimum ages of 26 and 33 years, respectively. The apparent minimum ages for reproductively active males (Form I) and females (active cement glands) were 15 and 28 years, and the apparent minimum age for the smallest ovigerous female was 27 years. The estimated minimum ages for the largest male and female specimens of *O. australis* in Hering Cave were 45 and 36 years, respectively (Fig. 9). The minimum projected ages for reproductively active females were 12 years, and the age of the smallest ovigerous female was projected to be 13 years. The minimum age of reproductively active males was estimated to be 13 years. Tony Sinks produced the least statistically robust data set, requiring the removal of one outlier prior to analysis (an individual with an anonymously low growth rate). The estimated minimum ages of the largest male and female specimens of *O. australis* from this cave were 19 and 50 years, respectively (Fig. 10). The minimum age for females with active cement glands was estimated to be 7 years and the minimum age for ovigerous females was 4 years. The minimum age for reproductively active males (Form I) in Tony Sinks was 5 years.

Discussion

In this study, we successfully applied a mark-recapture technique using VIT and VIE to multiple populations of *O. australis* and *C. tenebrosus*, and a single population of *C. hamulatus* in northeastern Alabama. This technique provided data required to construct growth models to estimate crayfish time-to-maturity and longevity. Due to low recapture rates, growth models for *C. tenebrosus* and *C. hamulatus* could not be produced. However, relatively robust growth models were developed for populations of *O. australis* in Limrock Blowing, Hering, and Tony Sinks caves (Fig. 8, 9, 10). The comparision of the results of these growth models provides valuable insight into potential “key” factors influencing the life history of *O. australis*.

A unique growth model was generated for each population of *O. australis* (Fig. 8, 9, 10). Differences in topologies among the models indicated potential differences in time-to-maturity and longevity of the different populations. The size-at-age curves produced by the growth model for Tony Sinks Cave showed the most rapid growth phase (~1-10 years required to reach asymptotic size), followed by an abrupt asymptote (~ >10 years, Fig. 8), while the models for Hering and Limrock Blowing caves produced size-at-age curves that gradually increased to an asymptote (Fig. 9, 10). Consequently, time-to-maturity estimates were lowest in Tony Sinks Cave, while Hering and Limrock Blowing caves had longer and similar estimates. The differences in time-to-maturity suggest that key factors affecting groath and developmental rates may vary among caves. Temperature has been shown to be a significant factor influencing growth rates (Strong 1972). However, temperature regimes among the three cave systems were similar, varying <3^{o}C. Another possibility may be the amount of available food resources (e.g., standing stock organic matter). Competition for limited food resources within cave environments is considered a significant driving force for cave species evolving life history characteristics associated with K-selection, such as slower growth rates, increased longevities, and reduced fecundities when compared to surface relatives (Culver 1982, 1995). Although organic matter supply—which provides food for the crayfish—was not quantified in this study, observations suggest that Tony Sinks Cave may have higher amounts of organic matter than Hering or Limrock Blowing caves. The higher organic matter supply may support higher growth and developmental rates for crayfish in Tony Sinks than the populations in Hering or Limrock Blowing caves. Thus, the variations among these populations suggest cave specific environmental factors may play a role in shaping the life history of different populations of *O. australis*.

The longevity estimates for *O. australis* varied widely among populations, with the estimates for the minimum age of the largest males ranging from 19 to 45 years, and the minimum age of the largest females ranging from 33 to 50 years depending upon cave (Fig. 8, 9, 10). These estimates, however, should be interpreted with caution for two reasons: i) the technique assumes that the growth model represents a random sample of the population. Consequently, individuals that have exceptionally fast growth rates and reach unusually large body sizes may not be represented in the model. Thus, estimating longevity from the largest individuals in the population may not be accurate; ii) The growth models in this study were extrapolated beyond the size range of marked and recaptured individuals to produce estimates of longevity for the largest individuals collected in the caves. As can be seen in Figures 8 and 10, the confidence limits in the models become broader (increased uncertainty) as projected age increases. This is due to the model being extended outside the range of the available data, which decreases the certainty of age estimates for very large individuals.

Our study was not the first to examine the life history characteristics of *O. australis*. A study by Cooper (1975) examined the life history of *O. australis* in Shelta Cave, located in Huntsville, AL. Cooper (1975) studied the population size, age structure, and growth of *O. australis* for 6 years. He marked over 900 *O. australis* and used mark-recapture techniques to estimate population size and growth rates. To predict the average time required for an individual to reach a given size, growth rates estimated from recaptured individuals were used to develop a growth model, which produced astounding results. Using maximum and average growth rates, time-to-maturity and longevity were estimated to be 16-105 years and 37-176 years, respectively. However, an important point must be stressed, Cooper himself appeared to have some doubts regarding his estimates: "This apparently extraordinary finding requires further comment. Two alternative approaches are open: (1) consideration of factors which could actually confer "immortality" on these populations, and (2) further search for the flies which are undoubtedly lurking in the ointment of growth records (based on carapace lengths) and rates inferred from them (pg 314; Cooper 1975)." As can been seen in Table 4, Cooper's(1975) results fall outside the bounds of what is known for other species of cave and surface crayfish. Our results, although also highly variable, are more similar to available estimates for other species of cave and surface crayfish (Table 4), suggesting that Cooper (1975) may have overestimated the longevity and time-to-maturity of *O. australis*.

Table 4. Longevity and time-to-maturity estimates for selected species of cave and surface crayfish. Ranges include males and females. Results from this study are in bold.

Species		Longevity (yrs)	Time-to-maturity (yrs)	Source
Cave	*Orconectes australis*	37-176	16-105	Cooper 1975
	Orconectes australis	**19-50**	**4-28**	**This study**
	Orconectes inermis	9-10	2-3	Weingartner 1977
	Procambarus erythrops	16+	n.a.	Streever 1996
Surface	*Procambarus clarkii*	1-2	0.5	Huner 2002
	Astacoides betsileoensis	20+	5	Jones et al. 2007
	Paranephrops zealandicus	16-25+	6-7	Whitmore & Huryn 1999
	Cambarus bartonii	13	5	Huryn & Wallace 1987

This study successfully applied a mark-recapture technique utilizing VIE and VIT to multiple species and population of cave crayfish in Alabama. The variability in life history attributes among the populations of *O. australis* so far detected suggest conservation personnel should consider cave-specific variation when developing management strategies for cave species in Alabama. We stress that our study—which is supported by the Alabama SWG program through 201—is ongoing and our estimates of longevity will continue to be refined as our sample size of crayfish growth rates increases. In addition to refining existing growth models for *O. australis* we are currently: ii) developing growth models for *C. tenebrosus* and *C. hamulatus*, iii) examining inter-annual variation among growth models, population sizes, and reproduction (i.e., fluctuations in the abundance of mature and immature males and females and occurrence of ovigerous females), and iv) attempting to correlate life history attributes of each population with cave specific environmental characteristics (e.g., standing stock organic matter). The resulting data will be beneficial for conservation and management efforts focused on the cave fauna of Alabama and will contribute to basic knowledge of cave ecosystem structure and function.

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