The utility of non-genetic data collected during genetic monitoring of proteus populations

Uporabnost ne-genetskih podatkov, pridobljenih med genetskim monitoringom močerila

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Proteus anguinus is the only obligate cave vertebrate of the European continent (Aljančič et al. 1993, Parzefall et al. 1999) and a Dinaric Karst endemic (Sket 1997). In the past decades, population declines of this charismatic species have been reported from a number of localities (Sket 1997, Hudoklin 2011). However, only recently initiatives have been undertaken to develop and establish schemes for monitoring population numbers and distribution. As a part of these initiatives, various new methodological approaches have been tested: a) *in situ* underwater animal tagging (Balázs et al. 2015), b) detection via environmental DNA (Aljančič et al. 2014, Gorički et al. 2017, Vörös et al. 2017), and c) genetic monitoring (Trontelj & Zakšek 2016).

Genetic monitoring as described in Trontelj & Zakšek (2016) requires catching substantial numbers of live proteus. In order to maximize the amount of scientific information obtained once an individual is caught, besides DNA, a number of other biological data are collected. These include body length and mass as well as observations on anomalies such as injuries, pigmentation, gravidity, parasites, etc. Body size can be used to assess population demography and, conjointly with data on injuries and parasites, even health of populations. Taking into account possible fixed differences in body size between proteus phyletic lineages, ecological conditions of cave systems can be inferred. Long term monitoring of animals' body size reveals life-history traits, which have been so far investigated mostly in captive individuals bred in cave laboratories (e.g. Briegleb 1962, Durand & Delay 1981, Juberthie et al. 1996, Aljančič & Aljančič 1998). In this contribution, we present first results based on non-genetic data obtained during the fieldwork for the genetic monitoring of proteus.

In 2015 and 2016, we extensively sampled proteus in several Slovenian caves. These were selected to represent populations from all major mitochondrial DNA lineages of proteus (Trontelj et al. 2009). Each year, we caught about 900 individuals, most of them in the Postojna-Planina Cave System. This is probably the largest dataset ever assembled for natural populations of proteus. Animals were caught using diving equipment and hand nets. First, a DNA sample was non-invasively taken from each individual using a skin swab. Then, body length and mass were measured, the animal was thoroughly examined for anomalies, photographed, and released back to the cave river.

The demographic structure of populations was tentatively assessed by estimating the proportion of reproductively capable individuals in a population. Relying on data from proteus bred in captivity (Durand & Delay 1981, Juberthie et al. 1996, Voituron et al. 2011), we assumed that 20 cm long animals are at least 18 years old and almost certainly already sexually mature. According to preliminary results, the proportion of reproductively capable individuals differed between our samples from different proteus populations. For example, in the Črna jama population we caught almost exclusively sexually mature animals, while in the cave Vetrovna jama pri Laški kukavi these represented only about one quarter of all individuals found.

The relationship between body length and mass was statistically modelled using a power function (see legend to Fig. 1 for more details). Preliminary results on the length-to-mass relationship in four proteus populations for which we had roughly similar sample sizes are shown in Fig. 1. Substantial differences were found between some of these populations. The populations from the subterranean Pivka and Reka Rivers differed most. At the maximal length of about 30 cm, animals

from the subterranean Reka River were almost twice as heavy as animals from the subterranean Pivka River. Whether this difference is genetically determined or the consequence of differences in food abundance and quality between cave systems, remains to be investigated.

Some individuals, whose mass was much lower than expected for animals of their length, were found to be infested with parasites. If a connection between infestation and low body mass is confirmed by additional cases, such outliers could be used to estimate population health. In Planina Cave, about 6% of individuals had some minor body injuries, mostly cuts in the tail fin and missing toes. This percentage was similar in both years of sampling, as was the percentage of individuals with at least some darkly pigmented patches of skin (about 7%).

Genetic identification of captured individuals provides an opportunity to assess changes in body size. Within a year, none of the 73 so far identified recaptured individuals from Planina Cave has grown more than one centimeter in length. Their change in mass was much more variable, showing increase and decrease of up to 8 g. As body mass can markedly change already after feeding or defecation, this variability needs to be taken into account before making conclusions about changes in body mass.

Taken together, the fieldwork undertaken in our genetic monitoring scheme produces, as a side product, a number of additional data that are relevant for the biology and conservation of this unique and enigmatic amphibian.

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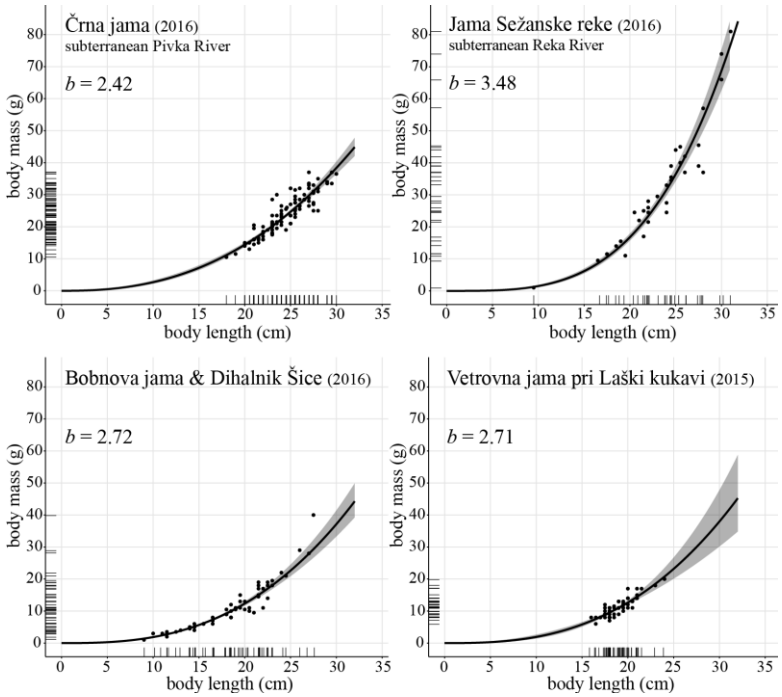

Figure 1. Relationship between body length and mass in four populations of *Proteus anguinus*. The power function $M = a L^b$ (M – mass, L – length, a – intercept, b – slope) was linearized and fitted to the log-transformed data. When length increases by 1%, mass increases by b%.

Slika 1. Odnos med telesno dolžino in maso pri štirih populacijah močerila (*Proteus anguinus*). Odnos opisuje potenčna funkcija $M = a L^b$ (M – masa, L – dolžina, a – presečišče, b – naklon), ki smo jo najprej linearizirali, nato pa ocenili vrednosti parametrov a in b za logaritemsko transformirane podatke. Pri povečanju telesne dolžine za 1 % se masa poveča za b %.