Project 7
Assessment of Population Dynamics for Cave-Inhabiting Crayfish in Alabama: a Request for Continuing Funds

Final Report – December 2011

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Summary

A six-year mark-recapture study was conducted on populations of the cave crayfish, *Orconectes australis* (3 populations) and *Cambarus hamulatus* (1 population) in four northeastern Alabama caves. A total of 5,406 crayfish were marked with unique, internal alphanumeric tags. Recapture rates ranged from 19 to 37% for *O. australis*, depending upon population, and 16% for *C. hamulatus*. By using this technique we were able to monitor the growth of some individuals for periods greater than 5 years. Size-distributions indicated that all populations were dominated by mature adults. A size-at-age model, based on growth rates of individuals that were free roaming for periods of >350 days, indicated that minimum longevity for *C. hamulatus* ranged from 5 years for males to 9 years for females; the estimated time-to-maturity was 3.5 years. No females with attached eggs of young were observed. The estimated longevity for *O. australis* ranged from 11 to 22 years for male and 15 to 22 years for females, depending upon population. Time to maturity ranged from 2.5 to 3.9 years for males and 3.5 to 5.1 years for females. Ten females that were carrying attached eggs or young were observed during autumn and early winter. Our estimates of longevity and time-to-maturity for *O. australis* are substantially lower than those previously reported. Our results indicate that accurate estimates of the longevity of *O. australis* are $\leq$22 years, with only a small proportion of individuals (<5%) exceeding this age. Regardless of our comparatively shorter life-span estimate for *O. australis*, its estimated longevity (~22 years) is relatively great compared with surface species in the same genus, indicating a K-selected life history and a high degree of specialization to cave habitats.

Introduction

Six species of cave-obligate crayfish (i.e., stygobitic crayfish, Peck 1998) have been reported from Alabama. Four species are endemic to the Tennessee River basin in Alabama (*Cambarus jonesi, C. veitchorum, C. pecki, Orconectes sheltae*,); the remaining species are more widespread, occurring in northern

Alabama and Tennessee (*C. hamulatus*, *O. australis*). None of these 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).

Analyses of population age structure and longevity are required for understanding patterns of recruitment and thus the viability of wildlife populations. Considering the high level of imperilment of cave crayfish, as indicated by their Heritage Ranks (ANHP 2003), it is striking that so little is known about the attributes of any population, not to mention the lack of knowledge about how these attributes vary among different populations. Knowledge of population age-structure, and longevity of multiple populations in particular will: 1.) provide insight into factors affecting population dynamics of crayfish among different cave systems (e.g. sources and quantities of water & organic matter, presence of bats, groundwater contamination), 2.) allow managers to focus conservation efforts on specific cave systems, and 3.) establish benchmarks for monitoring future trends for crayfish populations.

Because cave crayfish are long-lived (e.g., apparent longevity measured in decades; Cooper 1975, Culver 1982), the assessment of population viability based upon methods such as presence-absence surveys or simple counts without considering population age structure is problematical. This is because populations may persist for many decades without successful reproduction; consequently the decline of populations of cave crayfish may not be effectively detected by traditional survey approaches due to the limited span of observation and the lack of information about population age structure.

Prior to the funding of Alabama SWG T-03-02 in October 2005 (Huryn *et al*. 2008), the only populations of cave crayfish in Alabama that had been rigorously studied are those of Shelta Cave in Huntsville (Cooper 1975; Culver 1982). Since October 2005, however, 27 caves had been surveyed as potential locations for research (Huryn *et al.* 2008). Of these, four had sufficiently abundant populations of cave crayfish for study. Between November 2005 and December 2008 a total of more than 2,100 crayfish had been marked in these caves (Huryn *et al*. 2008). Due to the relatively large sizes of the populations and apparently high level of dispersal, however, the marking of sufficient individuals to ensure statistically sound recapture rates took longer than anticipated (~2 years). As a consequence, we were granted additional funds (Alabama SWG T-03-03) to continue our efforts, resulting in the marking of >5,500 crayfish (research conducted under both Alabama SWG T-03-02 and T-03-03) and a statistically robust rates of recapture.

In this report we summarize the results of our research conducted under Alabama SWG T-03-03 (a continuation of Alabama SWG T-03-02). The major objectives of this research were to:

- continue to develop mark-recapture methods to assess year-to-year variability in population size of *O. australis* in 3 cave systems and *C. hamulatus* in one cave system in Alabama.
- use individually marked crayfish to assess growth rates and year-to-year variability of growth rates for individuals in each cave.
- use size-at-age models to estimate year-to-year variability in longevity and age at first reproduction.
- combine size-at-age data with assessment of female reproductive status to estimate potential rates of recruitment.

Methods

Cave selection: Of 27 caves surveyed as part of Alabama SWG T-03-02, four caves with abundant populations of cave crayfish were selected for study: Tony Sinks Cave System (E-1) (N34^{o}46'57", W86^{o}18'21"), Herring Cave (N34^{o}37'28", W86^{o}24'06"), Limrock Blowing Cave (N34^{o}42'31", W86^{o}10'53") and Bluff River Cave (N34^{o}53'11", W86^{o}0'52"). *Cambarus hamulatus* occurs in Bluff River Cave; *O. australis* occurs in the remaining three caves. The cave-associated crayfish *Cambarus tenebrosus* also occurs in all four caves, as does the obligate cave salamander *Gyrinophilus palleucus*. The southern cavefish (*Typhlichthys subterraneus*) and the mottled sculpin (*Cottus bairdi*) occur in Limrock and Hering caves.

A phylogeographic study by Buhay and Crandall (2005) showed that the populations of *O. australis* in Limrock, Hering, and Shelta caves shared common mtDNA 16S haplotypes, indicating that at least these three populations share a common evolutionary history. While Tony Sinks Cave was not included in Buhay and Crandall's study, its close geographic proximity to the other caves (10-42 km) suggests that its crayfish population falls within the same well-defined clade, supporting their recognition as a single species.

Capture of crayfish: A study reach ranging from 327 to 1202 m containing a series of riffle and pool habitats with sand, gravel, and bedrock substrates was established in each cave. Sampling began in November 2005 in Hering, January 2006 in Limrock, July 2006 in Tony Sinks, and May 2007 in Bluff River caves and was conducted semi-monthly (conditions permitting) through August 2011. On each visit, study reaches were surveyed on foot and all crayfish encountered were collected using dip-nets.

Size, reproductive status, and condition of crayfish: For each crayfish captured, total carapace length (TCL) and ocular carapace length (OCL; posterior margin of ocular cavity to posterior center-margin of carapace) was measured (±0.1 mm) with dial calipers, and its reproductive status was assessed (e.g., Form I or II for males; presence of cement glands, ova, or young for females). Once these data were recorded and the crayfish was marked (see below), it was released at the point of capture.

Marking & recapture: Captured crayfish were marked using both internal tags [Visible Implant Alpha Tags (VIAT), Northwest Marine Technology, Shaw Island, WA, USA] and Visible Implant Elastomer (VIE; Northwest Marine Technology). VIATs are small (1.0×2.5 mm), fluorescent, uniquely numbered tags that are placed beneath the abdominal cuticle. The VIE was injected directly posterior to the VIATs to assess tag loss, which was infrequent.

Individual longevity and growth rates: Crayfish growth rates were estimated as the difference between OCL at initial marking and the OCL upon recapture divided by days elapsed (OCL was used rather than TCL to avoid errors due to damage to the acumen following release). This rate was then multiplied by 365 to acquire an annual growth increment. Since growth increments are "episodic" due to the molting cycle, annual growth increments were only calculated for individuals recaptured over intervals of 350 days or longer. Negative annual growth-increments were attributed to measuring error and were excluded from analyses. For crayfish recaptured multiple times, the annual growth increment was calculated using the recapture date closest to the 350-day minimum. Annual growth increments were regressed against average OCL to estimate the size-specific annual growth rate. To estimate size-at-age, the size-specific annual growth rate was first seeded with a 3 mm OCL individual (i.e., size of juveniles attached to the pleopods of a female collected from Hering Cave). This process was then iterated at annual intervals and growth trajectories bounded by 95% confidence limits were then constructed using a bootstrap technique (Whitmore and Huryn 1999). A significant difference in growth trajectories among caves was assumed when 95% confidence intervals did not overlap.

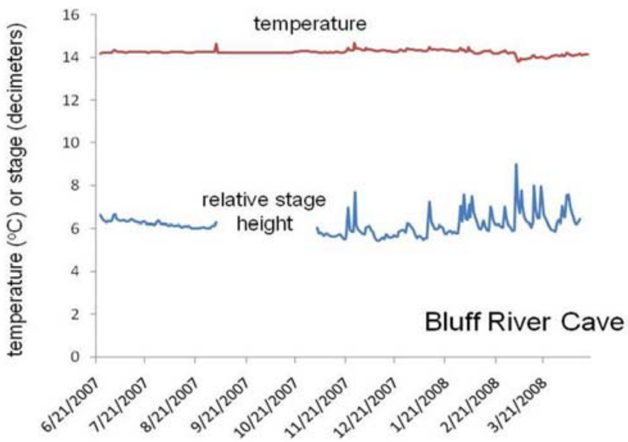

Fig. 1 Mean daily water temperature (°C) and relative stage height (water level, dm) for Bluff River Cave (21 June 2007 - 18 April 2008).

Time-to-maturity was estimated by plotting the smallest reproductive (Form I) male and female (presence of cement glands) onto the resulting growth trajectories. The largest male and female and the smallest female with ova or attached young were used to estimate minimum life span and age-at-first-reproduction, respectively. Ages constrained by 95% confidence limits represented the estimated range. Cumulative size-frequency distributions were constructed for each cave and then compared to examine for differences in size structure among populations using pairwise Kolmogorov-Smirnov (K-S) tests. K-S tests were performed in program R version 2.14.0 (R Development Core Team 2008).

<u>Ancillary measurements</u>: Recording water depth and temperature sensors (Solinst Barologger model 3001, Solinst Corporation, Georgetown, Ontario, Canada or HOBO Water Level Logger, www.onsetcomp.com) were installed in each cave stream. Due instrument malfunctions water depth was measured only from June 2007 to April 2008.

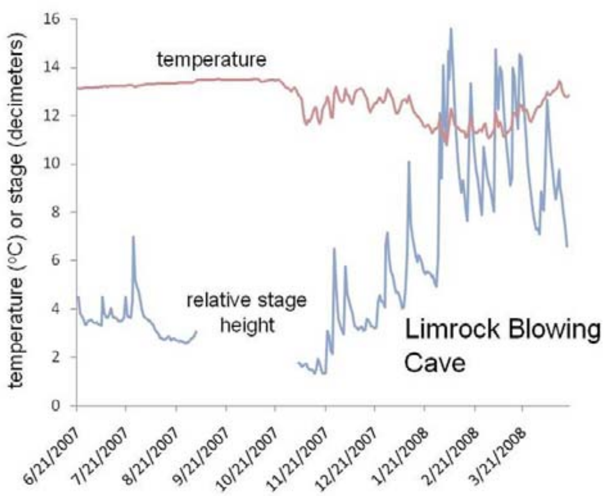

Fig. 2. Mean daily water temperature (°C) and relative stage height (water level, dm) for Limrock Blowing Cave (21 June 2007 - 18 April 2008).

Results

Description of study streams.
The lengths of sampling reach varied among the four caves (Tony Sinks, 324 m; Bluff River, 690 m; Limrock Blowing, 959 m; Hering, 1203 m. The sampling reach within Tony Sinks consisted entirely of pools. Stream reaches in Bluff River, Limrock Blowing and Hering caves consisted of 32%, 25%, and 15% riffle habitat, respectively.

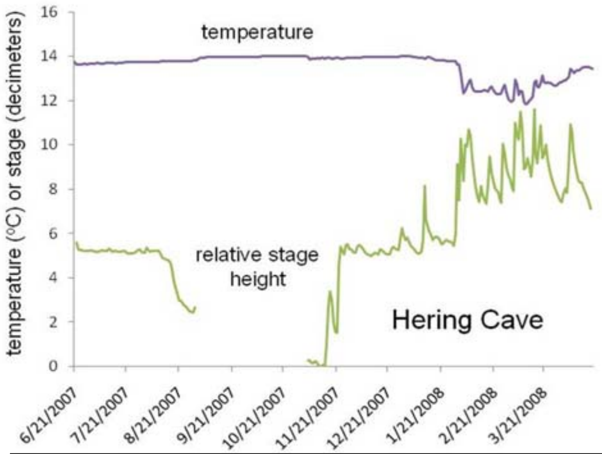

Fig. 3. Mean daily water temperature for Hering Cave (21 June 2007 - 18 April 2008).

Mean daily stream-water temperature, measured in each cave from 21 June 2007 to 18 April 2008 in Bluff River cave and from 21 June 2007 through July-August 2011 for the remaining caves, ranged from a maximum of 14.1°C in Bluff River Cave to a minimum of 12.7°C in Limrock Blowing Cave. Water temperature showed a relatively low level of temporal variability, with the annual range of mean daily temperatures being smallest in Bluff River Cave (0.9°C) and greatest in Limrock Blowing Cave (7.1°C). The temperature of the stream in Bluff River Cave (**Fig. 1**) showed little seasonality while the stream temperature in Limrock Blowing (**Fig. 2**) and Hering caves (**Fig. 3**) showed relatively marked decreases during winter. In contrast with the other caves, Tony Sinks showed an increase in water temperature during winter (**Fig. 4**). Seasonal shifts in water temperature appeared to be closely related to changes in relative groundwater flux. Mean daily air temperatures measured at Stevenson, AL, during this period was 14.6°C and ranged from -3.2 to 33.0°C (i.e., a range of 36.2 °C compared to a maximum range of 7.1°C measured for the cave streams).

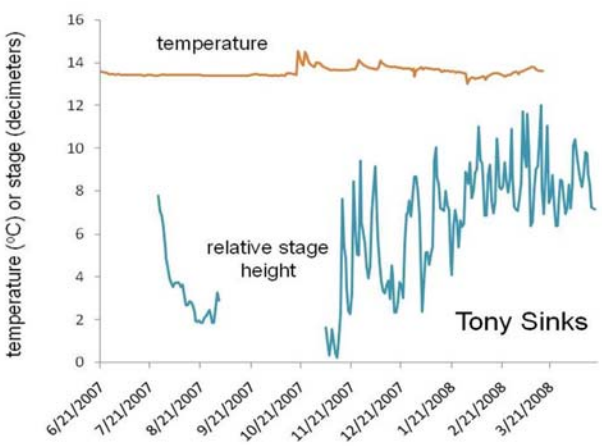

Fig. 4. Mean daily water temperature (°C) and relative stage height (water level, dm) for Tony Sinks (21 June 2007 - 18 April 2008).

Relative stage height, or water depth, provides information about the variability of flow of the stream habitat provided by each cave. As observed for the range of temperature fluctuation, the water depth 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 Sinks also showed changes in water level exceeding 100 cm (**Figs. 3, 4**).

Of the four caves, Bluff River Cave was 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 showed a seasonal pattern similar to those observed for Limrock Blowing and Hering caves, it differed by showing an increase in water temperature as water levels rose in early winter. Presumably this was due to a greater proportional influx of grpundwater.

Numbers of crayfish captured and tagged. Crayfish were captured and tagged in Bluff Cave at monthly intervals from 8 May 2007 to 1 September 2011; Tony Sinks at seasonal intervals from 27 July 2006 to 27 January 2007, and at monthly intervals from 24 June 2007 to 1 September 2011; Hering Cave at seasonal intervals from 18 November 2005 to 17 September 2006, and at monthly intervals from 6 April 2007 to 31 July 2011; Limrock Blowing Cave at seasonal intervals from 14 January 2006 to 17 September 2006, and at monthly intervals from 9 May 2007 to 18 July 2011.

From November 2005 to September 2011, a total of 5,875 crayfish were marked with VIAT tags and/or VIE implants in all four caves. All crayfish receiving VIAT tags were also marked with VIE (5,407 individuals), while crayfish too small to safely receive tags were marked with VIE only (460 individuals). A total of 1838, 865 and 920 specimens of *O. australis* were tagged with VIAT in Tony Sinks, Hering Cave and Limrock Blowing caves, respectively. A total of 327, 411, 36 and 292 *C. tenebrosus* were tagged with VIAT in Tony Sinks, Hering Cave, Limrock Blowing Cave and Bluff River caves, respectively. A total of 717 specimens of *C. hamulatus* were tagged with VIAT in Bluff River Cave.

Size and reproductive status of crayfish.
<u>*Orconectes australis*.</u> The mean total carapace lengths of reproductively active females (obvious cement glands) ranged from 25.5 mm in Tony Sinks to 30.3 mm in Hering Cave (**Table 1**). The proportion of reproductively active females, compared with the total number captured, was low, ranging from <0.5% in Limrock Blowing Cave to ~4% in Tony Sinks. The development of active cement glands showed no apparent pattern of seasonality for any population. Ten females that were either ovigerous or carrying attached young were collected: 3 in Tony Sinks (27 July 2006, 8 January 2008, 19 February 2011), 5 in Hering Cave (4 November 2007, 8 January 2008, 18 October 2008, 3

October 2009, 1 September 2010), and 2 in Limrock Blowing Cave (2 December 2007, 5 September 2009). Although numbers of ovigerous females observed was low, it is apparent that the laying of eggs and hatching of young occurs primarily in autumn and early winter.

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 – September 2011). Total ♂♂= total males observed. TCL(SE) = mean total carapace length in mm (standard error). F1 ♂♂= number of Form I (reproductively active) males. Total ♀♀=total females observed. $♀♀_{cement}$= numbers of females observed with active cement glands, $♀♀_{ovig}$=total females with attached eggs or young.

Males

Cave	Total ♂♂	TCL (SE)	F1 ♂♂	TCL (SE)
Bluff River Cave	---	---	---	---
Tony Sinks	907	19.3 (0.1)	157	22.7 (0.2)
Hering Cave	399	23.7 (0.3)	87	27.9 (0.4)
Limrock Blowing Cave	446	21.3 (0.2)	88	24.5 (0.4)

Females

Cave	Total ♀♀	TCL (SE)	$♀♀_{cement}$	TCL (SE)	$♀♀_{ovig}$	TCL(range)
Bluff River Cave	---	---	---	---	---	---
Tony Sinks	1057	19.9 (0.1)	43	25.5 (0.3)	3	25.5-32.2
Hering Cave	540	23.6 (0.3)	24	30.3 (0.6)	5	24.5-34.0
Limrock Blowing Cave	516	22.0 (0.2)	13	27.9 (0.9)	2	25.5-29.7

The mean total carapace-length of reproductively active (Form I) males of *O. australis* ranged from 22.7 mm in Tony Sinks to 27.9 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 17% in Tony Sinks to 22% in Hering Cave. The occurrence of Form I males showed no apparent seasonal pattern for any population. Sex ratios (females:male) were slightly biased toward females and ranged from ~1.2 (Tony Sinks, Limrock Blowing Cave) to ~1.4 (Hering Cave, **Table 1**). The largest female specimens had TCL of 32.5, 42.0 and 35.8 mm in Tony Sinks, Hering Cave and Limrock Blowing Cave, respectively, and the largest males had TCL of 30.9, 38.9 and 33.2 mm.

Cambarus hamulatus The mean total carapace length of reproductively active females (obvious cement glands) of *C. hamulatus* in Bluff River Cave was 17.2 mm (**Table 2**). The proportion of reproductively active females, compared with the total number captured was ~10%. The development of active cement glands showed no apparent pattern of seasonality for any population. No ovigerous females or females bearing young were observed.

Table 2. Size and reproductive status for specimens of *Cambarus hamulatus* captured in Bluff River Cave in Alabama (November 2006 – September 2011). Total ♂♂= total males observed. TCL(SE) = mean total carapace length in mm (standard error). F1 ♂♂= number of Form I (reproductively active) males. Total ♀♀=total females observed. ♀♀$_{cement}$= numbers of females observed with active cement glands, ♀♀$_{ovig}$=total females with attached eggs or young.

Males

Cave	Total ♂♂	TCL (SE)	M1 ♂♂	TCL (SE)
Bluff River Cave	423	15.8 (0.1)	99	17.1 (0.2)

Females

Cave	Total ♀♀	TCL (SE)	♀♀$_{cement}$	TCL (SE)	♀♀$_{ovig}$	TCL(range)
Bluff River Cave	444	15.8 (0.1)	43	17.2 (0.2)	---	---

The mean total carapace-length of reproductively active (Form I) males of *C. hamulatus* was 15.8 mm (**Table 2**). The proportion of reproductively active males, compared with the total number captured, was relatively high compared with females (~23%, **Table 2**). The occurrence of Form I males showed no apparent seasonal pattern. Sex ratios (females:male) for the total crayfish captured was ~1.1 (**Table 2**). The largest female specimen observed had a TCL of 24.5 mm, the largest male had a TCL of 21.5 mm.

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 with attached eggs or young.

Males

Cave	Total ♂♂	TCL (SE)	M1 ♂♂	TCL (SE)
Bluff River Cave	174	26.6 (0.6)	15	39.2 (0.9)
Tony Sinks	197	31.5 (0.7)	43	40.4 (0.6)
Hering Cave	216	39.3 (0.4)	112	47.0 (0.4)
Limrock Blowing Cave	20	37.4 (2.0)	3	42.9 (2.8)

Females

Cave	Total ♀♀	TCL (SE)	♀♀$_{cement}$	TCL (SE)	♀♀$_{ovig}$	TCL(range)
Bluff River Cave	132	24.0 (0.7)	6	37.5 (1.7)	---	---
Tony Sinks	143	28.1 (0.7)	6	40.5 (1.5	---	---
Hering Cave	202	28.9 (1.0)	63	42.0 (0.5)	6	40.0-44.6
Limrock Blowing Cave	15	36.2 (2.1)	---	---	---	---

Cambarus tenebrosus. *C. tenebrosus* was present in all four study caves. The mean total carapace lengths of reproductively active females (obvious cement glands) ranged from 37.5 mm in Bluff River Cave to 42.0 mm in Hering Cave (**Table 3**). The proportion of reproductively active females, compared with the total number captured was low in most caves, ranging from 0% in Limrock Blowing Cave to ~4-5-0% in Bluff River Cave and Tony Sinks. The proportion of reproductively active females in Hering Cave, however, was relatively high (31%). The development of active cement glands showed no apparent pattern of seasonality for any population. Six females bearing eggs or young were observed in Hering Cave (18 November 2005, 1 September 2007, 1 September 2010, 31 August 2011 (3 females)). Although observations are limited, it is apparent that egg laying and hatching of young occurs in late summer and early autumn.

The mean total carapace-length of reproductively active (Form I) males of *C. tenebrosus* ranged from 39.2 mm in Bluff River Cave to 42.9 mm in Limrock Blowing Cave (**Table 3**). The proportion of reproductively active males (compared with the total number captured) was relatively high, ranging from 9% in Bluff River Cave to 52% 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 biased toward males and ranged from ~0.7 in Tony Sinks and Limrock Blowing caves to 0.9 in Hering Cave (Table 3). The largest female specimens had TCL of 43.5, 54.0, 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 48.4, 57.0, 51.7 and 45.8 mm.

Recapture rates.
Recapture rates (i.e., percent of total VIAT-marked crayfish that were released and then recaptured at least one time) for *O. australis* ranged from 19 to 37%, depending upon cave (**Table 4**). Individuals were recaptured after free-roaming for periods exceeding 4 years for each population of *O. australis* (**Table 4**). The recapture rate of *C. hamulatus* in Bluff River cave was 16% and the longest period that an individual roamed between marking and recapture was 1256 days (**Table 4**). Compared with the cave-obligate crayfish, recapture rates for *C. tenebrosus* were low (6-16%). Nevertheless, the maximum period between mark and recapture was >2 years in all caves (**Table 4**).

Table 4. Recapture statistics for crayfish marked in Bluff River Cave, Tony Sink, Hering Cave and Limrock Blowing cave. # recap.= number of individual crayfish recaptured at least once. % recap. = the percent of all VIAT marked crayfish recaptured at least once. 365 recap. = the number of marked crayfish recaptured after a minimum of 365 days following the date of initial marking. Max d elapsed = the maximum number of days that an individual crayfish was free roaming between the initial date of marking and the final date of recapture.

Oconectes australis

Cave	# recap.	% recap	365 recap.	Max d elapsed
Tony Sinks	356	19%	94	1601
Hering Cave	292	34%	77	1920
Limrock Blowing Cave	337	37%	112	1826

Cambarus hamulatus

Cave	# recap.	% recap	365 recap.	Max d elapsed
Bluff River Cave	115	16%	28	1256

Cambarus tenebrosus

Cave	# recap.	% recap	365 recap.	Max d elapsed
Bluff River Cave	46	16%	10	1419
Tony Sinks	21	6%	5	963
Hering Cave	44	11%	13	1421
Limrock Blowing Cave	4	11%	2	1436

Growth rates.

Orconectes australis. The mean annual increase in carapace length (TCL) measured for the 5 most rapidly growing individuals among all three populations of *O. australis* studied ranged from 5.7 to 7.9 mm/yr (**Fig. 5**). The range of annual TCL increase measured for the 5 most slowly growing individuals among all caves ranged from 0.0 to 0.3 mm/yr (**Fig. 5**). The TCL of the 5 most rapidly growing individuals (12.5 to 24.0 mm) was generally smaller than those of the 5 slowest growing individuals (18.0 to 35.5 mm). There was a significant, negative relationship between OCL and growth rate (**Fig. 5).**

Cambarus hamulatus. The mean annual increase in carapace length (TCL) measured for the 5 most rapidly growing individuals of *C. hamulatus* ranged from 2.0 to 4.1 mm/yr (**Fig. 6**). The range of annual TCL increase measured for the 5 most slowly growing individuals ranged from 0.0 to 0.6 mm/yr. The TCL of the 5 most rapidly growing individuals (12.0 to 14.7 mm) was smaller than those of the 5 slowest growing individuals (15.2 to 17.2 mm). There was a significant, negative relationship between OCL and growth rate (**Fig. 6).**

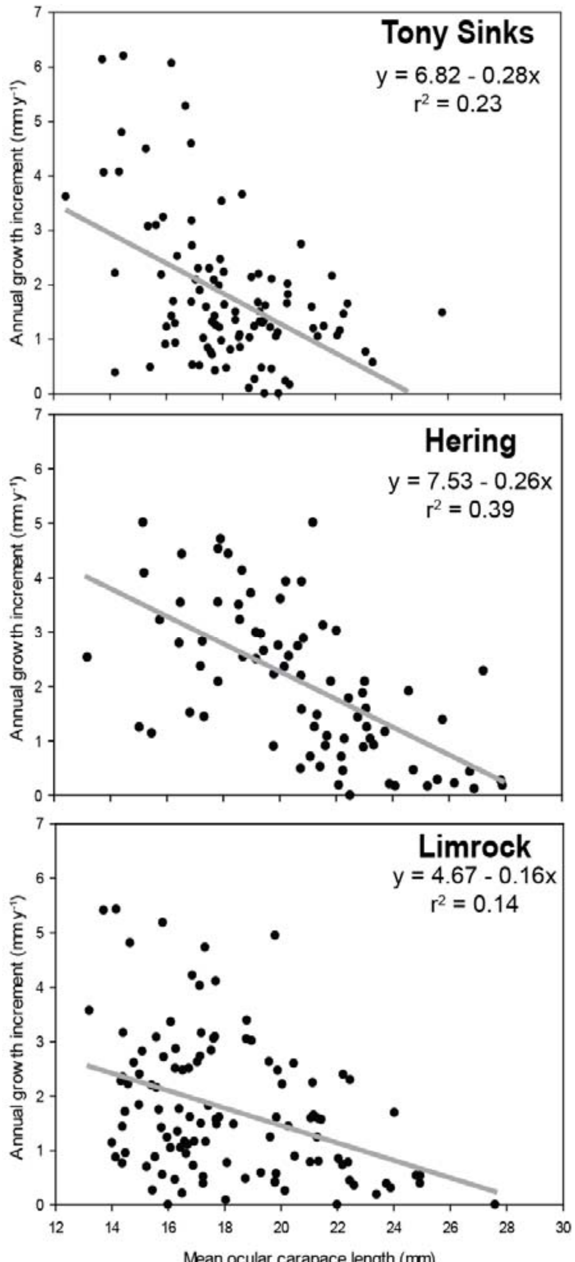

Fig. 5. Annual growth increment (mm y^{-1}) vs. mean ocular carapace length (mm) for *O. australis* in Tony Sinks (top), Limrock (middle), and Hering (bottom) caves. Lines are from least squared regression.

<u>*Cambarus tenebrosus*.</u> The mean annual increase in carapace length (TCL) measured for the 5 most rapidly growing individuals of *C. tenebrosus* among all caves ranged from 2.4 to 15.1 mm/yr. The range of annual TCL increase measured for the 5 most slowly growing individuals ranged from 0.0 to 5.1 mm/yr. The TCL of the 5 most rapidly growing individuals (15.0 to 52.9 mm) was similar to those of the 5 slowest growing individuals (19.2 to 57.2 mm).

Size-at-age and longevity. Statistically robust size-at-age models were possible for *C. hamulatus* in Bluff River Cave, and *O. australis* in Tony Sinks and Limrock and Hering caves. The production of size-at-age models for *C. tenbrosus* was not possible, however, due to low recapture rates and high variability of growth rates.

<u>*Cambarus hamulatus*</u>***.*** The 95% confidence envelope produced using the size-at-age model for *C. hamulatus* in Bluff River Cave (**Fig. 6**) indicated that the largest male and female crayfish observed in the cave had minimum ages of 5 and 9 years, respectively. The apparent minimum age for both reproductively active males (Form I) and females (active cement glands) was 3.5 years. No ovigerous females were observed during the study, so estimates of age-at-first reproduction were not possible. Approximately 84% of the population consisted of potentially reproductively active adults (**Fig. 6**).

<u>*Orconectes australis*</u>***.*** The 95% confidence envelopes produced using size-at-

age models for the different populations of *O. australis* (**Fig. 7**) indicated that minimum ages for males ranged from 11 years in Tony Sinks to 22 years in Hering Cave. Estimates of minimum age for females ranged from 15.5 years in Limrock Cave to 22+ years in both Hering Cave and Tony Sinks. The apparent minimum age for reproductively active males (Form I) ranged from 2.5 years in Tony Sinks to 3.3 years in Hering Cave. The age of potentially mature females (active cement glands) ranged from 3.5 years (Hering Cave) to 5.1 years (Limrock Cave). The estimate for age-at-first-reproduction (ovigerous) females ranged from 4.6 years (Hering Cave) to 6.4 years (Limrock Cave). A minimum of approximately 66% of each population consisted of potentially reproductively active adults (**Fig. 7**).

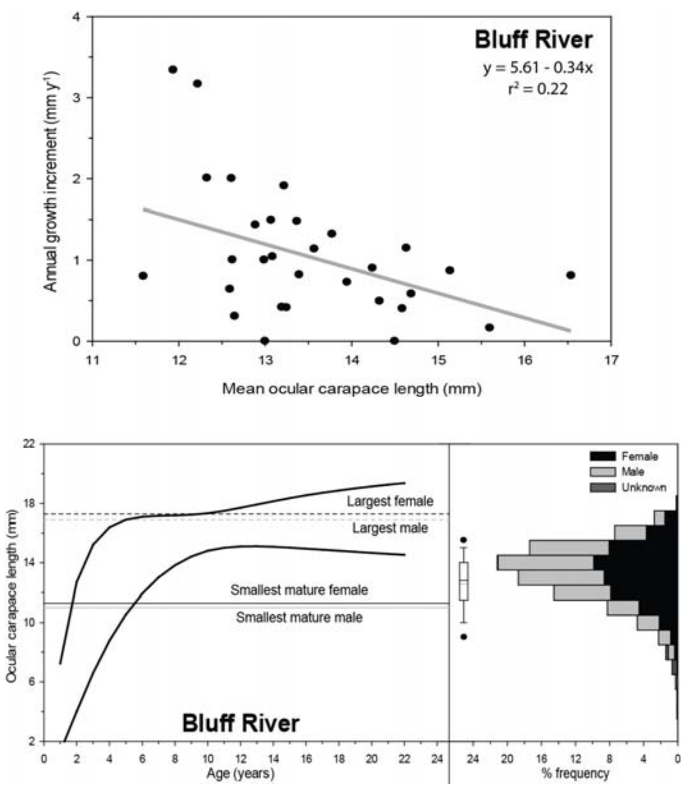

Fig. 6. (Top) Annual growth increment (mm y^{-1}) vs. mean ocular carapace length (mm) for *Cambarus hamulatus* in Bluff River Cave. Line is from least squared regression. (Bottom) Growth models for *C. hamulatus*. Dotted lines are upper and lower 95% confidence intervals. The pooled size-frequency distribution for the population is plotted to the right. To the left of the size-frequency distribution is a box and whisker plot. The box represents the 25th and 75th percentile containing the mean (dashed line) and median (solid line); whiskers are error bars; dots are the 5th and 95th percentiles.

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. By using this technique we were able to monitor the growth of single individuals for periods greater than 5 years. The data we acquired allowed the construction of statistically robust models for the estimating size-at-age, time-to-maturity, and longevity for populations of *O. australis* in Limrock Blowing, Hering, and Tony Sinks caves and for a population of *C. hamulatus* in Bluff River Cave (**Figs. 5, 6, 7**). The results of these models provide insight into potential “key” factors influencing the life history of these obligate cave crayfish.

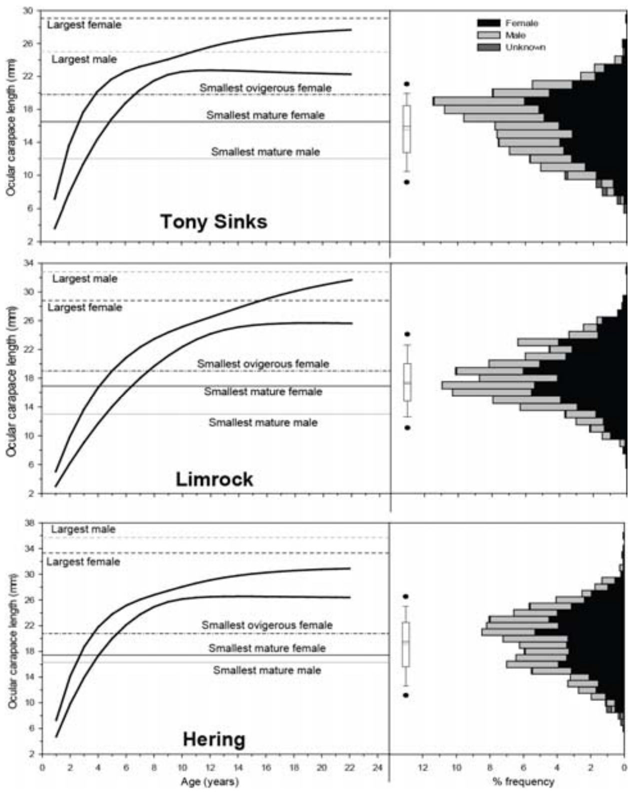

Fig. 7. Growth models for *Orconectes australis* from Tony Sinks (top), Limrock (middle), and Hering (bottom) caves. See caption for Fig.6 for further information.

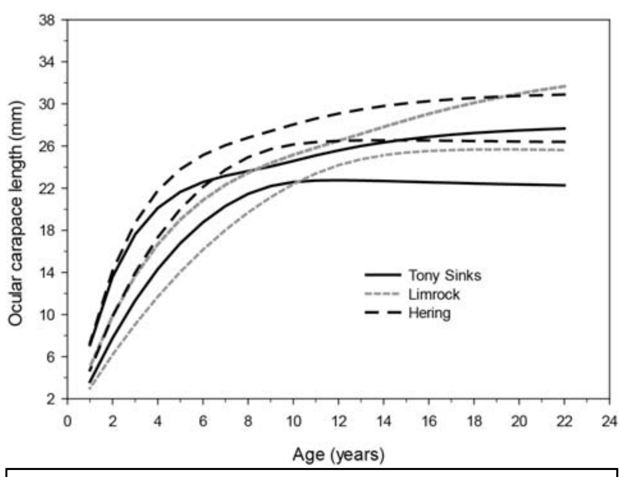

Fig. 8. Size-at-age models for *O. australis* from Hering (dashed black line), Limrock (dashed grey line), and Tony Sinks (solid black line) caves. Lines indicated the upper and lower 95% confidence intervals for each model.

Since a unique growth model was generated for each population of *O. australis* (**Fig. 7**), differences in growth patterns among populations could be analyzed. Nevertheless, we found that such differences were relatively small and not statistically significant, indicating that factors controlling growth and overall longevity among populations are similar among cave systems containing this species.

Interestingly, there was a large range in the relative hydrological and thermal stability of the different caves, with Bluff River Cave providing a relatively stable physical habitat with respect to seasonal fluctuations in water depth and temperature, while the other caves showed greater variability. Although any conclusions concerning factors underlying the distribution of the different crayfish species among caves must be speculative, the occurrence of *C. hamulatus* in Bluff River Cave and the occurrence of *O. australis* in the more seasonally variable systems poses a reasonable hypothesis for further study.

To our knowledge, this study is the first to assess the growth and longevity of *C. hamulatus.* Consequently, there is no information from other populations allowing our results to be placed in a broader context. This is not the case for *O. australis*, however. Cooper (1975) examined the life history of *O. australis* in Shelta Cave (Huntsville, AL) where he marked over 900 specimens of *O. australis* over a six-year period and used mark-recapture techniques to estimate growth rates in order to develop a size-at-age model. Coopers (1975) conclusions were astounding--time-to-maturity and longevity of *O. australis* in Shelta Cave were estimated to be 16-105 years and 37-176 years, respectively. Cooper (pg. 314, 1975) himself had some doubts regarding these 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." As can been seen in **Table 5,** Cooper's(1975) results fall outside the bounds of what is known for other species of cave and surface crayfish. We attribute this discrepancy to an overestimate of life span and age-at-first-reproduction by Cooper (1975) due to two factors. The first is the underrepresentation of young and rapidly growing crayfish in his marked sub-population. The second is the use of an iterative growth model that was based on mean growth rates rather than a model that captured the variation of growth rates among individuals.

Table 5. 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	**11-22**	**2-5**	**This study**
Orconectes inermis	9-10	2-3	Weingartner 1977
Procabarus erythrops	16+	n.a.	Streever 1996
Cambarus hamulatus	**5-9**	**3.5**	**This study**
Surface			
Procambarus clarkia	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

In summary, our estimates of life span for *O. australis* are substantially lower than Cooper's (1975) estimate of 37 to 176 years, indicating that his trepidation regarding these estimates was warranted. We suggest that a more accurate estimate of the life span for *O. australis* is $\leq$22 years, with only a few percent of the individuals of a given population exceeding this age. Our estimates for female time-to-maturity (4 to 5 years) and age-at-first-reproduction (5 to 6 years) are also substantially lower than the estimates of 16 to 35 years and 29 to 105 years, respectively, reported by Culver (1982) using Cooper's (1975) data. Our assessment of the longevity of *O. australis* is comparable to other estimates for both cave and surface species of crayfish. Weingartner (1977), for example, reported that the life-span and time-to-maturity for the obligate cave crayfish *Orconectes inermis* ranged from 9 to 10 years and 2 to 3 years, respectively, while Streever (1996) estimated a 16+-year life-span for the obligate cave crayfish *Procambarus erythrops* (**Table 5**). Life-span and time-to-maturity in surface crayfish vary widely, ranging from 1 to 60 years and 6 months to 14 years, respectively (Vogt, in press). In other species of *Orconectes*, life-span ranges from 1 to 5 years and time-to-maturity from 6 months to 2 years (Momot 1984). Regardless of our shorter life-span estimate for *O. australis*, its estimated longevity (~22 years) is relatively great compared with surface species in the same genus, indicating a K-selected life history and a high degree of specialization to cave habitats.

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