Shannon M. Knapp and Daniel W. Fong - Estimates of Population Size of *Stygobromus emarginatus* (Amphipoda: Crangonyctidae) in a Headwater Stream in Organ Cave, West Virginia. *Journal of Cave and Karst Studies 61*(1): 3-6.

ESTIMATES OF POPULATION SIZE OF *STYGOBROMUS EMARGINATUS* (AMPHIPODA: CRANGONYCTIDAE) IN A HEADWATER STREAM IN ORGAN CAVE, WEST VIRGINIA

SHANNON M. KNAPP[1] AND DANIEL W. FONG[2]
Department of Biology, American University, Washington, D.C. 20016 USA

Population sizes of Stygobromus emarginatus *were estimated using mark-recapture data at three sites each in two habitats of a headwater stream in the Organ Cave drainage. The stream channel habitat contained an average of 10 to 14 individuals per meter length and an estimated population size of 3000 to 4200 individuals. The pool habitat yielded very low recapture rates. We argue that the pool habitat represents a window into the epikarstic zone, and the low recapture rates indicate a large hidden population in the epikarst.*

A fundamental aspect of the ecology of organisms is population size. Small population size and small number of populations are typical reasons for a species to be listed as endangered or threatened. Although there are 50,000 to 100,000 obligate cave-dwelling species (Culver & Holsinger 1992), population sizes of only a few have been estimated using methods such as mark-recapture (see summary in Culver 1982; Simon 1997) that can provide more reliable results than simple sightings or one time counts. In this paper, we present initial results from a long term continuing study, initiated in September, 1994, to monitor fluctuations in population size of the crangonyctid amphipod crustacean *Stygobromus emarginatus* (Hubricht) in a headwater stream in Organ Cave, Greenbrier County, West Virginia.

STUDY ORGANISM AND STUDY SITE

We chose to monitor the population size of *S. emarginatus* for several reasons. First, as is typical of most troglobites, little is known about its basic ecology. Second, its large body size at maturity, up to 10 mm for males and 14 mm for females (Holsinger 1972), renders it amenable to mark-recapture studies. Third, one time count estimates indicate that populations of *S. emarginatus* occur at densities of 15/m² (Holsinger *et al.* 1976) to 30/m² (Culver *et al.* 1994), densities that may yield meaningful recapture rates in a mark-recapture study. Finally, Fong and Culver (1994) showed that within the Organ Cave system, *S. emarginatus* mainly occurs in small headwater streams, and is absent in large streams in the lower reaches of the cave drainage. They hypothesized that the primary habitat of *S. emarginatus* is not in the cave stream, but rather is in the interstitial water in the epikarst, the highly fractured limestone interface with the soil (Williams 1983). Their hypothesis predicts that the population size of *S. emarginatus* in cave streams near the epikarst should covary with the amount of water input from the epikarst into the cave stream. A long term study of the population size of this species should provide baseline data that eventually can be used to test this hypothesis.

Sively #2 is a headwater stream in the eastern section of Organ Cave (Stevens 1988). The stream originates as percolating water seeps and drips into the upstream portion of the cave passage, and flows for ~300 m before it sinks into breakdown. Two distinct habitats are associated with the stream: the rock-bottomed stream channel and mud-bottomed pools adjacent to the stream channel. The average width of the main channel is about 0.5 m. The pools occur in the upper reaches of the stream, are fed by seeps and also by water from the stream channel during high water, and frequently dry up completely. These pools average about 1.2 m in diameter. Depth of the water is typically less than 10 cm at all habitats. We picked this stream as the study site because it is easily accessible, it appears to have significant water input from the epikarst, especially in its upper half, and, based on complete dissection of 12 riffles, it contains *S. emarginatus* at a density of 11.5/ m² (Culver *et al*. 1994).

METHODS

We estimated the population size of *S. emarginatus* in late October, 1994 (OCT94), during the dry season; in mid-January and early April, 1995 (JAN95 and APR95), during the wet season; and in late June, 1995 (JUN95), during the beginning of the dry season. We designated six sampling sites in the Sively #2 stream, chosen primarily by the ease of access to the water. The sites were numbered sequentially from the downstream end. Sites S1, S2 and S3 were located in the stream channel at the lower half of the stream. Flow in the stream channel was intermittent in the upper half of the stream. Each stream site consisted of a 6-10 m length of the stream, and was separated from other sites by at least 15 m. Sites P4, P5 and P6 were mud-bottomed pools adjacent to the stream channel in the upper half of the stream. These pools ranged from 1.0 m to 1.5

[1] Present address: Department of Fisheries and Wildlife Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061
[2] Corresponding author.

m in diameter.

During each sampling period at each site, we took two samples of *S. emarginatus* at an interval of twenty-four hours. A sample consisted of all specimens collected individually with a turkey baster until the rate of finding additional animals had substantially diminished, a duration of about three to five worker-hours. After the first sample, we placed each specimen on a paper towel to absorb excess water, marked its tergum on the thorax with a blue or red Sharpie® permanent ink marker, and immediately put it in a dish of water. No animal was out of the water for more than 10 seconds. When all specimens had been processed, we confirmed that all individuals in the water dish were obviously marked and were able to walk or swim about before releasing them. After the second sample, we tallied the number of marked and unmarked individuals and released the specimens. We marked all specimens at all sites with blue ink in OCT94, and with red ink in JAN95. In APR95 and in JUN95, we marked individuals at odd numbered sites with blue ink and those at even numbered sites with red ink. Preliminary trials using this technique in the laboratory induced no mortality, with five of six specimens having retained the markings for over six months while the sixth lost its marking upon molting after four months.

We estimated population size using the Lincoln-Peterson index:

$$N = a\,n / r\ ,\ S.E. = [a^2\, n\,(n-r) / r^3 + 1]^{0.5}$$

or the Bailey correction for low numbers of recaptures ($r \leq 20$):

$$N = a\,(n+1) / (r+1),\ S.E. = [a^2\,(n+1)\,(n-r) / (r+1)^2\,(r+2)]^{0.5}$$

where N is the estimated population size and S.E. is the standard error of the estimate, a is the number of specimens marked and released in the first sample, n is the total number in the second sample, and r is the number of recaptures in the second sample (Begon 1979). For comparisons among sites and among seasons, density expressed as estimated population size per unit stream length (N/m) was used for stream sites. This was a more consistent index of density than estimated population per unit stream area (N/m²) because of the shallow nature of the stream channel, where a slight change in depth resulted in a drastic change in the surface area measured along the same length of stream.

RESULTS

We did not recapture any specimen marked during any previous estimation (Table 1). In APR95 and JUN95, when specimens from adjacent sites were marked with different colors, we did not recapture any color-mismatched specimens at any site.

Table 1. Mark-recapture data for *S. emarginatus* in Sively #2 stream in Organ Cave. a: number marked and released; n: number in second sample; r: number of recaptures; N and S.E.: estimated population size and standard error; N/m: estimated density per meter stream length.

		Stream Sites				Pool Sites			
Date		S1	S2	S3	Total	P4	P5	P6	Total
OCT94	a	29	10	15	54	-	-	-	-
	n	32	21	45	98	-	-	-	-
	r	14	3	5	22	-	-	-	-
	N	64	55	115	241	-	-	-	-
	S.E.	12	22	40	45				
	N/m	10	12	18	14				
JAN95	a	38	32	-	70	44	23	22	89
	n	42	35	-	77	27	17	14	58
	r	13	6	-	19	1	2	0	3
	N	117	165	-	273	616	138	-	1313
	S.E.	25	52		51	343	63	-	567
	N/m	6	16		10				
APR95	a	52	30	15	97	-	11	10	21
	n	55	33	20	108	-	6	2	8
	r	22	15	6	43	-	0	0	0
	N	130	64	45	244	-	-	-	-
	S.E.	22	11	13	29	-	-	-	-
	N/m	16	9	6	11				
JUN95	a	46	25	21	92	-	-	-	-
	n	48	31	10	84	-	-	-	-
	r	15	12	5	36	-	-	-	-
	N	141	61	38	215	-	-	-	-
	S.E.	28	13	10	27	-	-	-	-
	N/m	18	8	5	10				

STREAM SITES

We were able to estimate population sizes at all stream sites during all four sampling periods except one. In JAN95, we mistakenly sampled longer segments of the stream at S1 and S2 than originally planned, completely overlapping the original S3 site. The total distance sampled at S1 and S2 in JAN95, however, was similar to the total distance sampled at all three sites during the other three estimation periods.

Among stream sites, we marked an average of 28 specimens per site per estimation period, with a range of 10 to 52. The sizes of the second samples were generally similar, and ranged from 10 to 55 but averaged slightly higher at 34. Recapture rates (r/a in Table 1) ranged from 19% to 50%, with an average of 36%. Estimated population sizes ranged from 38 to 165 per site per estimation period, and averaged 90. Corresponding densities ranged from 5/m to 18/m, and averaged 11/m. Combining the data from all three sites for each estimation period resulted in estimated population sizes from 215 to 273 with an average of 254. The combined data yielded corresponding densities from 10/m to 14/m, with an average of 11/m which was identical to the average of the separate sites.

We used the estimated density per stream length to gauge the population size of *S. emarginatus* in the stream channel. Because of the apparent lack of migration of specimens among stream sites, we treated the values obtained from each site as independent estimates and calculated their mean. The average density values were 13.3/m, 11.0/m, 10.3/m, and 10.3/m, respectively, for OCT94, JAN95, APR95, and JUN95. These mean values closely matched the corresponding values based on combining the data from the three sites, at 14/m, 10/m, 11/m, and 10/m. Thus, assuming that the three sites are representative of the entire stream, we estimated that the population size of *S. emarginatus* in the channel of the Sively #2 stream, at a mapped length of about 300 m, was between 3000 and 4200 individuals.

POOL SITES

We were able to estimate population sizes at only two pool sites, P4 and P5, in JAN95. In OCT94, all three pools held little water and were not sampled. In JAN95, P6 yielded no recaptures from 22 marked specimens. In APR95, P4 was completely dry while P5 and P6 had shrunk to 20% of their sizes in JAN95, and we obtained no recaptures from 21 specimens marked in P5 and P6 combined. All three pools were completely dry in JUN95.

Among pool sites, we marked an average of 22 animals per pool per estimation period, with a range of 10 to 44. The second samples, however, were consistently smaller than the first samples, and ranged from two to 27 with an average of only 13. The recapture rates were extremely low, at 3% for all three pools combined in JAN95, and there were no recaptures in APR95. Estimated population sizes for sites P4 and P5 in JAN95 were 616 and 138, respectively. Combining the data from all three pools in JAN95 yielded an estimate of 1313. The standard errors for all these estimates were large due to the small numbers of recaptures.

OVIGEROUS FEMALES

We were unable to obtain sex ratios of the specimens because *S. emarginatus* was difficult to sex accurately without examination under the microscope. We were able to identify ovigerous females by the presence of developing embryos in their ventral marsupia. The proportion of ovigerous females among individuals captured at a site ranged from 3% to 18% from stream sites and from 4% to 25% from pool sites (Table 2). Combined data from stream sites showed the proportion of ovigerous females at 6%, 9%, 11%, and 4%, respectively, for OCT94, JAN95, APR95, and JUN95. Corresponding values for pool sites were 9% in JAN95 and 3% in APR95. There were too few values, however, to identify possible seasonal trends. Among stream sites, ovigerous females seemed to congregate disproportionately at one of the three sites. S1, the most downstream site, accounted for between 82% to 100% of the ovigerous females during any estimation period. Although ovigerous females were found in sufficient numbers among pool sites only in JAN95, nine of the 13 specimens were also concentrated in one site, P6.

Table 2. Occurrence of ovigerous females of *S. emarginatus* in Sively #2 stream in Organ Cave. O: number of ovigerous females; T: total number of different individuals sampled (= a + n - r of Table 1); p: ovigerous females as proportion of individuals sampled (= O/T).

		Stream Sites				Pool Sites			
Date		S1	S2	S3	Total	P4	P5	P6	Total
OCT94	O	8	0	0	8	-	-	-	-
	T	47	28	55	130	-	-	-	-
	p	0.17	0.00	0.00	0.06	-	-	-	-
JAN95	O	9	2	-	11	3	1	9	13
	T	67	61	-	128	70	38	36	144
	p	0.13	0.03	-	0.09	0.04	0.03	0.25	0.09
APR95	O	15	2	0	17	-	1	0	1
	T	85	48	29	162	-	17	12	29
	p	0.18	0.04	0.00	0.11	-	0.06	0.00	0.03
JUN95	O	6	0	0	6	-	-	-	-
	T	79	44	26	140	-	-	-	-
	p	0.08	0.00	0.00	0.04	-	-	-	-

DISCUSSION

Results from this study show that the mark-recapture technique used can provide accurate data on estimating population sizes of stygobites such as amphipods in caves. Some individuals could have lost their markings upon molting between the first and second samples during each estimation period. The magnitude of this error is likely small because of the short interval between samples and because molting in *Stygobromus* is infrequent, as molting is rarely observed among specimens kept over long periods in the laboratory. The lack of recapture of color mis-matched specimens when specimens from adjacent sites were marked with different colors indicates that, at least during the 24 hour sampling interval, there was no significant migration of individuals among sites. For stream sites, the sizes of the first and second samples were generally similar, indicating equal sampling efforts. These results, in combination with the high average recapture rate of 36%, indicate that the estimated values, of density between 10/m to 14/m and population size in the stream channel between 3000 and 4200, are reasonable.

For pool sites, low recapture rates inflated the standard errors of the population size estimates. We suggest that these low recapture rates, compared to the relatively high rates at stream sites, may reflect a complex three-dimensional nature of the pool habitat, that beneath the soft muddy substrate water in these pools communicates with water in the epikarst. These pools thus represent small windows into the epikarst, and fill when water in the epikarst expand during wet periods, and dry up when water in the epikarst contract during dry spells. Animals found in these pools may thus comprise a small sample of a larger population inhabiting the epikarst. Although our data indicate that *S. emarginatus* do not migrate far in 24 hours, marked specimens need only cover short distances to move out of these small and shallow pools. We did observe one marked specimen that burrowed and disappeared into the mud bottom of P5 in JAN95, and it did not reappear before we gave up after waiting for 20 minutes. The low recapture rates thus resulted from our having marked only a small portion of the population. We suggest that the estimated population size of 1313 from the pool habitat in JAN95, although not highly reliable, does point to a potentially large hidden population. Further study at these pool sites will need to employ different techniques, such as removal sampling over several days during the wet season, to estimate population size in this habitat.

The consistent preponderance of ovigerous females at S1 among stream sites during all four estimation periods, and the concentration of ovigerous females in P6 among pool sites in JAN95, indicate probable strong microhabitat preference by ovigerous females and uneven distribution of microhabitats among sites. Our experience during sampling was that all individuals were clumped within stream sites, and that usually several specimens were captured within short periods separated by long spans when no specimens were found, again suggesting strong microhabitat preference.

ACKNOWLEDGMENTS

We greatly appreciate the efforts of the following friends and colleagues in helping with the tedious task of capturing and marking specimens: Dave Culver, Diane Fong, Radu Popa, Kevin Simon, and Adam Switalski. We thank the former owners of Organ Cave, Lee Sively and the late George Sively, for kindly allowing us access to the cave. We thank Steve Taylor and an anonymous reviewer for their helpful comments on a previous draft of this paper. This research was partially funded by the Cave Conservancy of the Virginias.

REFERENCES

Begon, M. (1979). *Investigating Animal Abundance: Capture-Recapture for Biologists*. University Park Press. Baltimore, MD.

Culver, D.C. (1982). *Cave Life: Ecology and Evolution*. Harvard University Press. Cambridge, MA.

Culver, D.C. & Holsinger, J.R. (1992). How many species of troglobites are there? *National Speleological Society Bulletin 54*: 79-80.

Culver, D.C., Jones, W.K., Fong, D.W. & Kane, T.C. (1994). Organ Cave karst basin. In Gibert, J., Danielopol, D. & Stanford, J. [eds.]. *Groundwater Ecology*. Academic Press. San Diego, CA: 451-473.

Fong, D.W., & Culver, D.C. (1994). Fine-scale biogeographic differences in the crustacean fauna of a cave system in West, Virginia, USA. *Hydrobiologia 287*: 29-37.

Holsinger, J.R. (1972). *The Freshwater Amphipod Crustaceans (Gammaridae) of North America*. Biota of Freshwater Ecosystems Identification Manual No. 5. EPA. Washington, D.C.

Holsinger, J.R., Baroody, R.A. & Culver, D.C. (1976). *The Invertebrate Cave Fauna of West Virginia*. Bulletin No. 7, West Virginia Speleological Survey. Barrackville, WV.

Simon, K.S. (1997). Effects of organic pollution on an Appalachian cave: changes in macroinvertebrate populations and food supplies. *American Midland Naturalist 138*: 387-401.

Stevens, P.J. (1988). *Caves of the Organ Cave Plateau, Greenbrier County, West Virginia*. Bulletin No. 9, West Virginia Speleological Survey. Barrackville, WV.

Williams, P.W. (1983). The role of the subcutaneous zone in karst hydrology. *Journal of Hydrology 61*: 45-67.