

Estimating population size of the cave shrimp *Troglocaris anophthalmus* (Crustacea, Decapoda, Caridea) using mark–release–recapture data

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Abstract

Estimating population size of the cave shrimp Troglocaris anophthalmus (Crustacea, Decapoda, Caridea) using mark–release–recapture data.— Population size estimates are lacking for many small cave–dwelling aquatic invertebrates that are vulnerable to groundwater contamination from anthropogenic activities. Here we estimated the population size of freshwater shrimp *Troglocaris anophthalmus sontica* (Crustacea, Decapoda, Caridea) based on mark–release–recapture techniques. The subspecies was investigated in Vipavska jama (Vipava cave), Slovenia, with estimates of sex ratio and age distribution. A high abundance of shrimps was found even after considering the lower limit of the confidence intervals. However, we found no evidence of differences in shrimp abundances between summer and winter. The population was dominated by females. Ease of capture and abundant population numbers indicate that these cave shrimps may be useful as a bioindicator in cave ecosystems.

Key words: Mark–release–recapture, Population size, Dinarides, Vipavska jama, Sex ratio

Resumen

Estimación del tamaño de la población del camarón cavernícola Troglocaris anophthalmus (Crustacea, Decapoda, Caridea) mediante la utilización de datos de marcaje, liberación y recaptura.— Se desconoce el tamaño de la población de numerosos invertebrados acuáticos cavernícolas que son vulnerables a la contaminación de las aguas subterráneas provocada por las actividades antropogénicas. En este estudio estimamos el tamaño de la población del camarón de agua dulce *Troglocaris anophthalmus sontica* (Crustacea, Decapoda, Caridea) mediante las técnicas de marcaje, liberación y recaptura. La subespecie se estudió en la Vipavska jama (cueva de Vipava), en Eslovenia, y se calcularon la proporción de sexos y la distribución por edad. Incluso tras considerar el límite inferior de los intervalos de confianza, se halló un gran abundancia de camarones. No obstante, no se encontraron indicios de que haya diferencias en cuanto a la abundancia de camarón entre verano e invierno. La población estaba formada predominantemente por hembras. La facilidad de la captura y las elevadas cifras de población indican que estos camarones podrían utilizarse como bioindicadores en los ecosistemas cavernícolas.

Palabras clave: Marcaje, liberación y recaptura, Tamaño de población, Dinárides, Vipavska jama, Proporción de sexos

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Introduction

The freshwater shrimp genus *Troglocaris* (Dormitzer, 1853) consists of four subgenera distributed in the Western Balkans (fig. 1A; Sket & Zakšek, 2009; Matjašič, 1956; Babić, 1922; Jugovic et al., 2011) and the Caucasus (Sadovsky, 1930; Sket & Zakšek, 2009; Marin & Sokolova, 2014) and inhabits underground karst waters flowing to the surface.

Not much is known about the biology, ecology, distribution or habitat requirements of the European cave shrimp (Gottstein Matočec, 2003; Juberthie–Jupeau, 1974, 1975; Jugovic et al., 2010a). In this study we aimed to estimate population size for a population of *Troglocaris anophthalmus sontica* Jugovic et al., 2012, a subspecies of a type species of the genus. This subspecies is currently recorded from four subterranean localities (two in Slovenia and two in Italy) belonging to the Vipava–Soča River System (Jugovic et al., 2012). This river system is located on the north westernmost border of the distribution of subgenus *Troglocaris* s. str. (*sensu* Sket & Zakšek, 2009; fig. 1A). The Vipava River is a major water resource for the Vipava valley (SW Slovenia), with its headwaters arising from springs in Vipavska jama. The landscape behind the spring is an area of fractured and dissolved carbonate bedrock that stores significant quantities of groundwater.

As variation in daily and annual insolation is lacking in cave habitats, there is a significant regression of circadian (e.g., locomotor activity) and circannual (e.g., reproduction) patterns in troglobionts (Langecker, 2000; see also examples in Juberthie & Decu, 1994). Despite this, egg production of two species of Amphipods (Amphipoda), *Niphargus virei* and *N. rhenorodanensis*, exhibit reproductive patterns timed with hydrological fluxes that reach a maximum in summer and decrease in autumn. Similar annual variation in egg production has also been recorded in *Troglocaris planinensis* (Juberthie–Jupeau, 1974, 1975). Timed reproductive patterns are not clear for *N. rhenorodanensis* living in pools of percolated water, where the water does not flow, or for interstitial populations of the same species (Mathieu & Turquin, 1992). Fluctuations of population size were reported to be highly dependent upon water discharge rates that often vary considerably in correlation with conditions on the surface. In the case of *N. rhenorodanensis*, increased water discharge rates subject animals to drift and change population abundance (Essafi et al., 1992).

Population size estimates provide important information about rare and endangered species (Bueno et al., 2007). They are also used to identify species that may occur in sufficient numbers for use as bioindicators in environmental monitoring (Knapp & Fong, 1999). Mark–release–recapture (MRR) techniques are a popular choice for population size estimates; several methods have been developed to take into account the aim of the analysis and the type of species under investigation (Sutherland, 2006; Krebs, 1999; Seber, 1982). Non–commercial decapod crustaceans are rarely the object of population research (Rabeni

et al., 1997; Bueno & Bond–Buckup, 2000; Bueno et al., 2007) and to our knowledge, few MRR studies have been conducted in stygobionts (Hobbs, 1978, 1981; Culver, 1982; Simon, 1997; Knapp & Fong, 1999; Cooper & Cooper, 2009; Venarsky et al., 2012). Such studies are also rare in troglomorphic terrestrial invertebrates (Carchini et al., 1982, 1994; Bernardini et al., 1996). However, simple Lincoln–Petersen calculations have often been applied to cave beetles (Fejér & Moldovan, 2013).

The aim of our study was to estimate population size and sex ratios in a population of *T. a. sontica* from Veliko jezero (Large lake) in Vipavska jama. Using MRR techniques, we estimated population size and sex ratio in summer and winter.

Material and methods

Study site and field work

Veliko jezero is accessed through a 239 m man–made passage (fig. 1B), originally excavated for mercury ore. The lake is ellipsoid, with a surface area of approximately 180 m² and maximum dimensions of approximately 10 X 18 m. The lake is surrounded by steep, almost vertical walls and is accessible at only one point. The sampling area covered by the current study was approximately 6 m². Four square meters were reached in the lake from the access point; a further two square meters were accessible from the same point but situated along a narrow crevice (fig. 1B).

As these cave shrimps are omnivorous/detritivorous animals (Gottstein Matočec, 2003) and little is known about their diet, the development and testing of baits was outside the scope of the current study. Hence, animals were caught by hand–net. Hand nets of 1 mm mesh size were used for both capture and recapture. A net with a 1.5 m handle was used for specimens collected in deeper water. Although animals are always present in the lake, long periods of time (≥ 8 hours) were spent in the field as much patience was needed to catch the shrimps.

During the summer estimate, four sampling sessions were carried out, from 22–29 IX 2012. Three additional sampling sessions were carried out in winter from 18–24 II 2013 (table 1). Sampling was carried out by two people on a single day, over a period of 8–10 hours. If fewer than 25 animals were caught on a single day, the session was extended to the following day to ensure sufficient sample sizes. When an extended sampling session was required, animals caught on the first day were kept in a plastic tank with water over night. This approach was adopted in order to strengthen the equality of sampling among the sampling sessions. For both normal and extended sampling, a single day was left before the next sampling session.

By cutting off the tips of the uropods, telson or rostrum, animals were occasion–specifically marked (see an example of broken telson, fig. 1C). The possible negative impact of the marking procedure has been investigated previously on rostra (Jugovic et al., 2010a), revealing no noticeable impact on survival

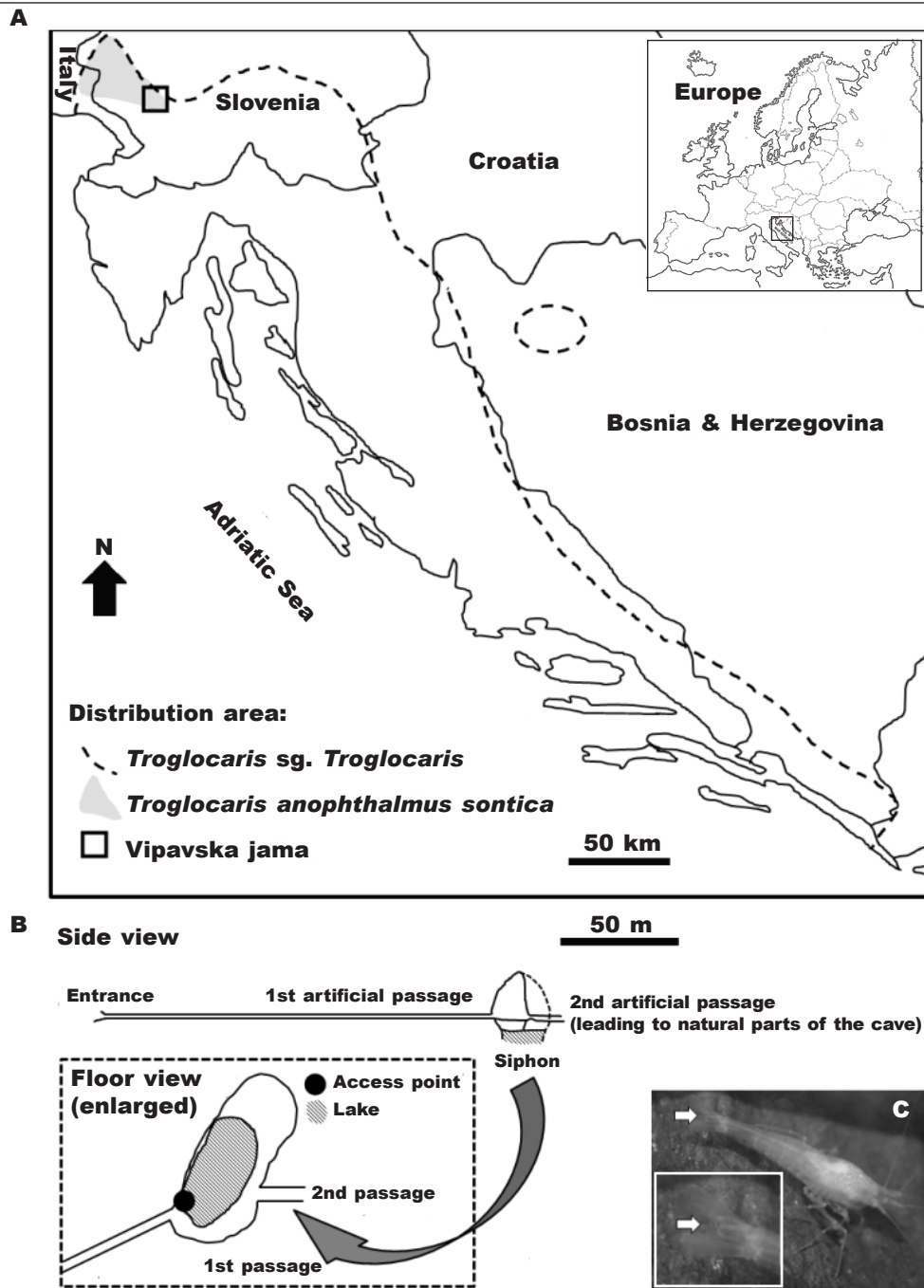


Fig. 1. A. Approximate geographic distribution of *Troglolaris* sg. *Troglolaris* showing *T. anophthalmus sontica* distribution area and geographic position of Vipavska jama; B. Sketch of Vipavska jama from entrance to second artificial channel (redrawn after JDDR; <http://www.jddr.org/kataster/vipavska/>) with depiction of Veliko jezero and access point to the lake where shrimp sampling was conducted; C. A shrimp with broken telson; this specimen was kept alive in a laboratory for over two years (photo: J. Jugovic).

Fig. 1. A. Distribución geográfica aproximada de *Troglolaris* sg. *Troglolaris* donde se aprecia el área de distribución de *T. anophthalmus sontica* y la localización geográfica de la cueva de Vipava; B. Esquema de la cueva de Vipava desde la entrada hasta el segundo canal artificial (modificación del dibujo de la sociedad espeleológica eslovena JDDR, <http://www.jddr.org/kataster/vipavska/>) donde se muestra el lago Veliko y el punto de acceso al mismo en el que se llevó a cabo el muestreo del camarón; C. Un camarón con un telson roto; este espécimen se mantuvo vivo en un laboratorio durante dos años (fotografía: J. Jugovic).

Table 1. Data on shrimp sampling during late summer (September 2012) and winter (February 2013): n_i , Number of animals caught in the i -th sampling occasion; m_i , Number of recaptures in the i -th sampling occasion; $u_i = n_i - m_i$, Number of unmarked animals in the i -th sampling occasion; M_i , Number of animals marked prior to the i -th sampling occasion; m_i/n_i , Ratio of marked animals in the i -th sampling occasion; * 47 animals were caught but only 44 were released (three were kept for morphometrics); no deaths regarding the manipulation of animals were observed.

*Tabla 1. Datos sobre el muestreo de camarones a finales de verano (septiembre de 2012) y a finales de invierno (febrero de 2013): n_i , Número de animales capturados en cada muestreo; m_i , Número de recapturas en cada muestreo; $u_i = n_i - m_i$, Número de animales no marcados en cada muestreo; M_i , Número de animales marcados antes de cada muestreo; m_i/n_i , Proporción de animales marcados en cada muestreo. * Se capturaron 47 animales, pero solo se liberaron 44 (tres se utilizaron para realizar estudios morfométricos); no se observaron muertes relacionadas con la manipulación de los animales.*

| Sampling occasion | n_i | m_i | u_i | M_i | m_i/n_i |
|-------------------------|-------|-------|-------|-------|-----------|
| Summer (September 2012) | | | | | |
| 22 IX * | 44 | 0 | 44 | 0 | 0.000 |
| 24 IX | 53 | 2 | 51 | 44 | 0.038 |
| 26–27 IX | 60 | 6 | 54 | 95 | 0.100 |
| 29 IX | 42 | 4 | 38 | 149 | 0.095 |
| Sum (summer) | 199 | 12 | 187 | | 0.060 |
| Winter (February 2013) | | | | | |
| 18 II | 27 | 0 | 27 | 0 | 0.000 |
| 20–21 II | 25 | 1 | 24 | 27 | 0.042 |
| 23–24 II | 29 | 1 | 28 | 51 | 0.034 |
| Sum (winter) | 81 | 2 | 79 | | 0.025 |
| Sum (total) | 280 | 14 | 266 | | 0.050 |

of marked animals. During the past samplings (for other studies), individuals with broken parts of telson, uropods or other appendages were commonly found alive and in good condition (field observations, see also Jugovic et al., 2010a). Moreover, one shrimp with a heavily injured telson (fig. 1C) remained alive in a laboratory for over two years.

Capture history for each animal was made in the following order by the subsequent markings for four sampling sessions during the summer: (1) left uropod exopodite, (2) right uropod exopodite, (3) left uropod endopodite, (4) rostrum and for two sampling sessions during the winter, (5) right uropod endopodite, and (6) the telson. Animals were not marked in the last sampling session.

Whenever samples were taken, the temperature of the air and water was recorded. For each individual, sex and age group (adult male, adult female, ovigerous female, juvenile, see Jugovic et al., 2010b for age groups) were determined.

Data analysis

Different models assume either an open or closed population. When the assumptions of closed popula-

tion models are met, they may provide more precise estimates of population size than open models can. Care must be taken when choosing the best models. Knowledge of the biology and ecology of the target population should guide the choice of appropriate models, and methods exist for relaxing some of the common assumptions of MRR (Greenwood & Robinson, 2006). MRR models can provide more reliable estimates than simple sightings or once-off counts (Knapp & Fong, 1999; Cooper & Cooper, 2011). Two different approaches to population size estimation were used within the Programme MARK (White & Burnham, 1999) to test closed population models.

Closed models were tested within the module 'Closed captures' and with 'Capture' available within MARK. Data were coded as individual encounter histories and entered into the input file (.inp). The two approaches differ in model selection. Models within MARK were selected based on AICc criterion. Four models were tested for each season, with different parameterization of capture probability (p), recapture probability (c), and the mixture parameter (π): M_i , M_b , M_h and M_0 (Chao & Huggins, 2005). Presence of individual heterogeneity within the population could cause non-identifiability of population size (Link, 2003;

Table 2. Analysed close population models in MARK closed population module for summer and winter with model selection criteria. Selected model is in bold: ML. Model likelihood; NP. Number of parameters; D. Deviance; $N \pm SE$. Estimated population size; CI. Confidence interval; * Model M_b was ranked high but parameter estimates, SEs and CIs were unrealistic; ** Model M_h with two mixture groups was especially considered, but estimates of π were not reliable.

*Tabla 2. Modelos de población cerrada analizados en el módulo de MARK para las poblaciones cerradas durante el verano y el invierno con criterios de selección de modelos. El modelo seleccionado se destaca en negrita: ML. Modelo de probabilidad; NP. Número de parámetros; D. Desviación; $N \pm SE$. Tamaño poblacional estimado; CI. Intervalo de confianza; * El modelo M_b obtuvo buenos resultados pero la estimación de los parámetros, las desviaciones estándar y los intervalos de confianza no fueron realistas; ** El modelo M_h con dos grupos de mezcla se estudió exhaustivamente, pero las estimaciones de π no fueron fiables.*

| Model | AICc | Δ AICc | WAICc | ML | NP | D | $N \pm SE$ | 95% CI |
|-------------------------|-----------------|---------------|----------------|----------|----------|----------------|-----------------------------------|------------------|
| Summer | | | | | | | | |
| M_0 | -960.564 | 0 | 0.55966 | 1 | 2 | 14.3276 | 1,196 \pm 322 | 735–2,043 |
| M_t | -958.837 | 1.7268 | 0.23602 | 0.4217 | 5 | 9.9896 | 1,188 \pm 319 | 731–2,029 |
| M_b^* | -958.548 | 2.0153 | 0.20432 | 0.3651 | 3 | 14.3268 | 1,245 \pm 1848 | 291–10,939 |
| M_h^{**} | -956.526 | 4.0377 | 0.06919 | 0.1328 | 4 | 14.3276 | 1,196 \pm 322 | 735–2,043 |
| Winter | | | | | | | | |
| M_0 | -336.129 | 0 | 0.61097 | 1 | 2 | 8.2864 | 1,064 \pm 727 | 350–3,668 |
| M_b^* | -334.091 | 2.0376 | 0.22058 | 0.3610 | 3 | 8.2724 | 2,802 \pm 40,789 | 107–260,905 |
| M_t | -332.312 | 3.8168 | 0.09062 | 0.1483 | 4 | 7.9822 | 1,062 \pm 726 | 349–3,662 |
| M_h^{**} | -332.008 | 4.1211 | 0.07783 | 0.1274 | 4 | 8.2864 | 1,065 \pm 727 | 350–3,668 |

Holzmann et al., 2006). We have therefore especially considered modelling the population with heterogeneity in capture probability (model M_h). Capture models were tested with the selection of the 'Appropriate' function, which is based on a discriminant function analysis procedure.

Equality of frequencies of males and females (ovigerous and non-ovigerous pooled together) was tested by χ^2 -test. Juveniles were excluded from the test. For the calculations, Excel 2007 was used.

Results

Population size estimates

All marks on the recaptured animals were clearly visible and easily distinguished from injuries of other origin. On four sampling occasions during the summer period, 199 animals were marked. On the first occasion, we caught but did not release an additional three animals that were kept for laboratory analysis. Only 81 animals were marked during the three winter sampling occasions. The average number of marked individuals recaptured was low, 6.0% for summer and 2.5% for winter, with no animals marked during the summer recaptured in the winter period (table 1).

Water temperature was almost constant, at 10°C in winter and 11°C in summer; air temperature was constant across seasons at 9°C.

Both approaches, MARK and Capture, selected M_0 model (with constant and equal capture and recapture probabilities, $p = c$) over other models for summer and winter capture periods (tables 2, 3; fig. 2). Most probably, this model was selected due to sparse data in terms of recaptures. Results based on models M_t and M_h were also comparable (table 2), despite our data showing less statistical support for those models. Noticeable differences in abundance estimates, with unrealistic standard errors and confidence intervals, resulted for M_b model, and were not taken into account as relevant (table 2). The summer M_0 estimate of population size was 1,196 individuals (SE = 322; 95% CI = 735–2043), with $p = 0.042$ (SE = 0.012; 95% CI = 0.024–0.071) within MARK's 'Closed captures' (table 2, fig. 2). In winter the population size estimated was 1,064 individuals (SE = 727; 95% CI = 350–3668), with $p = 0.025$ (SE = 0.018; 95% CI = 0.006–0.095; table 2, fig. 2). Capture estimates with model M_0 for summer were 1,195 individuals (SE = 320; 95% CI = 736–2,038), with $p = 0.042$ (no SE or CI reported in Capture), while in winter the estimates were 1,064 individuals (SE = 720; 95% CI = 353–3628), and $p = 0.0254$ (no SE or CI reported in Capture; fig. 2).

Table 3. Models analysed in programme Capture and model selection criteria (model selected has maximum value). The selected model is in bold.

Tabla 3. Modelos analizados en el programa Capture y criterios de selección de modelos (el modelo seleccionado tiene el valor máximo). El modelo seleccionado se destaca en negrita.

| Model | M_o | M _h | M _b | M _{bh} | M _t | M _{th} | M _{tb} | M _{tbh} |
|-----------------|----------------------|----------------|----------------|-----------------|----------------|-----------------|-----------------|------------------|
| Summer criteria | 1.00 | 0.81 | 0.18 | 0.52 | 0.00 | 0.35 | 0.26 | 0.65 |
| Winter criteria | 1.00 | 0.83 | 0.38 | 0.68 | 0.00 | 0.45 | 0.32 | 0.73 |

Sex ratio and distribution of age groups

In all sampling occasions within both seasons, the proportion of females (mean = 76.5%; range = 55.1–86.8%) was much higher than that of males (mean = 19.6%, range = 9.4–37.9%) and unsexed juveniles (mean = 3.1%, range = 0–4.8%; fig. 3). Adult females were more numerous than adult males ($\chi^2 = 37.463$, $p < 0.001$). Only two ovigerous females were caught, one in each season, each carrying approximately 10 eggs (counted *in vivo*).

Discussion

Population size and structure are basic demographic parameters that allow ecologists to evaluate the current status of a species, and may also serve for bioindication (Knapp & Fong, 1999; Praprotnik et al., 2013). In crustaceans, several MRR approaches have been used to estimate population size (Knapp & Fong, 1999; Bueno et al., 2007). We chose to apply the MRR technique for cave shrimps from Veliko jezero in Vipavska jama for the following reasons: (1) the

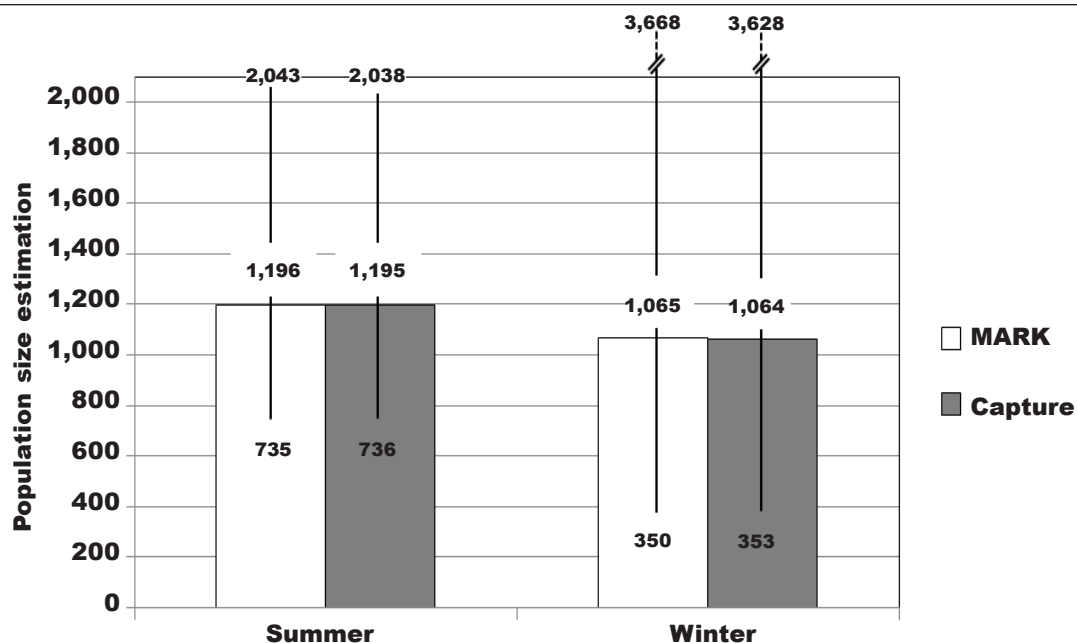


Fig. 2. Summer and winter population size estimates derived from model M_o with upper (above the estimates) and lower (below the estimates) 95% confidence limits.

Fig. 2. Estimaciones del tamaño de la población en verano y en invierno obtenidas con el modelo M_o con los límites de confianza del 95% superior (por encima de las estimaciones) e inferior (por debajo de las estimaciones).

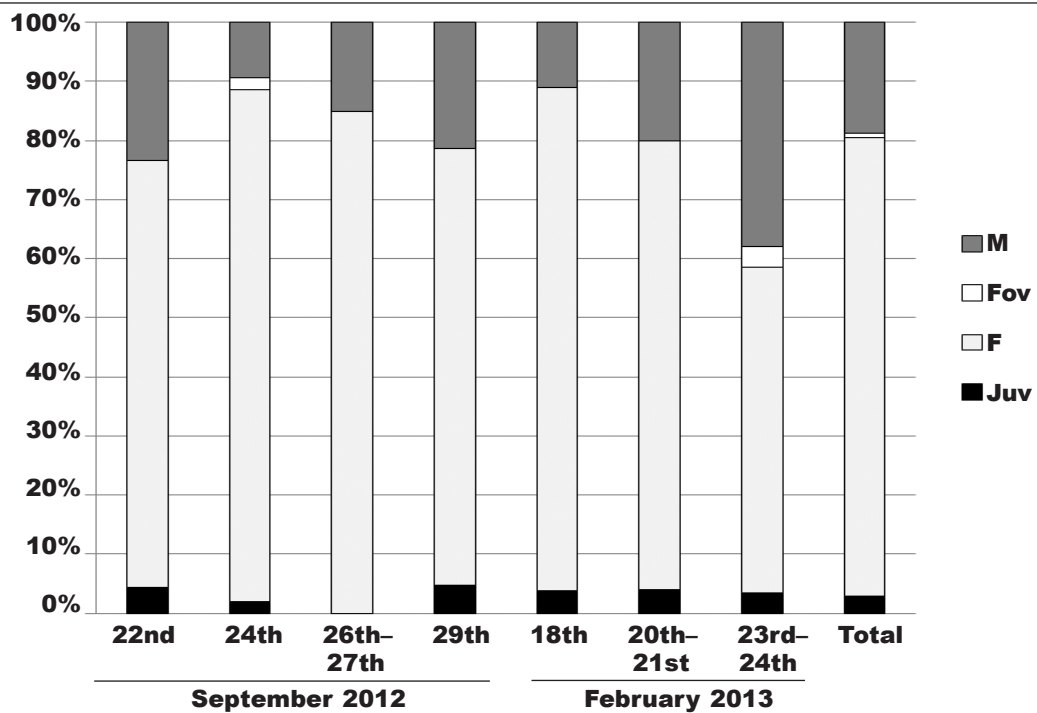


Fig. 3. Population structure (adult males (M), adult ovigerous females (Fov), adult non-ovigerous females (F) and juvenile animals with no sex identified (Juv)) for *Troglolaris anophthalmus sontica*. The data were collected on seven sampling occasions in summer (September 2012, five sampling days, four occasions) and winter (February 2013, five sampling days, three occasions). In total, 283 individuals were recovered across all sampling occasions.

Fig. 3. Estructura de la población (machos adultos (M), hembras adultas ovígeras (Fov), hembras adultas no ovígeras (F) y animales jóvenes de sexo no identificado (Juv)) de *Troglolaris anophthalmus sontica*. Los datos se recogieron en siete muestreos llevados a cabo en verano (septiembre de 2012, cinco días de muestreo, cuatro muestreos) y en invierno (febrero de 2013, cinco días de muestreo, tres muestreos). En todos los muestreos se recuperaron 283 individuos en total.

chosen site was appropriate for its relatively static nature compared to underground streams or rivers; the calmer waters in this lake environment are a preferred habitat to study organisms as they can reach high population densities (Jugovic, personal observations; see also Praprotnik, 2014) and (2) *Troglolaris* cave shrimps with adult sizes ranging from 15–25 mm are large enough to be marked using occasion-specific identifiers. Although injuries made in marking procedure can disrupt the shrimps' movement to some extent, we assumed (3) that these small injuries do not significantly affect their survival, considering that shrimps can successfully survive injuries of the chitinous parts of the body due to cave salamander (*Proteus anguinus* Laurenti 1768) attacks (Jugovic et al., 2010a). Salamanders were present during our field work in Vipavska jama. We are also aware that markings could be lost through successive moults, but (4) laboratory observations over a three-year period indicated that moulting occurs only rarely (Praprotnik, 2014; Jugovic, unpublished data).

We obtained the first rough estimates of *T. a. sontica* population size in the ground water system of Vipavska jama. In spite of the large confidence intervals, our estimates appear relatively high for such a small sampling area. As access to most cave ecosystems is difficult, it is not easy to estimate population sizes of cave taxa, and no such sampling procedures that are needed for multi-occasion MRR analyses have been conducted so far in such small stygobitic invertebrates. Nevertheless, some attempts have been made in larger stygobionts or troglophilic terrestrial invertebrates. Simple Lincoln–Petersen calculations have often been applied to cave beetles (see references in Introduction).

Although there are no data of cave shrimp lifespan in the literature, we assumed low mortality rates between sampling seasons. These organisms are *k*-strategists with low metabolic rates, and they produce a small number of relatively large eggs. They are capable of depositing extensive extracellular lipids (oleospheres) as a reserve for at least two years of

starvation (Jugovic, pers. observ. in laboratory; see also Vogt & Štrus, 1999). Despite recorded longevity, we did not have recaptures from the first season. Hence we treated these two seasonal samples as separate populations.

The population size estimates were mostly similar across the two seasons and their wide confidence intervals overlapped. The low rate of recaptures should not be neglected, indicating a large population, along with the presence of many places for shrimps to avoid capture; shrimps are also capable of movement beyond the surveyed area (Zakšek et al., 2009). Although low capture probabilities generally tend to overestimate population size due to the estimator structure (Chao & Huggins, 2005), this is true for the surveyed area of 6 m² exclusively. Therefore, we believe our estimations represent the minimum number of shrimps living in the wider area of the suitable habitat within the lake. Individuals present in samples may thus comprise only a small portion of a large population that cannot be easily detected. A larger part of a population in Vipavska jama is probably present in other parts of the lake, or in the siphon itself from where shrimps can move to other waters of the cave system. In the cave amphipod *Stygobromus emarginatus* (Hubricht, 1943), researchers indicated that the pool habitat represents a window into the epikarst zone, and the low recapture rates indicate a large hidden population in the epikarst (Knapp & Fong, 1999).

We found no evidence of statistical differences between summer and winter regarding shrimp abundance. Moreover, we detected the presence of ovigerous females during both seasons, but their low frequency of occurrence together with only two sampling periods did not allow for conclusions about possible annual rhythmicity for egg laying. Juveniles were also present during both seasons. According to the literature, ovigerous females are present in *Troglocaris planinensis* throughout the year, with a peak in late autumn (Jubertie–Jupeau, 1975). It should be noted that the estimated number of eggs in *T. a. sontica* from Vipavska jama is much lower and not consistent with data given by Juberthie–Jupeau (1974) for its closer relative *Troglocaris planinensis* (20–45 eggs). The estimated number instead resembles data for *Gallocaris inermis* (Fage, 1937) (8–12 eggs, Jubertie–Jupeau, 1974), another European cave dwelling shrimp species from southern France.

The small proportion of males contradicts the expected equal frequencies of males and females in invertebrates, but numerous exceptions have been reported (see Hodgson in EOLSS). The observed proportion of approximately 20% males is even lower than was estimated from random samples collected over the past years (*i.e.*, approx. one male per three females; Jugovic et al., 2010b). A low proportion of juveniles may be the result of either heterogeneity in capture probabilities or the relatively small proportion of a lifespan that can be recognized as juvenile (*i.e.*, excluding larval stages that were not sampled). The short period of the juvenile stage in many cave dwelling animals has been reported previously (for cave shrimps, see Matjašič, 1958). The accelerated juvenile

development is considered to be a result of a rather small number of large eggs with lots of nutrients (Juberthie–Jupeau, 1975; Matjašič, 1958).

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