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Trabajo Fin de MÁSTER

LONG-TERM ASSESSMENT OF THE POPULATION TRENDS AND BODY CONDITION OF *CARABUS VARIOLOSUS NODULOSUS* IN SLOVENIA

ESTUDIO A LARGO PLAZO DE LAS TENDENCIAS POBLACIONALES Y LA CONDICIÓN CORPORAL DE *CARABUS VARIOLOSUS NODULOSUS* EN ESLOVENIA.

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RESUMEN

Para evaluar las tendencias poblacionales actuales de *Carabus variolosus nodulosus* en Eslovenia y analizar la condición corporal de los individuos, se emplearon datos obtenidos a lo largo de 17 años en cinco regiones geográficamente diferenciadas. Se registró el número de escarabajos capturados por trampa y se tomaron mediciones de individuos vivos con el fin de calcular tanto las tendencias poblacionales a nivel nacional como regional, junto con un índice de condición corporal. Para evaluar el posible efecto del sexo y las perturbaciones sobre la condición corporal, así como para explorar la relación entre esta y la densidad poblacional, se emplearon modelos lineales de efectos mixtos. Los resultados revelaron una tendencia poblacional general estable, con diferencias significativas entre las distintas regiones. No se encontró correlación entre la condición corporal y la densidad poblacional ni las perturbaciones, aunque sí se observaron variaciones significativas en la condición corporal según el sexo. Este estudio subraya la importancia de realizar un seguimiento a largo plazo y a diferentes escalas para detectar factores de estrés y posibles extinciones locales que, de otro modo, podrían pasar desapercibidas, y propone esfuerzos de muestreo para futuros planes de seguimiento con el fin de realizar evaluaciones fiables de las tendencias poblacionales de la especie.

ABSTRACT

To gain insight into the current population trends of *Carabus variolosus nodulosus* in Slovenia and assess the body condition of individuals, data from 17-year monitoring program were analyzed across five geographically distinct regions. The number of beetles captured per trap was recorded, and measurements of live specimens were taken to calculate both national and regional population trends, along with a body condition index. Additionally, linear mixed-effects models were used to examine the potential impact of sex and disturbances on body condition, and to explore the correlation between body condition and population density. The results indicated an overall stable population trend with marked regional differences. No correlation was found between body condition and population density or disturbances, though significant differences in body condition between sexes were observed. This study underscores the importance of long-term and multi-scale monitoring to detect stress factors and potential local extinctions that might otherwise remain undetected and proposes sampling efforts for future monitoring schemes for reliable trend assessments of the species.

Key words: body condition, scaled mass index, long-term monitoring, carabids, ground beetle, Natura 2000.

INTRODUCTION

Long-term monitoring constitutes an indispensable tool for advancing the comprehension and management of intricate ecological systems, serving as baseline data to evaluate potential threats, formulating conservation priorities and assessing the impact of management actions (Lindenmayer et al., 2012; Reif, 2013). It involves systematic and standardized collection of data over extended periods, enabling scientists to identify and understand trends, patterns and lagged responses to environmental changes that may be overlooked in short-term studies (Lindenmayer & Likens, 2009; Magurran et al., 2010).

Although long-term data are of great significance for biodiversity conservation, they are strongly biased in terms of the species represented. Those that are locally abundant, easily identified (Boakes et al., 2016) or with a societal preference are largely over-reported (e.g. large terrestrial mammals and birds), while other animal taxa, such as invertebrates, are under-represented in biodiversity databases (Monsarrat & Kerley, 2018; White, 2018), and proper long-term population trend assessments are usually lacking.

Insects are well known for being the most diverse group of organisms (Stork, 2018). They represent the largest proportion of macroscopic terrestrial animal life, both in terms of species richness and biomass (Lamarre at al., 2020) and play vital roles in ecosystems, contributing to the four main types of ecosystem services, providing a food source for higher trophic levels and participating in processes such as pollination and nutrient cycling (Hallmann et al., 2017; Didham et al., 2020).

Nevertheless, several studies have corroborated the global decline of this group, predominantly as a consequence of habitat loss and fragmentation, pollution, insecticides, land-use intensification and the frequency of extreme climatic anomalies (Sánchez-Bayo & Wyckhuys, 2019; Cardoso et al., 2020). Conversely, other studies have been unable to substantiate this trend due to the restricted availability of long-term data on insects (Schowalter et al., 2021).

As indicated by the International Union for Conservation of Nature (IUCN), only 0.06% of the described insect species have been evaluated. This paucity of data represents a substantial and remarkable environmental concern, as it hinders the identification of trends and potential drivers of population changes (Conrad et al., 2004), obscuring the determination of the extent of population declines and their underlying causes and consequences (Gregory et al., 2005; Loh et al., 2005), but on the other hand conservative approach in nature conservation might also produce apparent but wrong conclusions about insect population development (Regan et al., 2005; Rondinini et al., 2006).

Among insects, ground beetles, belonging to family Carabidae, are a crucial component of insect predator diversity (Pizzolotto et al., 2018) and are considered potential bioindicators of ecosystem stability or stress, as they exhibit strong relationships with environmental conditions and respond to ecosystem changes at both fine and broad scales (Allegro & Sciaky, 2003; Kotze et al., 2011). The study of the morphological and biometric traits of these species can provide valuable information about a population, such as habitat quality and niche requirements (Michalcewicz & Ciach, 2012), as well as the nutritional or body condition of the individuals.

Some carabid species has been designated by the European Habitats Directive (Council Directive 92/43/EEC) as key species for nature conservation in Europe. This study focuses on one of these species, *Carabus variolosus* (Creutzer, 1799). It is considered an endangered carabid species currently protected under the European Habitats Directive (Council Directive 92/43/EEC). The taxonomic status remains unresolved and open to discussion, with some authors proposing the existence of two subspecies (*C. variolosus nodulosus*, and *C. variolosus variolosus*) (Breuning, S. 1926; Matern, et al, 2007), while others regard them as distinct species on the basis of the male morphological difference (Turin et al., 2003). This paper employs the context of the EU Habitats Directive, wherein the taxon is identified as *Carabus variolosus nodulosus* (Creutzer, 1799). The taxon is regarded to be highly endangered and even extinct in Italy and Switzerland, and strongly declining in France, Germany and Austria (Turin et al. 2003), with the population stronghold being in the Balkans, especially in Slovenia (Vrezec et al. 2020). The *Carabus variolosus* exhibits a nocturnal and semi-aquatic lifestyle, with a restricted distribution to wet areas such as brook banks, puddles and floodplains within a limited European range (Matern et al., 2007; Vrezec et al. 2021). It is and extremely hygrophilous and stenotopic species, with a poor dispersal power and a habitat preference for mountainous and hilly areas characterized by sparse soil vegetative cover and the presence of decaying tree stump (Turin et al., 2003; Matern et al, 2008).

There is no published long-term trends for the species yet, and only local assessments exists (Vrezec et al. 2020; Bedjanič et al. 2021). This study aims to achieve four primary objectives: (1) to analyze the population trends and dynamics of *Carabus variolosus* across Slovenia at both national and regional scales; (2) to analyze population trends according to conservation measures and population size; (3) to analyze range shifts, (4) to investigate the variability of a body condition index of in relation to population dynamics as a monitoring tool in future population assessments, and (5) to estimate the minimal number of required sites and years for conducting monitoring of *Carabus variolosus* for reliable population trend assessment in selected area.

METHODS

Study sites

In the study we have used long-term data of regular and periodical national monitoring of *Carabus variolosus* in Slovenia (Vrezec at al., 2009; Vrezec et al., 2021). The term 'regular monitoring' was used to describe the yearly monitoring, whereas 'periodical monitoring' was used to describe the monitoring that occurred every 5 years. The former was conducted across 20 different locations over a 16-year span (2008-2023), while the latter took place between 2007 and 2023 at 180 distinct sites across Slovenia. The sampling sites were distributed over the whole territory in Slovenia, which was divided into five regions according to geographic regionalization of Slovenia that was used in further data analysis (Figure 1), and which are characterized by specific habitat features (Table 1).

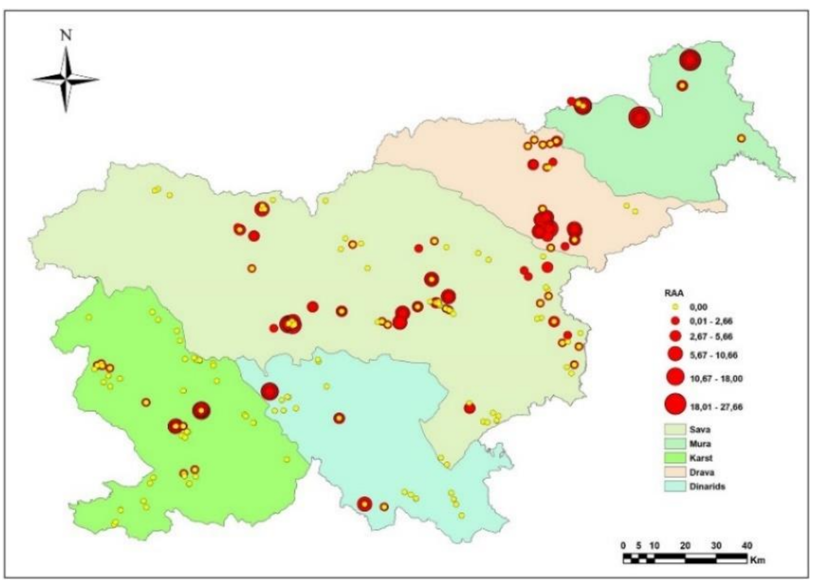

Figure 1. Map of sampled regions and localities in Slovenia with relative abundances of *Carabus variolosus* per locality. Relative abundances were calculated as a number of trapped specimens in 10 trap nights. See Monitoring and field methods for further details.

Table 1. Characteristics of the regions in Slovenia analyzed in the study. The total level of hydrological network of level 2 represents running waterbodies smaller than river, which are suitable habitat for *Carabus variolosus* (Horton, 1945; Turin, 2003).

Region	Karst	Mura	Dinarids	Sava	Drava
Minimum altitude [m]	0	143	125	125	176
Maximum altitude [m]	1795	559	1286	2863	1543
Area size [km^2]	3778,2	2144,2	2895,1	9147,5	2307,8
Forest cover [%]	18,8	6,4	14,4	48,7	11,7
Total length hydrological network of level 2 [km]	5203	5929	1820	21594	60635
Coordinates of the center	45.775, 14.000	46.620, 16.065	45.728, 14.843	46.179, 14.688	46.461, 15.495
Urbanized areas (%)	14,0	13,0	8,3	49,8	15,0

Monitoring field methods

The species was sampled using pitfall traps using vinegar as a bait (Vrezec & Kapla, 2007) between May and June, when there is a peak of activity of the species (Matern et al., 2007b; Vrezec et al., 2012).

Pitfall trapping is an effective standard technique frequently employed for the characterization of ground-active arthropod assemblages (Prasifka at al., 2007). In this study, 0.5L plastic pots were buried and filled with approximately 0.5 dL of wine vinegar. A total of 15 traps (life traps) were set at each site for regular monitoring and 5 traps (lethal traps) for periodic monitoring. They were left from 2 to 17 days exposed in the field. At the field the beetles have been counted by trap and relative abundance was calculated as number of caught specimens per 10 trap nights (Vrezec & Kapla, 2007). The beetles caught in life traps were weighted, sexed and photographed. The photographs of each individual were taken on a millimeter grid and later loaded into Merilec ver 1.10 program (Vrezec et al., 2011). This program allowed us to take the measurements with an accuracy of 0.1 mm. Subsequently, the total body and elytra length, as well as the head and thorax width, were measured and recorded for calculation of the scaled mass index. Additionally, at each location the possible disturbance in the habitat of the *Carabus variolosus* where detected. The sites where we detected waterflow regulation, urbanization or wood cutting were considered as disturbed in a current year, and others as non-disturbed.

Statistical analysis

Population trends

The calculation of population trends was conducted using TRIM (TRends and Indices for Monitoring data) implemented in the rtrim library for R. TRIM is a software tool designed for the analysis of temporal variation in biodiversity monitoring data (Pannekoek & van Strien, 2005). This program allows the calculation of time-series models using a Generalized Estimating Equation (GEE) approach, thereby enabling the prediction of indices and population trends from data sets with missing counts (Gregory et al., 2005; Węgiel et al., 2021). The overall slope for long-term trends was assessed using multiplicative parameters. Population trends were classified into six categories, as follows: 'Strong increase' for an increase exceeding 5% per year; 'Moderate increase' for an increase below 5% per year; 'Stable' for trends without significant change; 'Moderate decrease' for a decline under 5% per year; 'Strong decrease' for a decline exceeding 5% per year; and 'Uncertain' when no clear trend could be established, although slight decreases or increases could be discerned (Garcia et al., 2015; Sereno-Cadierno et al., 2023). The initial estimate of abundance is fixed at a value of one, and each subsequent annual index is calculated in comparison to this baseline value (Conrad et al., 2004).

Count data were transformed by the square root method (Gonçalves & Ghosh, 2022) and Poisson distributions were checked prior the calculation of every population trend, as the models assumed independent distributions for all counts. Weights were calculated according to the number of trap nights, dividing the total of traps by the number of them for that year and location, so higher values mean less sampling effort. Time-effect models were also used and evaluated by the goodness-of-fit, considering chi-square > 0.05. Following Pannekoek & van Strien (2001), overdispersion and serial correlation were also taken into account, with values < 3 and < 0.4 respectively (Sereno-Cadierno et al., 2023).

Several trends were calculated in this study. An overall trend was first calculated including regular and periodical monitoring data. Furthermore, based on geographic and geological variables of the area, we considered the existence of 5 different regions, and trends were calculated for each one to check the current situation of the population within regions (Table 1, Figure 1). The population trends of Mura and Drava was calculated with a gap of 1 year, since the monitoring was interspersed in these two regions.

In order to test if the dynamics of the population were influence by its size, we used the initial state data for each monitored location. The initial state data are defined as samples from the first year of monitoring between 2007 and 2012, and relative population size was estimated by multiplying the relative abundance by the habitat size of each location. Relative population size is comparable between locations but cannot be used as approximate of the absolute population size. The habitat size was defined as the total length of the II. level streams (Horton, 1945) within the buffer of 3 kilometers around the sampling site. The data were obtained from hydrological map of Slovenia (Ministrstvo za naravne vire in proctor, 2024) using GIS program (ESRI, 2011). To establish thresholds that differentiate between large, medium and small populations, Kernel densities were calculated, and two thresholds were defined with distinct quantile values. The first threshold was set at Q1, while the second was set at Q3, with populations falling between these thresholds classified as medium-sized (Jones, 1992; Lindblad, 2000).

Finally, we calculated trends based on site classification according to Natura 2000 network and were divided into distinct categories: (1) Natura 2000 sites (sites were the species is qualification and certain measures are taken for its conservation) and (2) non-Natura 2000 sites (including non-Natura 2000 sites and Natura 2000 sites where the species is not qualifying so no specific conservation measures are taken for the species here). Similar procedure was employed for "high-disturbed" locations, defined as those with over 50% of years with detected disturbance, and "low-disturbed" locations, which exhibited 50% or less years with detected disturbance. Finally, population trends were calculated for each group and then compared to ascertain the potential impact of disturbance and protection (Natura 2000) on population dynamics of *Carabus variolosus*.

Range change dynamics

In order to ascertain whether the current population is expanding or shrinking in its range monitoring years were divided into two periods: 2007-2012 and 2013-2023. Only sites present in both periods, and with at least one presence in either, were considered. Consequently, the locations were divided into three distinct scenarios: (1) sites with recorded presence in the first period but none in the second were classified as disappearance; (2) sites with no recorded presence in the first period but at least one presence in the second were categorized as new appearance; (3) sites with recorded presence in both periods, or any presence in either period, were defined as stable.

Scaled mass index

By definition, the scaled mass index (SMI) is a non-destructive and powerful alternative CI method that standardizes body mass to a fixed value of a linear body measure based on the scaling relationship between mass and length (Peig & Green, 2009). It is based on the formulation of Lleonart et al. (2000) for comparative morphology, whose aim was to describe a normalization technique to scale data that exhibit allometric growth using the Thorpe-Lleonart model (Thorpe, 1975).

Thus, according to Peig & Green (2009) and 'scaled mass index' of body condition can be computed as follows:

$$SMI = M_i [\frac{L_0}{L_i}]^{bSMA}$$

In this model, the body mass (Mi) and linear body measurement (Li) of individual i are the dependent variables. The scaling exponent (bSMA) is estimated by the SMA regression of M on L. L_0 is an arbitrary value of L, which may be the arithmetic mean value for the study population. Finally, SMI is the predicted body mass for individual i when the linear body measure is standardised to L_0.

Prior to using parametric statistics, we tested for outliers based on body mass (English et al., 2018). A total of 6 outliers were removed following the generalized S estimator method (GSE) (weight < 5e-05) (Temple et al., 2019). All the measurements collected were then ln-transform and tested in Pearson correlations to see which measurement (L) was the best predictor for body mass. Finally, SMA regression was calculated to determine the scaling exponent of the power function/the slope of the fitted line (bSMA) (Maceda-Veiga et al., 2014).

Factors with a potential effect on body condition

In order to test the factors influencing body condition, we used general linear mixed models (GLMM) to control the effect of location and year and test for the effect of different variables. We also used a stepwise reduction method, whereby all explanatory variables were initially considered (i.e. the full model) and then gradually removed in order to ascertain which variables contributed the least to explaining the result (Whittingham et al., 2006). This process involved the exclusion of the variable with the highest p-value until only those with a p-value of less than 0.05 remained.

A GLMM was first fitted with sex and disturbance with two levels, disturbed and no disturbed - as fixed factors, location and year as random factors, and the scaled body length mass index as the dependent variable. The interaction between disturbance and sex was also included.

As it was mentioned before, body condition can be considered an important determining factor of the fitness of the species. Therefore, we tested the possible correlation between density of species and scaled mass index by GLMM. Density was set as the dependent variable, SMI as factor, and year and location as random effects.

Estimation of minimal number of required monitoring sites

A multi-step approach was employed in order to identify a strategy for minimizing sampling effort while ensuring the maintenance of accurate population trends. The dataset was initially filtered to retain only those locations that had been consistently sampled across all years. Subsequently, a specified number of these locations were randomly selected, and population trend models were unpacked iteratively, with 100 iterations conducted (Brashares & Sam, 2005). Once the minimum regular sites were established, models were evaluated using subsets of these locations, where some locations were sampled periodically (every 2 years). For the purpose of this study, a definition of 'reliable' sample effort was proposed as one that reliably detected true increases and decreases in populations with at least 95% certainty.

To determine the minimum number of years required, a similar process was applied. A set of random consecutive years was selected, with the starting points distributed across the timeline, and resulting population trends were then tested for significant differences with the original trends. Only those interactions that exhibited stable trends were considered 'successful'.

RESULTS

Overall population trend in Slovenia

Analysis of the overall population trend was found to be stable (Figure 2). Notable changepoints were identified with significant shifts in trend observed at several points, including 2009, 2013, 2018, and 2020 ($p < 0.05$) suggesting that these periods experienced significant changes in the trend dynamics.

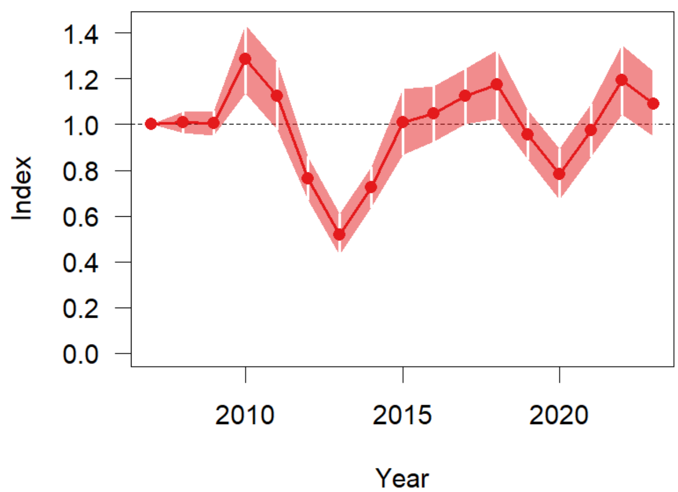

Figure 2. Overall population dynamics of *Carabus variolosus* in Slovenia from 2007 to 2023. The population trend is stable, with a +0.43 of annual change rate, significant variation ($\chi^2 = 321.3$, $p = 0.998$) and appropriate values for overdispersion (0.803) and serial correlation (0.272).

Population trends by regions

Regional trends showed considerable variability between regions (Table 2). However, all the models fitted a log-linear distribution (χ^2 and LR p-values > 0.05), and the overdispersion and serial correlation values were in accordance with the TRIM recommendations previously outlined.

Just Mura and Sava regions showed opposite trends (Figure 3). The population in Mura region suffered important decreasing between 2010-2014 (-53%) and 2018-2020 (-58%). In contrast in Sava region significant fluctuations were detected in 2011, with a notable decrease of about -32%, followed by a increase of +39% from 2013 to 2015.

Table 2. Modelled annual population trends of *Carabus variolosus* populations in different regions in Slovenia.

	Mura	**Sava**	**Drava**	**Dinarids**	**Karst**
No. sites	9	94	25	23	49
No. data points	41	294	83	131	189
Goodness-of-fit (Chi-square, p)	17.4, p > 0.05	108.3, p > 0.05	29.8, p > 0.05	31.6, p > 0.05	48.0, p > 0.05
Likelihood ratio (LR, p)	21.04, p > 0.05	133.20, p > 0.05	37.54, p > 0.05	39.10, p > 0.05	56.18, p > 0.05
AIC	-34.96	-112.80	-68.46	-76.90	-81.82
Overdispersion	0.6205	0.8803	0.5618	0.5454	0.6959
Serial correlation	0.3496	0.2504	-0.2197	0.0950	0.2143
Annual change rate [%]	-12.9	+3.2	+2.2	-4.2	+3.9
Trend estimation	Moderate decreasing	Moderate increasing	Uncertain	Uncertain	Uncertain

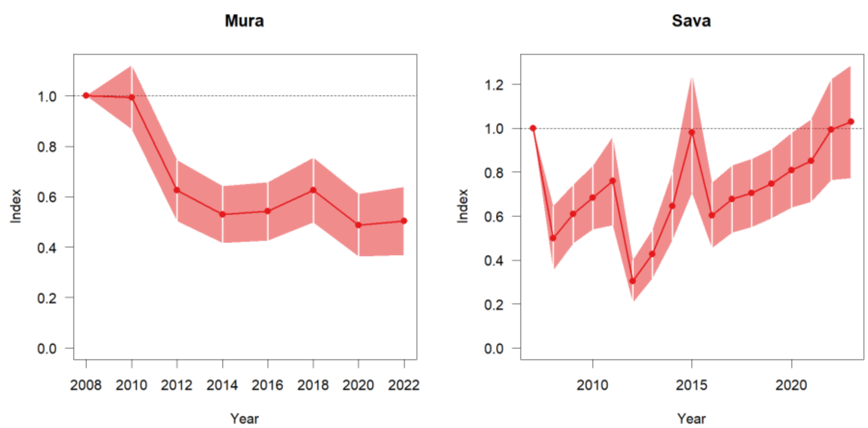

Figure 3. Models of population dynamics of *Carabus variolosus* in the regions of Mura and Sava.

However, the regions of Drava, Dinarids and Karst exhibited uncertain trends, with a theoretical decline in Dinarids and an increase in Drava and Karst (Figure 4). For instance, both the Karst and Dinarids regions showed notable decreases around 2014, while the Drava region displays significant changes in the early 2010s, reflecting similar patterns of fluctuation.

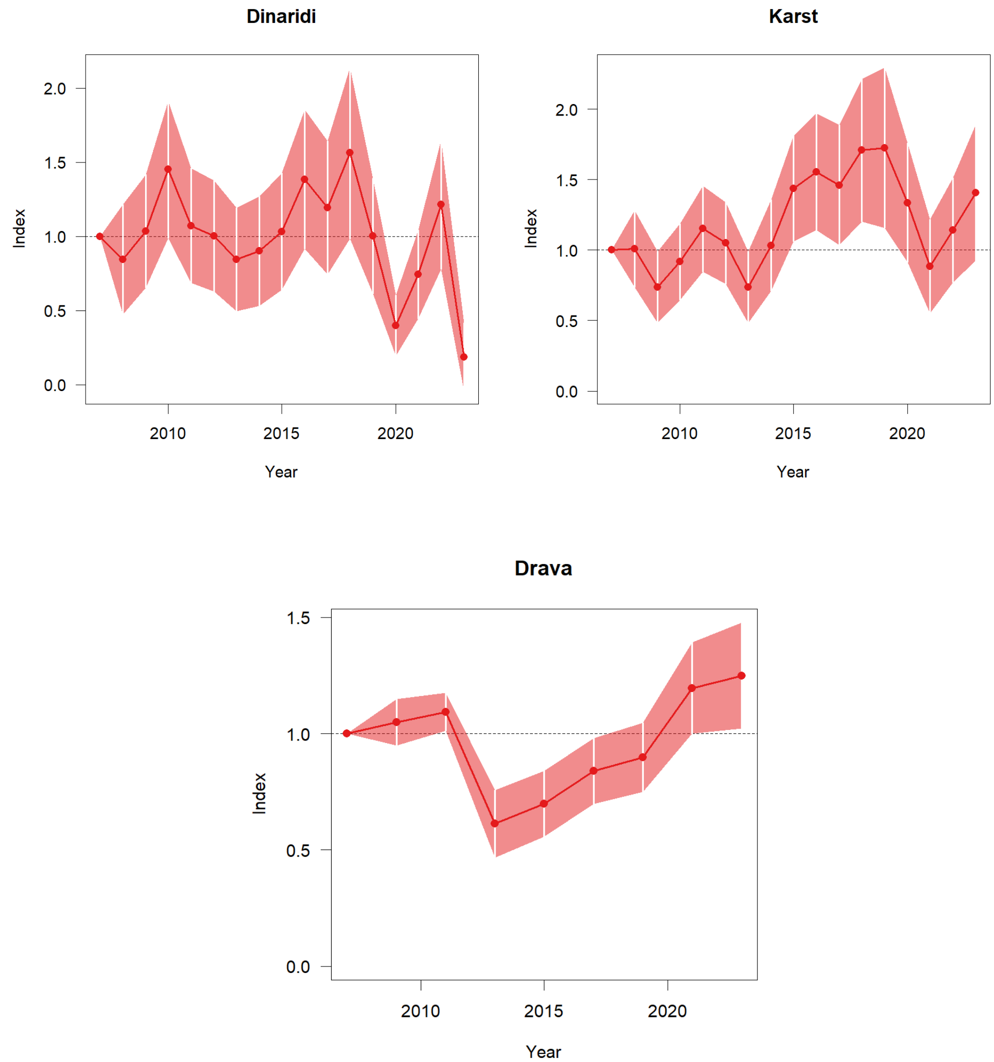

Figure 4*:* Models of population dynamics of *Carabus variolosus* in the regions of Dinarids, Karst and Drava.

Trends according to population size

A total of 41 populations were classified as small, while only two were identified as large, with the remaining two classified as medium. The calculated trends indicated a stable trend for small populations, whereas trend for medium populations was uncertain, and a moderate decline in larger populations (Table 3, Figure 5). The Wald test indicates that the trend deviations from linearity are marginally significant for the medium population (Wald = 12.14, p=0.06).

Table 3. Modelled annual population trends of *Carabus variolosus* populations in different sized populations in Slovenia.

	Large populations	Medium populations	Small populations
No. data points	11	16	308
Goodness-of-fit (Chi-square, p)	1.04, p > 0.05	4.99, p > 0.05	188.43, p > 0.05
Likelihood ratio (LR, p)	0.65, p > 0.05	5.92, p > 0.05	241.47, p > 0.05
AIC	-11.35	-8.08	-260.53
Overdispersion	0.1736	0.7125	0.7507
Annual change rate [%]	-7.53	- 3.37	- 0.34
Category	Moderate decline	Uncertain	Stable

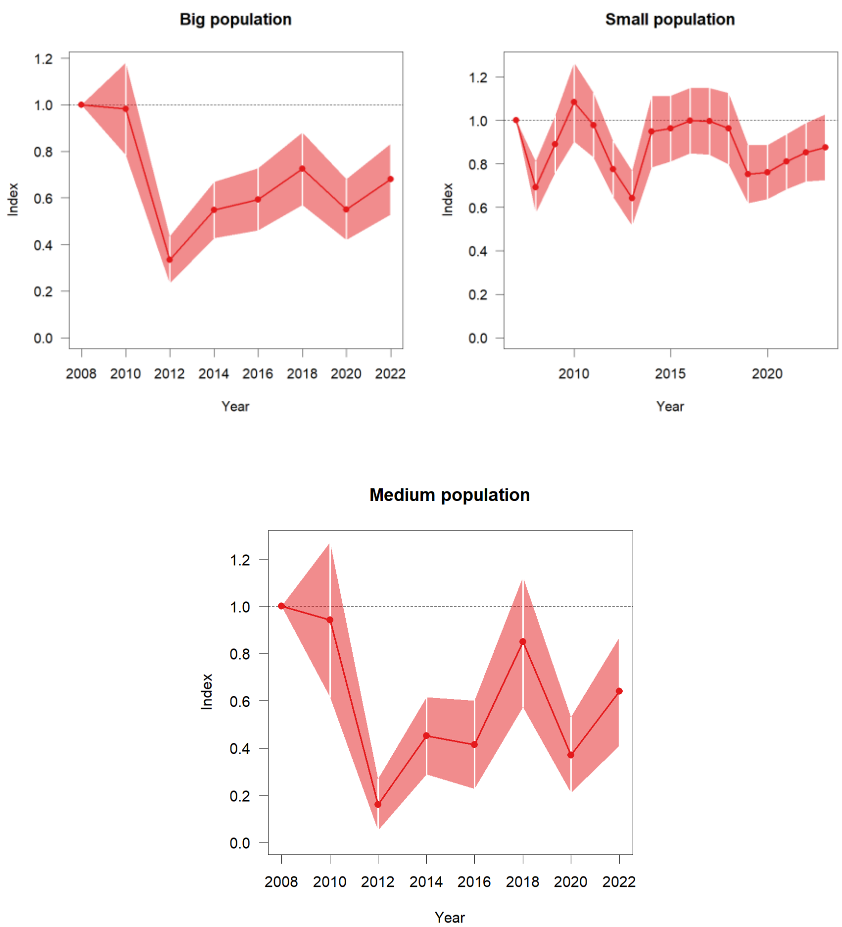

Figure 5: Models of population dynamics of *Carabus variolosus* in large, medium and small populations.

Trends according to the level of protection (Natura 2000)

The analysis of both the Natura 2000 and non-Natura 2000 sites models demonstrates that the population trends are overall stable (Table 4).

Table 4. Modelled annual population trends of *Carabus variolosus* populations within and outside Natura 2000 sites in Slovenia.

	Natura	**No Natura**
No. data points	318	466
Goodness-of-fit (Chi-square, p)	161.1, p>0.05	142.8, p>0.05
Likelihood ratio (LR, p)	194.3, p > 0.05	180.1, p > 0.05
AIC	-209.7	-201.9
Overdispersion	0.797	0.748
Serial correlation	0.299	0.08
Annual change rate [%]	+0.69	+0.51
Category	Stable	Stable

Trends according to the level of disturbance

Population trends for sites subject to and not subject to disturbance demonstrated generally stable population trends (Table 5)

Table 5. Modelled annual population trends of *Carabus variolosus* populations high- and low-disturbed sites in Slovenia.

	High disturbance	**Low disturbance**
No. data points	123	93
Goodness-of-fit (Chi-square, p)	39.9, $p > 0.05$	37.8, $p > 0.05$
Likelihood ratio (LR, p)	48.2, $p > 0.05$	46.6, $p > 0.05$
AIC	-159.8	-107.4
Overdispersion	0.3840	0.491
Serial correlation	0.269	0.391
Annual change rate [%]	-0.12	+0.62
Category	Stable	Stable

Range dynamics

A total of 16 locations (26.2%) the species was absent in the first and appeared in the second period, while just nine locations (14.7%) demonstrated disappearance in the second period. In 98 sites, no presence was recorded in any of the periods, and 36 locations (22.6%) registered at least one presence in both periods.

Body condition

The strongest body measure correlated with mass was found to be the whole length ($r = 0.68$, $p < 0.0001$) closely followed by elytra length ($r = 0.67$, $p < 0.0001$), with a regression slope of 2.8. Therefore, for the calculation of the SMI, measures of the whole body were used. It was therefore calculated as follows:

$$SMI = M_i \times (29.02/L_i)^{2.8}$$

The linear mixed model revealed a statistically significant influence of sex on body condition (Table 6) whereas the level of disturbance exhibited no statistically significant influence. However, the level of disturbance did not show a statistically significant impact. The interaction between sex and disturbance indicated that males in non-disturbed locations exhibited a modest increase in body condition (Table 6). The random effects analysis showed minimal variability across locations and dates, with no significant variability attributable to the unique identifier of the individual (Table 7). The ANOVA confirmed the significance of sex ($F = 6.17$, $p < 0.05$), while disturbance was found to have a negligible effect ($F = 0.20$, $p > 0.05$). Furthermore, the interaction between sex and disturbance was found to be moderately significant ($F = 3.57$, $p \approx 0.06$). Only 0.73% of the variance is explained by the fixed effects, while 11.4% of the variance is explained when both fixed and random effects are included.

Table 6. Effects of fixed variables of GLMM in body condition index. The DHARMa nonparametric dispersion test shows no significant overdispersion in the model's residuals ($p > 0.05$).

Fixed variables	Estimate	Std. Error	t value
Sex	-0.023	0.007	-3.115
Level of disturbance	-0.007	0.016	-0.455
Sex x Level of disturbance	0.022	0.011	1.890

Table 7. Effects of random variables of GLMM in body condition index. The DHARMa nonparametric dispersion test in the model's residuals ($p > 0.05$).

Random variables	Variance	Std. Dev.
Location	0.0007	0.0264
Year	0.0005	0.0218
Residual	0.0098	0.0989

The Spearman correlation coefficient between SMI and density was -0.0406, indicating a very weak negative correlation between the variables. The linear mixed model evaluating the impact of body condition on density revealed that SMI had a negligible effect ($p > 0.05$). The fixed effects accounted for an extremely small amount of variance in density ($r2 < 0.0001$), indicating minimal explanatory power. However, the model including both fixed and random effects explained 51.6%

of the variance in density (r2 = 0.516). DHARMa nonparametric dispersion test showed no significant overdispersion (0.938) or misfit ($p > 0.05$).

Estimation of minimal number of required monitoring sites

The minimum sampling effort required for reliable population monitoring was determined to be four regular sites and thirteen years of continuous sampling, as shown in Tables 8 and 9.

Table 8. Variations in sampling efforts and their reliability across different sites (regular and periodical). No. S = number of successful iterations. Significant difference between sampling sites ($X^2 = 187.89$, p-value < 2.2e-16)

Regular	Periodical	No. S	95% CI	Reliability
2	0	0.61	[0.51, 0.71]	Not Reliable
3	0	0.87	[0.79, 0.93]	Not Reliable
4	0	0.98	[0.93, 1.00]	Reliable
2	2	0.46	[0.36, 0.56]	Not Reliable
1	3	0.15	[0.09, 0.24]	Not Reliable
3	1	0.67	[0.57, 0.76]	Not Reliable

Table 9. Variations in sampling efforts and their reliability across different years. No. S = number of successful iterations. Significant difference between sampling years ($X^2 = 42.95$, $p < 0.001$)

Years	No. S	95% CI	Reliability
10 years	38	[0.28, 0.48]	Not Reliable
11 years	71	[0.61, 0.80]	Not Reliable
12 years	81	[0.72, 0.89]	Not Reliable
13 years	100	[0.96, 1.00]	Reliable

DISCUSION

Our study is the first assessment of long-term and large-scale population trends of endangered carabid beetle *Carabus variolosus nodulosus* in its global population stronghold in Slovenia. As postulated by Günther & Assmann (2004b), the estimation of fluctuations in population abundance data requires the analysis of consecutive years, as insects often undergo large yearly fluctuations in population size (Tscharntke, 2007). Our study has followed these guidelines, and we revealed that overall population was stable in the period 2007-2023, but with marked regional differences with on one hand declining and on the other increasing local populations. This is consistent with the knowledge that species response can differ locally due to complex biotic and abiotic interactions (Schmeller et al., 2015).

In *Carabus variolosus nodulosus* we have found that population fluctuations and trends are locally specific and cannot be generalized. Although this study has not focused on the causes of the trend fluctuations, the decline of the population in Mura (easternmost region) could be explained by the geology of the region, with mainly clay, silt, and sand, while other regions are characterized by calcareous soils (Hrvatin et al., 2020). The sandy soils are known to present an acidic pH and low water retention (Huang & Hartemink, 2020), with a preference of *Carabus variolosus nodulosus* for soil with high moisture and circumneutral pH range (Matern et al., 2007). Mura region was also affected by high temperatures and low flows (Tadić et al., 2022). On the other hand, species population sizes appeared to be the largest in Mura region indicating that habitat conditions at least were very favorable for the species. Although this study did not include climate variables in its analysis, we cannot exclude climate change effects although negative climate change effects were not evident in other regions in Slovenia. In general, we found that larger populations were more prone to decline than smaller, what could have detrimental effects in the future although the species still have moderate local recolonization power as shown by our range dynamic data. It is widely acknowledged that small populations are typically the most vulnerable, due to the increased risk of inbreeding, genetic impoverishment and elevated extinction probability (Matern et al., 2007). However, the stability of these populations indicates that these populations are not significantly diminished, thereby precluding the possibility of genetic drift between them, especially in areas in extensive hydrological network. In examining the declining trend among larger populations, it is essential to consider the regional context. The Mura region initially recorded the highest population densities, which, when considered alongside the aforementioned reasons for the decline in Mura, may provide a potential explanation for the subsequent decline.

Our results have also shown that local population fluctuations are not dependent on local conservation measures or the effects of habitat disturbance. These findings were unexpected, given the well-documented recognition among conservationists of the growing concern surrounding human disturbance and its impacts on wildlife (Whitfield et al., 2008). However, the findings of evident patterns in the response to disturbances is frequently inconclusive or contradictory, particularly with regard to the impact of human disturbance on animal fitness parameters or population trends (Price, 2008). This could be explained by the incomplete understanding of the mechanisms linking animal responses to human disturbance, and the difficulty of a robust assessment of the effects (Tablado & Jenni, 2015; Pirotta et al., 2018). Therefore, in order to conclude that disturbance and conservation management had no or marginal effect on the population, a detailed study of the responses and influencing variables should be undertaken since we have also found that the species can quite efficiently recolonize previously disturbed areas, but we believe that this is possible only, where populations in hinterland are still vital, strong and possibly undisturbed.

Body condition data, however, had no power to interpret or forecast population dynamics in *Carabus variolosus*. Although it was expected to see a correlation between body condition and density, either positive (Kamimura et al., 2021) or negative (Landa & Skogland, 1995; Flajšman et al., 2017), the response of these two variables may differ under different factors (e.g. climate, competition, disturbances). Some studies affirm that intrinsic processes influenced local population density more than extrinsic factors, possibly due to the temporal lag in the response of population density to changes (Trzcinski & Reid, 2009), while individual body condition of beetles seems to be highly susceptible to environmental conditions (Villada-Bedoya et al., 2019).

Regarding to the factors tested for body condition it was anticipated that notable discrepancies would be observed between the sexes. This hypothesis was corroborated by the findings, with males generally having lower values compared to females, which can be attributed to the energetic costs associated with reproduction, leading to the accumulation of higher energy reserves in females (Rödel et al., 2015). With regard to the influence of disturbance, no discernible impact was discerned on this occasion, which can be attributed to the lack of understanding of perturbation responses as previously mentioned. The interaction between sex and disturbance, on the other hand, suggests that the effect of disturbance varies by sex, particularly benefiting males in non-disturbed environments.

Although *Carabus variolosus nodulosus* is strongly declining and endangered insect in Europe (Matern et al., 2007), its population is still stable in its population stronghold indicating strong and vital populations of the species, which might serve as a baseline for reintroductions and revitalization of populations in western and northern parts of its range.

Population monitoring perspectives

Understanding and managing population dynamics is fundamental to effective species conservation. While this study has included an examination of certain parameters, it is believed that the inclusion of additional factors may assist in elucidating the underlying causes of population fluctuations. Therefore, we have proposed a list of factors for inclusion in future studies:

(1) The age of the individuals is an important parameter for studying the survival rates as a ratio between fresh (young) and old imagoes and to monitor fertility and mortality factors in the population (Tyndale-Biscoe, 1984). Although it is not straightforward to ascertain in beetles, a number of methods have been proposed. These include measuring the water content, which has been observed to decrease with increasing age (Perez-Mendoza et al., 2004), checking the accumulation of follicular relics in females (Grodowitz and Brewer, 1987), examining the differences between the cuticular hydrocarbon patterns of different development stages (Steiger et al., 2014, Kartika et al., 2021) or using techniques of mark–release–recapture like thoracic notching (Guzman et al., 2012).

(2) Environmental parameters of the habitat, such as water pH, soil moisture content, soil organic matter content and leaf litter, can determinate habitat quality and they have been found to influence carabid abundance and diversity and they could be a measure of habitat quality (Vician et al., 2018).

(3) Climate variables should also be considered when studying the population trends of this species, due to its highly hygrophilous condition (Matern et al., 2007). Furthermore, these variables can assist in establishing the environmental threshold of the species, with the aim of

predicting the potential impact of climate change on future population dynamics (Tyszecka et al., 2023)

(4) The physiological and behavioral responses of individuals to a known or potential stressor (disturbance) warrant further study, given *Carabus variolosus'* heightened sensitivity to such perturbations, acting as a bioindicator for future environmental stressor (Makwela et al., 2023; Chowdhury et al., 2023).

(5) The genetic structure of *Carabus variolosus* is currently a topic of debate. Some studies have identified an exceptional level of genetic divergence even at close distances, and the existence of five distinct genetic clusters within the species, although the causes of this remain unclear (Matern et al., 2009; Mossakowski et al., 2020). In the sense of monitoring, it is thus important to monitor all relevant genetic lineages, which may occur in the population since there are significant differences in population trends in local populations as shown within this study.

(6) Finally, the examination of additional bioindicator species within the habitat of the study species can facilitate a more comprehensive understanding of habitat quality and the formulation of more effective conservation strategies (Bonada et al., 2006). In this instance, we propose the inclusion of two additional species of European conservation concern that coexist with *Carabus variolosus*: the Stone Crayfish (*Austropotamobius torrentium)* and the Balkan Goldenring (*Cordulegaster heros*) (Klobučar et al., 2013; Boda et al., 2015) in comprehensive monitoring scheme of small forest waterbodies.

Population conservation perspectives

In light of the findings of our study, we put forth a series of guidelines for future monitoring programs. We consider that is of the outmost importance to first identify the genetic unit under study, in order to gain a full understanding of the genetic structure and taxonomy of the species in question and ascertain whether any potential subspecies or cryptic species may have been overlooked.

Regarding the sampling strategy, we recommend that sampling should extend beyond merely assessing species abundance. It should also include data on age and sex distribution within the population. Understanding these demographic factors is key to evaluating the impact of various threats on different segments of the population. Age data, in particular, could enable the calculation of survival rates important for assessing threat factors at certain developmental stages of the species.

This study has also highlighted the significance of a long-term monitoring commitment (Council Directive 92/43/EEC). While the IUCN criteria specified 10 years for assessing rates of change, (IUCN, 2024) some authors affirm that it is essential making time series longer than 15 years to detect underlying trends (Pollard et al. 1995; Thomas, 2005).

Our results align more closely with the latter statement, as it was found that at least 13 years of population monitoring are required to assess reliable population trends. Regarding sampling sites, only the configuration with 4 regular sites achieved a proportion of success of 0.98, while the remaining configurations failed to satisfy the 95% reliability threshold. At each site, it is recommended that 15 traps be deployed over a two-day period, resulting in a total of 30 trap-nights per sampling site. The sampling sites should be defined in advance, taking into account the geographical extent of the species' habitat, genetic units and the scale of the study (national, regional or local).

Finally, the limited insights afforded by body condition have been demonstrated. It is therefore proposed that further time and resources be allocated to the addition of further sites in regions that are currently under-represented, rather than to the recording of measurements, which have not yielded any significant results useful for conservation measures of this species.

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