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MORPHOPHYSIOLOGICAL INDICES OF INTERNAL ORGANS OF THE DICE SNAKE *NATRIX TESSELLATA* (REPTILIA, COLUBRIDAE) OF THE DNIEPER RIVER IN THE STEPPE

We studied morphophysiological indices of organs of the dice snakes, *Natrix tessellata* (Laurenti, 1768), from three populations in the Steppe Zone of Ukraine along the Dnieper River. The specimens were collected in 2013–2016 in three areas: Prydniprovska Thermal Power-Station (Dnipro City), the National Nature Park "Velykyi Luh", Zaporizhia region, and Majorova Balka (the ravine closed to Majorka village, Dnipro district, Dnipropetrovska region). Studied biotopes differ in some abiotic parameters and the level of anthropogenic load. Significant differences in kidneys and liver indices between sexes were noted. The relative mass of lung and heart diverges between animals from different studied sites.

K e y w o r d s: reptiles, organ relative mass, morphophysiology, sexual dimorphism.

Introduction

It is known that a complex of environmental factors influence on morphophysiological processes in living organisms (Shvarts et al., 1968). Environmental pollution is deemed as one of powerful factors of such morphophysiological alterations (Burraco et. al. 2016).

Several studies have shown that the relevant morphophysiological index can be used as a bioindication parameter. At the same time, it is necessary to take into account the ecological features and intersexual specificity of species. The variability of morphophysiological characteristics of poikilothermic animals is known to be higher than in homeothermic ones (Schwartz et al., 1968, Kruglova, 2011). Such types of studies were practically not conducted on snakes.

Dice snake *Natrix tessellata* (Laurenti, 1768) is one of the most common reptile species in the Dnieper valley within the Steppe zone (Bulakhov et al., 2007). Under conditions of strong anthropogenic

influence *N. tessellata* populations are able to maintain their numbers, but, at the same time, they acquire a number of morphological, physiological and other traits (Yermolenko et al., 2016). Our study provides further information on animal biodiversity as an element of natural and disturbed ecosystems as well as of the ecosystem services.

Materials and methods

Our studies were carried out in August–September, which is non-reproductive period for the local snake species, in 2013–2016. We studied adult specimens of *N. tessellata* from three populations, which occupied ecosystems of the Dnieper River's banks differentiated by anthropogenic load. The specimens were collected in the biotopes adjoining Prydniprovska Thermal Power Station (48.400716 °N 35.113721 °E, 7 males and 5 females (1)), in the ecosystems of the National Nature Park "Velykyi Luh" (47.447652 °N 35.133827 °E, 21 males and 9 females (3)), and in Majorova Balka (the ravine closed to Majorka village, Dnipro district, Dnipropetrovsk Oblast, 48.262769 °N 35.169007 °E, 20 males and 18 females (2), fig. 1). Deposited sulfur dioxide, nitric oxides, carbon oxides and dust particles affect the biotopes closed to Prydniprovska TPS. The TPS discharges about 1,055,747 thousand m^3 of liquid wastes into the Dnieper River annually (DTEK, 2015). As a result, the water in close vicinity of the TPS is polluted with petrochemical products and phosphates. Their levels exceed maximum permissible concentrations 5–6 times (Choban, Choban, 2008; Kroyik, Patskova, 2011; Striletz, 2014).

There are no significant differences in hydrochemistry between studied biotopes. They have purely comparable water concentrations of humic and fulvic acids (Osadcha, 2012) as well as other parameters (Hydrology, 1989; Stas, Kolesnyk, 2016).

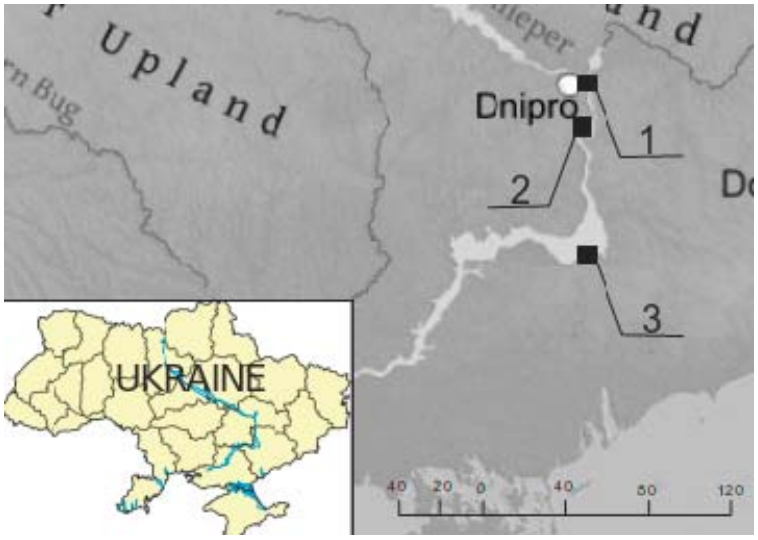


Fig. 1. Location of the studied habitats along the Dnieper River. Explanation is in the text.

For morphometric analysis, we studied the dice snakes' internal organs, which actively participate in metabolic processes, notably liver, lung, kidneys and heart. The relative weight (index) of the organs was calculated by the following formula:

$$C = \frac{P_1}{P_0} \times 1000\ (‰),$$

where C is the relative weight of the organs; P_1 is the weight of the organ (g); P_0 is the weight of the animal body (g) (Schwartz et al., 1968).

The research was carried out according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, 1986 with Amendments, 2005, and 2009.All the experiments were performed under the supervision and permit of the Committee on Bioethics of Oles Honchar Dnipro National University. The captured snakes were anesthetized and used in a wide spectrum of different physiological and biochemical studies.

The differences between the studied samples were evaluated using a multifactor analysis of variance (MANOVA) performed in the Statistica 10 (StatSoft, Inc., the USA, 2011). The sex, habitat and their combined effect were used as factors for the analysis. The differences were considered as significantif $P < 0.05$.For each characteristic, the diagrams demonstrate the average values (small square in the center of the rectangle and horizontal lines for each population as a whole), the first and third quartiles (upper and lower bounds of the rectangle), 95 % confidence interval (vertical lines above and below the rectangle), outliers (shown by asterisks or small circles).

Results and discussion

Liver and kidneys are multifunctional organs that perform protective processes in an organism of vertebrates. It is assumed that the more toxic substances enter the organism and the longer their influence, the higher the liver index should be (Misyura, Marchenkovskaya, 2007). On the other hand, it is considered that intensifying metabolites excretion from the body can cause growing kidneys relative weight (Thammachoti et. al, 2012). The strengthening of exertion of an organ that is capable of growth should affect its morphophysiological index.

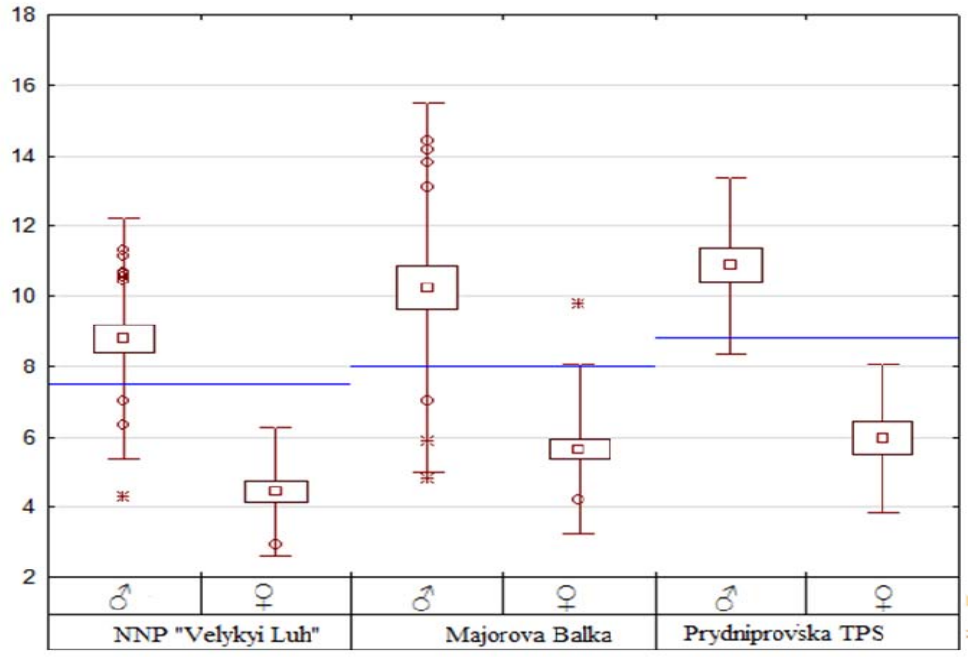


Fig. 2. The liver index of *N. tessellata* from the studied populations.

Table 1. **Multifactor analysis (MANOVA) of morphophysiological indices in dice snakes from studied populations**

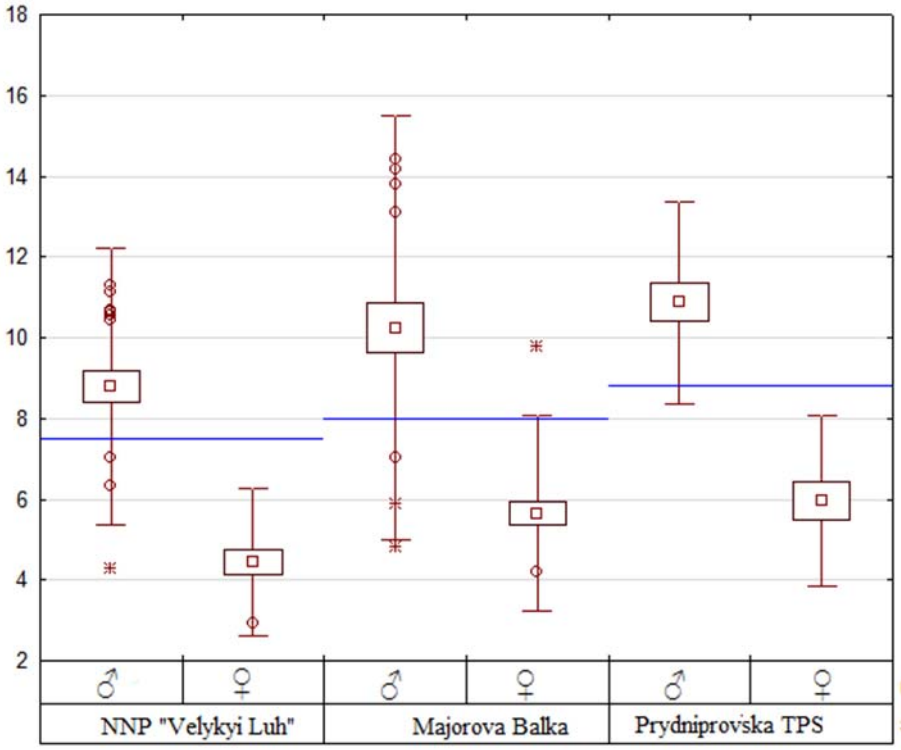
Organ	Factor	Sum of squares	Number of degrees of freedom	Mean square	F-criterion	P
Liver	ecosystem	278,1	2	139,1	1.08	0,34
	sex	564,4	1	564,4	4.39	0,04
	ecosystem × sex	492,5	2	246,3	1.91	0,15
Lung	ecosystem	465,1	2	232,6	14.7	4.11×10^{-6}
	sex	36,1	1	36,1	2.27	0,13
	ecosystem × sex	153,6	2	76,8	4.84	0,01
Right kidney	ecosystem	12,1	2	6,03	1.76	0,18
	sex	234,8	1	234,8	68.5	$1,00\times10^{-7}$
	ecosystem × sex	3,27	2	1,64	0.48	0,62
Left kidney	ecosystem	21,8	2	10,9	3.10	0,06
	sex	259,1	1	259,1	73.8	$1,00\times10^{-7}$
	ecosystem × sex	0,21	2	0,11	0.03	0,97
Heart	ecosystem	21,1	2	10,5	8.98	$3,26\times10^{-4}$
	sex	0,66	1	0,66	0.56	0,46
	ecosystem × sex	8,93	2	4,47	3.81	0,03

The results of a multifactor analysis of the studied organs' indices are presented in the tabl. 1.

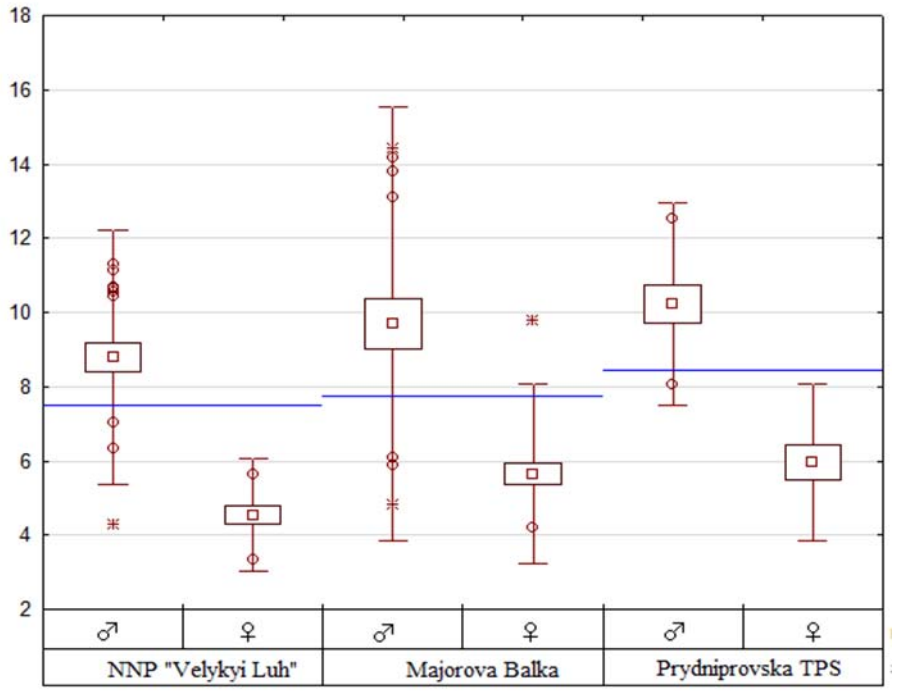
The liver index was not significantly affected by the ecosystem factor, but the differences between sexes were revealed (fig. 2). Bonnet et al. (1998) reported the intersexual differences in relative mass of different organs in three snake species. The sexual dimorphism in liver index was different in studied species. In spite of heavier liver in females of *Vipera aspis*, the other species (*Elaphe*sp. and *Coluber*sp.) had no such differences. Presumably, other snake species may have distinct ratio of the liver index between sexes.

It should be noted that the tendency in increasing the liver index of snakes from polluted site was observed. That was proved bystudies on amphibian and small mammal populations from contaminated biotopes (Misyura, Zalipukha 2006, Zemlianoy, 2007).

Dice snakes in all the studied populations have the indices of both left and right kidneys in males higher than in females, which is shown in fig. 3 a and 3 b. The differences is proved by probability value of 1×10^{-7} for both kidneys (tabl. 1).



3 a



3 b

Fig. 3. The kidney indices of *N. tessellata* in the populations from the studied areas (3 a — right kidney, 3 b — left kidney).

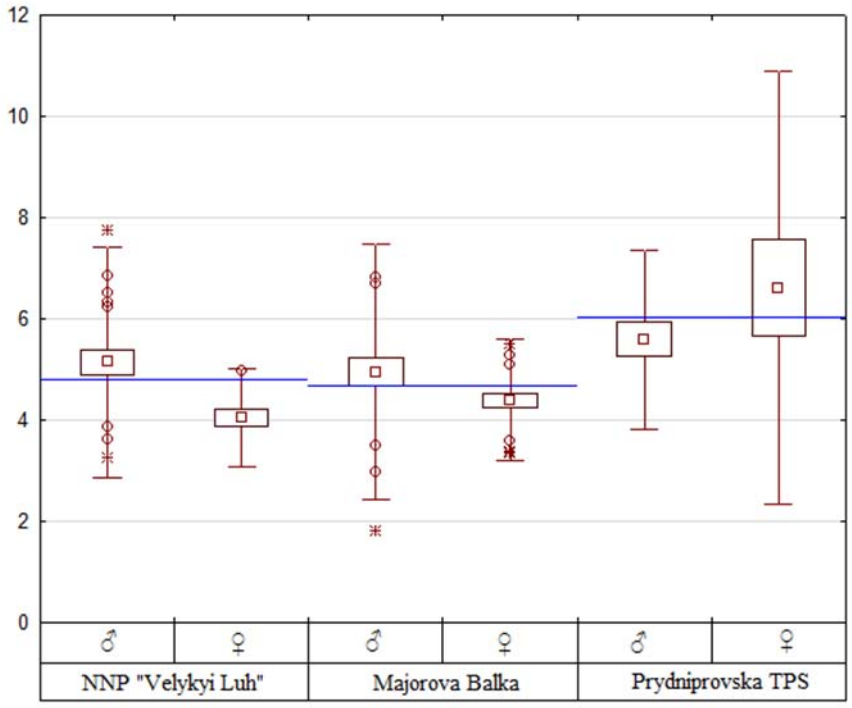


Fig. 4. The heart indices of the *N. tessellata* in populations from the studied areas.

Fig. 5 shows the same differences. The population in the vicinity of Prydniprovska TPS has the highest average index of the lung.

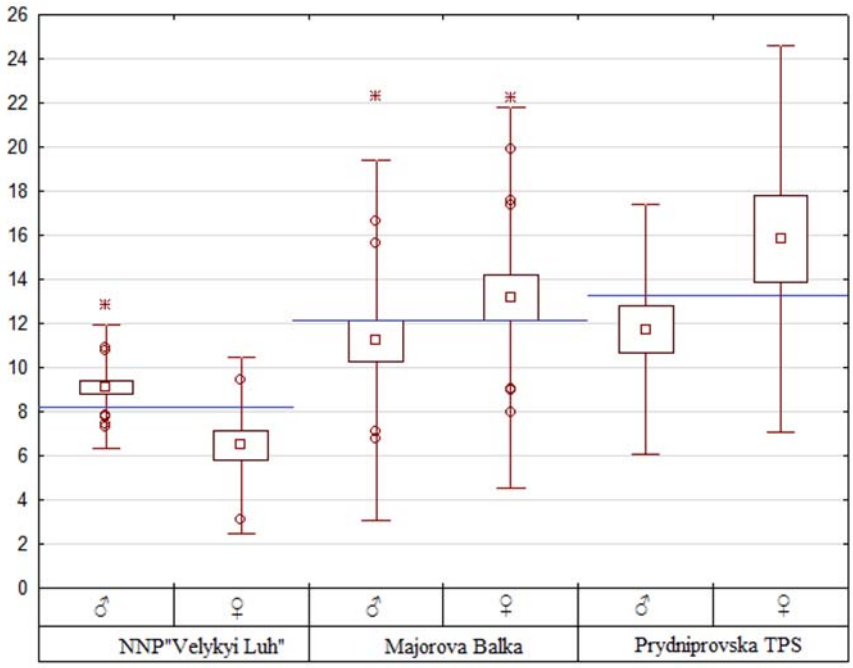


Fig. 5. The lung indices of *N. tessellata* from the studied populations.

The same kind of differences was found in other snake species (Bonnet et al., 1998) The MANOVA showed no statistically significant differences in kidney indices between the habitats as well as under combined ecosystem × sex factor. Despite the fact that ecosystems did not demonstrate their effect on the kidney indices, the slight tendency in increase of that index in snakes from polluted biotopes was also noticed. The ageing could not cause any differences between genders as no involution of organs was found (Petter-Rousseaux, 1953).

Morphophysiological index of a heart is usually associated with the intensity of energy expenditure of an organism in the process of locomotor activity (Shvarts et al., 1968; Sychev, Georgiev, 2005). The populations of *N. tessellata* inhabited both the NNP "Velykyi Luh" and Majorova Balka are characterized by the lower average values of the heart index in comparison with the animals from polluted site closed to Prydniprovska TPS (fig. 4). According to multifactor analysis, the relative weight of the snake heart in a population depends both on the habitat ($P \sim 3 \times 10^{-4}$)and on the combined effect of ecosystem and gender ($P = 0{,}03$) (tabl. 1).

The increased liver and lung indices in the snakes from the ecosystem closed to Prydniprovska TPS can be probable entailed by specificity of the site. The biotope is characterized by 10–30 meters deep. The other studied river parts are only of 2–5 meters. Moreover, just around the TPS the high current velocity and turbulence due to water discharge were observed. *N. tessellata* is predominantly ichthyophagous (Bulakhov et al., 2007) and in the studied area prefers gobies mostly. These both factors demand much physical efforts for movement from the bottom to the riverside (e.g., for swallowing) and back. Probably, it is logical to assume that industrial pollution may cause the decrease in number of feedstuff species of the dice snakes. That should make a valuable contribution to the energy costs and underwater time for trophic activity with the following increasing of the heart and lung indices. However, the dice snakes feed on the short-cycle fish mostly. The number of such fish (gobies, etc.) changes quickly and their populations are able to restore fast (Bulakhov et al., 2008). Thus, the factor of feedstuff number needs further research.

Besides, according to the literature data the invasion of parasites causes inflammatory processes and histological disturbances in the affected organs (Nurtazin et al., 2012). It can entail a change in morphophysiological indices of relevant organs. That issue requires further studies of the consequences of intensity and localization of parasitic infestations in snakes.

Conclusions

Dice snakes in studied populations are characterized by sexual dimorphism in relative mass of liver and kidneys. Significant differences in heart and lung indices were found between populations (ecosystem factor). These morphophysiological parameters are higher in snakes from the biotopes subjected to the Prydniprovska Thermal Power Station influence

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МОРФОФИЗИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ВНУТРЕННИХ ОРГАНОВ ВОДЯНОГО УЖА, *NATRIX TESSELLATA* (REPTILIA, COLUBRIDAE), РЕКИ ДНЕПР В ПРЕДЕЛАХ СТЕПНОЙ ЗОНЫ

Изучены морфофизиологические характеристики органов водяного ужа *Natrix tessellata* (Laurenti, 1768) из трех популяций, обитающих в условиях степного Приднепровья. Материал собран в период 2013–2016 гг. на территории, прилегающей к Приднепровской ТЭС (г. Днипро), в биотопах Национального природного парка «Великий Луг» (Запорожская обл.) и Майоровой балки (с. Майорка, Днипровский район, Днепропетровская обл.). Исследования проводились в биотопах с разными абиотическими условиями и антропогенной нагрузкой. Выявлены достоверные межполовые отличия индексов почек и печени. Относительный вес легкого и сердца различался у животных из разных мест обитания, в которых проводились исследования.

К л ю ч е в ы е с л о в а: пресмыкающиеся, относительный вес органа, морфофизиология, половой диморфизм.

С. В. Єрмоленко, Г. М. Гагут, В. Я. Гассо

МОРФОФІЗІОЛОГІЧНІ ОСОБЛИВОСТІ ВНУТРІШНІХ ОРГАНІВ ВОДЯНОГО ВУЖА, *NATRIX TESSELLATA* (REPTILIA, COLUBRIDAE), РІКИ ДНІПРО В МЕЖАХ СТЕПОВОЇ ЗОНИ

Вивчено морфофізіологічні характеристики органів водяного вужа *Natrix tessellata* (Laurenti, 1768) з трьох популяцій, що мешкають в умовах степового Придніпров'я.

Дослідження проводилися в біотопах з різним рівнем антропогенного навантаження. Матеріал зібраний в період 2013–2016 рр. на території, прилеглій до Придніпровської ТЕС (м. Дніпро), в біотопах Національного природного парку «Великий Луг» (Запорізька обл.) і Майорової балки (с. Майорка, Дніпровський район, Дніпропетровська обл.). Дослідження проводилися в біотопах з різними абіотичними умовами і антропогенним навантаженням. Виявлено достовірні міжстатеві відмінності індексів нирок і печінки. Відносна вага легені і серця відрізнялася у тварин з різних місць проживання, в яких проводилися дослідження.

К л ю ч о в і с л о в а: плазуни, відносна вага органу, морфофізіологія, статевий диморфізм.