Rocznik Świętokrzyski. Ser. B – Nauki Przyr. 34: 65–75, 2013

Polska Akademia Nauk – Oddział w Krakowie,
Kieleckie Towarzystwo Naukowe

Assessment of the use of *Triopis* in the control of atmospheric environment

Możliwości wykorzystania przekopnic do kontroli środowiska atmosferycznego

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Streszczenie. Obiektem prowadzonych badań była przekopnica australijska (*Triops australiensis*) należąca do skorupiaków z gromady skrzelonogów (*Branchiopoda*) rzędu tarczowców (*Notostraca*).

Cel badań to analiza dynamiki liczebności populacji przekopnicy w zróżnicowanych warunkach środowiskowych wynikających ze zmian w składzie chemicznym wody. Badania rozwoju przekopnic w wodzie o zróżnicowanym składzie chemicznym mają dowieść ich wartości bioindykacyjnych i wykazać znaczenie tych skorupiaków w sezonowej ocenie stanu środowiska atmosferycznego. Środowisko atmosferyczne wpływa na kwasowość opadów, co przekłada się na skład chemiczny kałuż podeszczowych – środowiska życia przekopnic.

Skorupiak ten występuje w zbiornikach wodnych okresowo wysychających. Larwy w czasie suszy przechodzą w stan anabiozy, tworząc cysty. Przerwanie tego stanu następuje w momencie występowania opadów deszczu. Na ontogenezę tych skorupiaków wpływają takie czynniki, jak: dostępność wody i jej pH, oraz temperatura. Wyniki badań wykazały, że najszybszy rozwój obserwowany jest w wodzie lekko zasadowej (pH > 7), optymalne warunki termiczne to 14–20°C. Wzrost stopnia zanieczyszczenia powietrza atmosferycznego powoduje zakwaszenie gleby i obniżenie pH wody, w której cystują przekopnice. W zależności od kwasowości środowiska przebieg ontogenezy odbywa się bez zakłóceń, zostaje spowolniony lub całkowicie ustaje. Zbyt duże nagrzanie wody powoduje zmianę warunków, co również wpływa na spadek liczebności populacji i wydłużenie stanu anabiozy cyst.

Słowa kluczowe: skład chemiczny wody, środowisko atmosferyczne, przekopnice australijskie, cysty.

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INTRODUCTION

Bioindication describes changes in the environment through the use of biological indicators. It is one of the basic methods used in biological monitoring. It makes use of specific reactions of organisms to the factors which limit physiological and biochemical reactions of cells, as well as sensitivity of indicative plant and animal species, sensitivity of the population and biocoenose. The use of bioindication to control the condition of the environment is one of the most modern study methods. As this method uses living organisms, the main argument in favour of it is its high effectiveness, the possibility of quick interpretation of environmental changes on the basis of the reaction of the used bioindicator organisms, the opportunity to take protective measures, as well as cost-effectiveness of studies. If the bioindicator reacts to a toxin, it is an alarm that warns against contamination and makes it possible to assess the toxicity of a given pollutant. Contrary to some physical and chemical methods that only allow to detect the substance we expect to find, bioindicators react to the presence of such substances. Due to the fact that a great number of environmental toxins work synergically, we can assess the total toxicity of all harmful substances. Until now, there have not been constructed instruments which would enable the study of growing negative impact of pollutants. Only living organisms make it possible to assess the growing accumulation of toxins in the studied samples. The reaction of a given organism and advancement of changes serve as the basis for determining biological activity of a given system. Ecology treats bioindication as a method to assess the condition of ecosystems. It helps to determine the capacity of ecosystems against toxins and assess the interactions as well as cause and effect relations between toxins and between a given toxin and the environment. Bioindicators have been introduced in numerous countries as part of routine studies. The organisms used to assess the environment must have specific features, i.e. wide spreading, low mobility, availability all year long, easy sample collection, high pollutant concentration ratio and sensitivity to toxins. Such organisms include, among others, the semaphore crab (*Heloecius cordiformis*). The studies on this crustacean made it possible to isolate heavy metals from its internal organs. Another example is the noble crayfish (*Astacus astacus*) – which is sensitive to pH changes; it is a fresh-water species of decapoda order (*Decapoda*). Indicative crustaceans also include species from the *Artemia*, genus, e.g. (*Artemia salina*), the bioindicator of salt lakes (Lowry, 2013) mysidae of *Mysidacea*, order, detritophagous indicators occurring on the territory of Poland in the lakes: Lubie, Drawsko, Żerdno and Mamry (Engelhardt et al.,

1998) and species (*Hemimysis anomala* and *Neomysis integer*) of estuary water. *Asellus aquaticus* is used as a bioindicator in still water. It is a crustacean of isopoda order (*Isopoda*). It lives on organic remains and its development depends on water temperature. As a bioindicator, it is as good as the species listed earlier (Castiglioni, Bond-Buckup, 2007). *Daphnia pulex* is a proven species of bioindicator of chemical contamination of water. It is also a model organism for environmental genomics, which studies the interactions between organism genes and the environment (Anteau, Afton, 2008; Witt et al., 2006; Baldinger, 2004). Moreover, from all invertebrates sequenced so far, it shares the greatest number of genes with human beings. It is used e.g. in enzymatic tests, showing how quickly the bioindicator reacts to a given toxin.

The object of the studies is to analyse the dynamics of the size of *Triops cancriformis* population in diverse environmental conditions arising out of changes in chemical composition of water. The studies of *Triops cancriformis* development in water of diverse chemical composition are to prove their bioindicative value and the significance of these crustaceans in the seasonal evaluation of the condition of the atmosphere.

MATERIALS AND METHODS

The breeding of *Triops cancriformis* was performed three times, repeated every two months from April to September. The studies were conducted in laboratory conditions, in two faunaboxes of 10-litre capacity. The proper temperature was retained. The testing containers were filled with a 3 cm layer of fine-grained sand and water plants in order to introduce cysts, and then they were filled with municipal and rain water.

There were 60+-5 cysts in each faunabox. During both tests, the applied lightning followed the 24-hour cycle. Water temperature was controlled in 24-hour periods. We noted the time of the occurrence of larvae, the number of ecdyses, and the size of *Triops cancriformis* body after its final stabilisation. The breeding was maintained until the natural death of organisms. The cysts laid in the container were secured in order to use them for the next breeding. We measured the conductivity of water, pH, water hardness, and the content of iron, manganese and ammonium ions; we also compared the final size of *Triops cancriformis* in each breeding and the number of adult forms hatching from cysts with larvae.

In order to determine the birth ratio (R) of both larvae hatching from cysts and adult forms developing from larvae, we used a formula which determines the total number of born individuals and the total population,

$$R = \frac{b}{N}$$

where: b – number of offspring;
N – average size of population.

After each month, N value was calculated using the formula:

$$N = \frac{N_0 + N_t}{2}$$

where:
N_o – initial number;
N_t – final number (after time t) (Banaszak, Wiśniewski 2004).

CHARACTERISTIC OF THE OBJECT OF STUDIES

Triops australiensis crustacean of the family Triopsidae. The length of *T. armour* is up to 6.5. They occur in male, female and hermaphroditic form (Wilanowski 2007). The most popular populations are without male individuals, whose scutella are smaller, flatter and more round than those of females (Photo 1) and hermaphrodites. Females and hermaphrodites are similar to each other (Cothran, 2008, 2004; Cothran et al., 2010). The back of the body is covered with a carapace adherent only in the head part, with a pair of compound eyes and an naupulis eye and of visible dark green colour. This population is common in water tanks which become periodically dry (Väinölä et al., 2008). It multiplies by means of parthenogenesis. It nourishes with protozoa, *Chironomidae* larvae, daphnia, while larger individuals nourish with *Tubifex tubifex* and dead insects as well as water plants (Jura, 2004).

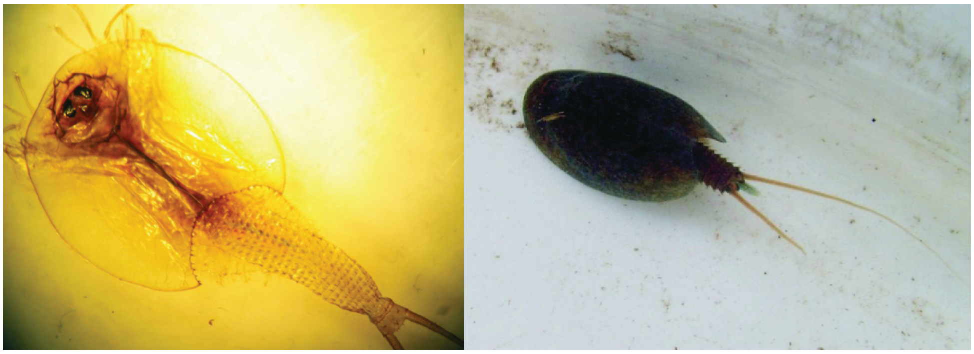

Photo 1. Female *Triops australiensis* from laboratory culture (Photo K. Sadko)

Fot. 1. Samice przekopnicy australijskiej (Triops australiensis) pochodzące z hodowli laboratoryjnej (fot. K. Sadko)

RESULTS

The analysis of average temperatures and the number of larvae hatching from cysts and adult individuals hatching from larvae points to a relation between the mentioned parameters. The drop of temperature of the breeding which used municipal water (Table 1) caused the increase of the number of hatching larvae of, respectively, 27, 21 and 41 individuals (Table 5). The increase of temperature of the breeding which used rain water (Table 1) did not have a significant influence on the number of hatching larvae and amounted to 56, 56 and 60 individuals respectively (Table 5).

Chemical analysis of water used in the experiment as regards pH, general hardness, electrolytic conductivity, the content of iron, manganese and ammonium ions shows that the values of these parameters are much higher in municipal water than in rain water (Table 2, 3). These factors influence the number of hatching larvae and the adult forms that survive the successive months of breeding (Table 5). It should also be emphasised that the average size of the individuals, measured from the tips of even furcae, achieved by imago forms was on average two times larger than in populations bred in rain water (Table 4).

Table 1. The average results of the measurement of water temperature in three successive breedings in municipal and rain water

Tabela 1. Średnie wyników pomiarów temperatury wody w trzech kolejnych hodowlach w wodzie wodociągowej i deszczowej

No of culture/*Nr hodowli* Environment / *Środowisko*	1	2	3
Average temperature in three subsequent cultures *Średnie temperatury w trzech kolejnych hodowlach* [°C]			
Waterwork water / *Woda wodociągowa*	14,3	13,9	13,6
Rainfall water / *Woda deszczowa*	11,3	11,9	12,2

Table 2. Physic-chemical analysis of rainfall water from three subsequent cultures

Tabela 2. Analiza fizykochemiczna wody deszczowej z trzech kolejnych hodowli

No of trial *Nr próby* Analysed parametre *Analizowany parametr*	Culture 1 *Hodowla 1*	Culture 2 *Hodowla 2*	Culture 3 *Hodowla 3*	Method *Metoda*	Acceptable value/ *Wartość dopuszczalna*
pH	7,9+-0,1	8,2+-0,1	8,3+-0,1	PN-90C-04540/01	6,5–9,5
Twardość ogólna *General hardness*	10 mg CaCO3/l	19 mg CaCO3/l	27 mg CaCO3/l	PN-ISO6059:1999	60–500
Przewodność *Conductivity*	8,29 µS/cm	8,91 µS/cm	9,09 µS/cm	PN-EN27888:1999	2500
Żelazo ogólne (Fe) *General iron*	15 µg/l	19 µg/l	21 µg/l	PNISO-6332:2001	200
Mangan (Mn)	< 50 µg/l	< 50 µg/l	< 50 µg/l	PN-92C-04590/02	50
Jon amonowy (A) *Amon ion*	–	–	–	PN-C-04576-4:1994p.6a	0,50

Table 3. Physic-chemical analysis of waterworks water from three subsequent cultures
Tabela 3. Analiza fizycznochemiczna wody wodociągowej w trzech kulturach

No of trial / *Nr próby* — Analysed parametre / *Analizowany parametr*	Culture 1 *Hodowla 1*	Culture 2 *Hodowla 2*	Culture 3 *Hodowla 3*	Method *Metoda*	Accepta-ble value *Wartość dopusz-czalna*
pH	6,6 +-0,1	6,9 +-0,1	7,8+-0,1	PB/OB-Ś/34wyd.1z01.12.2010	6,5–9,5
General hardness *Twardość ogólna*	31 mg CaCO3/l	37 mg CaCO3/l	39 mg CaCO3/l	PN-ISO 6059:1999	60–500
Conductivity *Przewodność*	369 µS/cm	8,91 µS/cm	9,09 µS/cm	PNEN 27888:1999	2500
General iron *Żelazo ogólne* (Fe)	153 µg/l	192 µg/l	190 µg/l	PNISO-6332:2001	200
Mangan (Mn)	20 µg/l	31 µg/l	37 µg/l	PB/OBŚ/03wyd.8.11.2004	50
Amon ion *Jon amonowy* (A)	Poniżej 0,07 mg NH4/l	Poniżej 0,07 mg NH4/l	Poniżej 0,07 mg NH4/l	PN-C-04576-4:1994p.6a	0,50

Table 4. Average size of *Triopis* after growth stabilization from three subsequent cultures in waterworks water and rainfall water

Tabela 4. Średnia wielkość przekopnicy australijskiej (Triops australiensis) po stabilizacji wzrostu z trzech kolejnych hodowli w wodzie wodociągowej i wodzie deszczowej

Average size of *Triopis* *Średnia wielkość przekopnic*	Culture 1 in waterworks water/ *Hodowla 1 w wodzie wodociągowej*	Culture 2 in waterworks water/ *Hodowla 2 w wodzie wodociągowej*	Culture 3 in waterworks water/ *Hodowla 3 w wodzie wodociągowej*
	6,5cm	4,5	5,0
	Hodowla 1 w wodzie deszczowej 7,5 cm	Hodowla 2 w wodzie deszczowej 8,0 cm	Hodowla 3 w wodzie deszczowej 8,0 cm

Table 5. The number of larvae and adult specimen in subsequent cultures of *Triopis australiensis*
Tabela 5. Liczba larw i postaci dorosłych w kolejnych hodowlach Triops australiensis

The number of larvae leaving cysts *Liczba larw opuszczających cysty*	Culture 1 in waterworks water *Hodowla 1 w wodzie wodociągowej*	Culture in waterworks water *Hodowla 2 w wodzie wodociągowej*	Culture 3 in aterworks water *Hodowla 3 w wodzie wodociągowej*
The number of adult specimen after the first month of culture *Liczba postaci dorosłych po pierwszym miesiącu hodowli*	1 doba 19 2 doba 7 3 doba 6 Razem 32 27	1 doba 11 2 doba 14 3 doba 14 Razem 39 31	1 doba 15 2 doba 15 3 doba 11 Razem 41 41
The number of adult specimen after the second month of culture *Liczba postaci dorosłych po drugim miesiącu hodowli*	22	19	38
The number of larvae leaving cysts *Liczba larw opuszczających cysty*	Culture in rainfall water/*Hodowla 1. w wodzie deszczowej*	Culture 2 in rainfall water/*Hodowla 2. w wodzie deszczowej*	Culture3 in rainfall water/*Hodowla 3. w wodzie deszczowej*
The number of adult specimen after the first month of culture *Liczba postaci dorosłych po pierwszym miesiącu hodowli*	1 doba 21 2 doba 22 3 doba 13 Razem 56 56	1 doba 21 2 doba 24 3 doba 11 Razem 56 56	1 doba 19 2 doba 24 3 doba 17 Razem 60 57
The number of adult specimen after the second month of culture *Liczba postaci dorosłych po drugim miesiącu hodowli*	51	46	51

Table 6. The rate of births of larvae and adult specimen
Tabela 6. Współczynnik urodzeń larw i postaci dorosłych

	Larvae *Larwy*				Imago		
					Culture 1 Hodowla 1 Culture 2 Hodowla 2 Culture 3 Hodowla 3		
The rate of births in waterworks water *Współczynnik urodzeń (R) w wodzie wodociągowej*	Hodowla 1	Hodowla 2	Hodowla 3	I[a]	0,59	0,64	0,95
	0,695	0,787	0,811	II[a]	0,48	0,38	0,75
The rate of births in rainfall water *Współczynnik urodzeń (R) w wodzie deszczowej*	0,965	0,965	1,025	I[a]	1,21	1,13	1,12
				II[a]	1,10	0,93	1,00

[a] Month of culture
[a] *Miesiąc hodowli*

The analysis of birth ratio points to higher R values both for larvae and the imago for breeding conditions in rain water (Table 6).

CONCLUSIONS

The obtained results indicate that the larvae develop most quickly in rain water. The larvae achieved sizes similar to those in which they occur in the natural habitat. In municipal water where the amount of iron is multiplied 10 times (from 15 μg/l to 153 μg/l) ontogenesis of *Triops cancriformis* was less dynamic, and bred populations were 2 times smaller in terms of the number of individuals. An important breeding parameter for these crustaceans are pH conditions. The obtained results point to the faster development of cysts, higher birth ratio and greater survival of the studied crustaceans in rain water of pH 7.9 to 8.3 as compared with municipal water of pH 6.6 to 7.8. The element found in physical and chemical analyses of rain and municipal water is manganese. Manganese has an influence on animal growth and, indirectly, also on the transformation of calcium and phosphorus. Hygiene norms specify the allowed manganese concentration in drinking and tap water at 0.05 mgMn*dm^{-3}. Such high requirements are the consequence of numerous negative water features caused by a higher amount of manganese. It has a negative effect on organoleptic water features. It can be sensed in water only when it contains several mgMn*dm^{-3}; however, even if the amount of manganese is small, it still may lead to the proliferation of manganese bacteria, which results in unpleasant taste and smell of water. When the concentration of manganese is higher, both the bacteria and oxidising substances used do disinfect water lead to the emergence of dark brown manganese oxides, which change water colour. If manganese occurs in water-pipe network, a film of manganese bacteria is formed. Excessive accumulation of manganese in the organism disturbs the metabolism of other elements, e.g. iron, hindering the production of haemoglobin. Furthermore, there are some mentions related to neurotoxic symptoms and the possible carcinogenic properties of manganese. However, there are no convincing proofs of such adverse effect of manganese. Manganese usually accumulates in liver and it is expelled by gastrointestinal tract.

Manganese of lower oxidation level has toxic properties. This pertains especially to the oxides of divalent manganese. The dust of manganese compounds, which emerges while manganese ores are mined, is also very dangerous. The deficiency of manganese in plants hinders their growth and leads to the loss of chlorophyll.

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