Motor activity of *Daphnia magna* (Crustacea, Cladocera) during thermal selection: Peculiarities of search reactions in the non-uniform environment

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Received 19.04.2018
Received in revised form 09.05.2018
Accepted 10.05.2018

We conducted a study of the peculiarities of the motor activity of juvenile and adult individuals of *Daphnia magna* in the process of thermal selection. On the example of *Daphnia*, we experimentally proved the assumption that the decrease in the motor activity of the ectotherms in the selected temperature range is a behaviour mechanism typical for thermal selection. The experiment was conducted on the offspring of one parthenogenetic female (pure line) of a laboratory culture maintained over several years at room temperature. The *Daphnia* individuals had been beforehand acclimated over several generations to 23.4 °C, and then were placed in a thermogradient apparatus. The control individuals were maintained at room temperature. The final temperature preference was determined using the so-called "chronic" method, when the tested organisms are maintained in a thermogradient apparatus over several days. The positions of the *Daphnia* individuals were recorded daily during 24 days. As a parameter which would characterize the motor activity of *Daphnia*, we used the parameter of average relative shifting, which was calculated as a difference (in cm) between the position they were found in two sequential records, divided by time (in min) between these records. Over the first 3 days, in the conditions of the temperature gradient, the *Daphnia* mostly selected heightened temperatures (24–28 °C). During the period from the 4th to 24th day, 74% of the *Daphnia* selected the range of 18–23 °C and 85% – 17–24 °C. Thus, the range of the ultimate selected temperatures decreased by 4 °C compared to the initially selected temperatures and enlarged by 3 °C towards the lower temperatures. In the thermogradient apparatus, the parthenogenetic females had the lowest value of the mean relative movement. In the control, at room temperature, this parameter of the adults was by 45% higher compared to the gradient. The pattern of the dynamic of the indicator in both variants of the experiment was similar for the periods and phases of fluctuations. With the juveniles the value of the parameter of mean relative shifting in the gradient was higher by 40% compared to the adult *Daphnia*, but close to the parameter of the adults in the control. We determined a relationship between the juveniles selecting the higher temperatures and higher mean relative shifting, which indicates a relationship between the behavioural selective reaction of juvenile *Daphnia* and general physiological condition of their organism. During the absence of the temperature gradient, the *Daphnia* were observed to show symmetry in their motor reaction. Occurrence of the asymmetry of the motor reaction of the *Daphnia* in the condition of the thermogradient, manifesting in the prevalence of shifting to lower or higher temperatures, can indicate more clearly manifested search reaction in the condition of a non-uniform environment.

**Keywords**: behaviour reactions; ultimate temperature preferendum; temperature gradient; acclimation

**Introduction**

The ability for search behaviour in the conditions of a thermally heterogeneous environment occurs due to the peculiarities of the physiology of ectotherms, which allow them to sense insignificant temperature differences. Therefore, for example, it was determined that fish can feel a difference in the temperature, that equals 0.03 ºC (Bardach & Bjorklung, 1957; Steffel et al., 1976). For large crustaceans, the parameters are determined to be 1.0 ºC for the American lobster (*Homarus americanus*) (Jury, 2000), to 1.3–2.0 °C for the European crayfish (*Astacus astacus*) (Kivivuori, 1994) and 2.1–4.2 °C for *Procambarus clarkii* (Espina, 1993). For *Daphnia*, according to the results of the experiments in thermogradient devices, it was determined that they react to average changes in the temperature which equal 0.2–0.5 °C (Gerritsen, 1982).

No thermoreceptors or thermoregulating centres have been identified in crustaceans. Therefore, it is considered that their thermal behaviour is based on non-directed behaviour mechanisms (orthokineses and klinokineses) which are caused by changes and differences in the temperatures (Lagerspetz & Vainio, 2006). However, there are no articles known to us, apart from the abovementioned, which include research on the behavioural mechanisms of the formation of the final temperature selection of Cladocera and, particularly, the peculiarities of their behaviour in the conditions of a temperature gradient.

The other completely unstudied aspect is whether the lower crustaceans (Branchiopoda) have symmetry or asymmetry in their behavioural selective reactions. The lower vertebrates (Anamnia) exhibit bilateral asymmetry in their reactions, but this is usually considered to be related to the functional asymmetry of the CNS: the animals usually focus on an object with a certain eye – left or right (Andrew, 2009). Using various experimental methods, such asymmetry has been found for birds, reptiles and amphibians (Vallortigara & Bisazza, 2002). It is now being actively studied for teleosts (Andrew et al., 2009; Milosi et al., 1997, 2001; Milosi & Andrew, 1999; Nepomnyashchikh & Ivezkov, 2007; Takeuchi et al., 2010; Ivezkov & Nepomnyashchikh, 2013). Therefore, the example of the larvae of zebra fish demonstrates the
forming of series of rotations directed to one side, and the sequence of
these series occurs spontaneously under the impact of the central
oscillator, and not as a response to external stimulation (Romanovski et
al., 2014). A similar sequence of series of rotations to the right and left
was found in barrel flagtail Kuhlia magil Forster (Kuhlilidae).
(Gautrais et al., 2009). Also, it was determined that in a uniform
environment, the spontaneous organizing of behaviour manifests as
so-called interrupted search which consists of two interchanging
phases – moving and exploring (Nepomnyashchikh, 2000). Such
reactions have never been studied for the lower crustaceans. All that is
assumed that the decrease in the activity of the Daphnia is a general
behavioural mechanism which occurs during thermal selection.

The objective of the paper is the study of the peculiarities of the
motor activity of juvenile and adult Daphnia magna Straus which were
maintained in a thermogradient device over a long period
compared to individuals which were maintained in water at room
temperature.

Material and methods

The final selected temperature (FTS) was determined using the
so-called "chronic" method (Reynolds & Casterlin, 1979; Rosetti
et al., 1989; Verbitsky & Verbistskaya, 2012; Golovanov, 2013), when
the tested organisms are kept in a thermogradient device for several
days. For the analysis, we used the data obtained in the course of
identifying the final selected temperature (FTS) among the offspring
of one parthenogenetic female Cladoceran D. magna (pure line) of
laboratory culture which were kept over several years at room tempe-
trature. The Daphnia were beforehand acclimated to the constant
temperature of 23.4 ºC over several generations.

The device for studying the selected temperature was a Herter’s
tray with a metal bottom and walls of transparent acrylic glass (Fig. 1).
The size of the trays was 180 x 1.5 x 2.5 cm. The horizontal gradient
(0.1 ºC/cm) was made by maintaining different temperatures on the
opposite sides of the tray (4–8 to 28–30 ºC) with a UTP-1 thermore-
gulating device, a heating element of 0.8 kW power and a cooling
aggregate. For preventing the convective flow and vertical gradient,
the water thickness in the tray was 10–12 mm. This allowed creation
of a sufficiently gradual horizontal temperature, gradient which equa-
lated about 0.1 ºC/cm. For recording the water temperature along the
tray at fixed points, we installed thermometers at 10 cm intervals.

The temperature measurement was accurate to 0.1 ºC. The values of
the temperature were recorded every hour. The difference between
the two sequential records could equal ~0.0–0.7 ºC. After the experi-
ment, using these data, the crustaceans’ positions were converted
from linear to temperature values. The tray was divided by lateral
non-transparent barriers into three sections, each containing 1 adult
parthenogenetic D. magna female. The Daphnia were put into the
sections at the temperature of acclimation.

Three control Daphnia from the same clone as the experimental
individuals, also provisionally acclimated to 23.4 ºC, were put into the
control tray divided into three sections and filled with water at room
temperature. On different days, the water temperature equaled 18–
22 ºC. At the end of each day, after the end of taking observations, a
suspension of chlorella (Chlorella sp.) was added in equal portions
the full length of each tray to the amount of 5.0–7.5·10^5 cell/ml and
filtrated river water diluted with a distillate in the proportion of
1/2 was added for achieving a constant level. Before the addition of
fresh food, the sediment on the bottom of the trays was removed
using a pipette.

The tray of the apparatus was uniformly illuminated by 8 lamps
(40 W each) of the daylight. Two were placed along the tray at 46 cm
height and 6– on the ceiling at 1.4 m distance from the tray. The illu-
minance above the water surface was ~700 lux. The experiments
were conducted at the photocycle of 9/15 h (light/dark).

The indications of Daphnia positions were recorded daily in a
periodicity of 20–30 min from 8:30 to 16:40 during 24 days. The
young born by the females over the experiment were left in the trays
for 3–5 days and then removed. In total, during the experiment, we
recorded 702 observations of the adult Daphnia from the control and
experimental trays and 1,826 observations of the young individuals
from the experimental trays.

Results

Three adult Daphnia females had similar dynamics of selected
temperatures. The calculation of the coefficients of Spearman rank
 correlation indicated the absence of statistically reliable differences
between the sequences of data ($R_{23.9} = 0.75$ at $P < 0.001$; $R_{22.3} = 0.48$
$P = 0.23$; $R_{22.3} = 0.67$ at $P < 0.001$). This allowed the data on these
three individuals to be united into one sum and further to be analysed
as three replications of one experiment.

After the Daphnia were put in conditions of temperature gradient,
over the first 3 days, they mostly chose high temperatures of 24.0–
27.9 ºC (88.5% of records). Starting from the 4th day, the temperature
selection (TS) shifted to the area of lower temperatures. From the 4th
to 15th day, the level of TS manifestation reduced, and the range
enlarged, the values of TS fluctuated from 19 to 25 ºC (86.2% of the
records). From the 16th day and to the end of the monitoring, the
values of TS fluctuated from 16 to 22 ºC (89% of records). On the
whole, over the period from the 4th to 24th day, 74% of the Daphnia
selected the range of 18-23 ºC and 85% – the range between 17–
24 ºC. Thus, the range of the final selected temperatures decreased by
4% compared to the initially selected temperatures and enlarged by
3% towards lower temperatures (Fig. 2).

After comparing the curves of distribution of mature Daphnia in
the control and experimental trays over the period from the 5th to

Fig. 1. Thermogradient apparatus: 1, 2, 3 – the sections of the gradient
apparatus; 4, 5, 6 – the sections of the control tray

As a parameter which characterizes the motor activity of Daph-
nia, we used the parameter of mean relative shifting (ΔSaver), which
was calculated as the difference (in cm) in the position of an indivi-
dual during two sequential observations divided by the time (in min)
between these records. The presence and reliability of the relation
between the different parameters were evaluated using the coeffici-
ents of Spearman rank correlation. The statistical analysis of the data
was conducted in the R statistical pack, version 3.2.2 (R Development
Core Team, 2015).

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24th day, developed using the average data for every 10 cm, we can see that at room temperature, the *Daphnia* were distributed relatively uniformly along the tray (at each point – 3.9–6.7% of the records) (Fig. 3a). In the gradient of temperatures, we observed clearly manifested selection in the range of 40–110 cm, where 69.1% of the records were concentrated (at each point – 5.5–12.9% of the records) (Fig. 3b). Outside the zone of selection, the number of the records at each point fluctuated from 0.2% to 4.9%.

The young individuals chose the highest temperatures. At the same time, the curve of distribution had two maximum values – at 27°C and 23°C. The values of their mean relative shifting (1.1 ± 0.2 cm/min) were 43.4% higher than in the gradient. However, Figure 1 shows that the pattern of dynamics in the control and experimental trays was similar in the period and phases of fluctuations. The amplitude of the fluctuations, measured in absolute values, was higher in the control than in the gradient. However, the differences between the mean minimum and maximum values of shifting in relative values equaled 35.6–37.4%, i.e. this parameter ranged within the same values as in the experimental variant (Table). The coefficient of Spearman rank correlation between the two variants (0.68 at P = 0.007) also supports the statistical significance of the similarity in the pattern of dynamics.

The juvenile individuals demonstrated the highest values of ΔS_{max} in the conditions of temperature gradient (1.7 cm/min), which exceeded the ΔS_{max} in the control, 2.1 times higher in the gradient. The adult individuals in the gradient had motor activity approximately the same as among the adults in the control and by 40.2% higher than the adults in the gradient.

Table

<table>
<thead>
<tr>
<th>Periods of the observation</th>
<th>Parthenogenetic females in the gradient</th>
<th>Parthenogenetic females in the control</th>
<th>Juvenile individuals in the gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, day</td>
<td>ΔS_{max} ± standard deviation, cm/min</td>
<td>ΔS_{max} ± standard deviation, cm/min</td>
<td>ΔS_{max} ± standard deviation, cm/min</td>
</tr>
<tr>
<td></td>
<td>0.77 ± 0.03 0.51 ± 0.07 0.90 ± 0.05</td>
<td>1.39 ± 0.03 0.87 ± 0.12 1.35 ± 0.11</td>
<td>1.67 ± 0.06 0.41 ± 0.03 1.35 ± 0.06</td>
</tr>
<tr>
<td>Difference between the periods, %</td>
<td>33.8 43.3</td>
<td>37.4 35.6</td>
<td>142 95.7 92.9</td>
</tr>
</tbody>
</table>

The juvenile individuals showed a higher motor activity than the mature individuals. However, the changes in the mobility of the juvenile individuals of *Daphnia* were much more active than the adult *Daphnia*. The pattern of the dynamic of relative shifting of the young individuals was also fluctuating, with the same period as the mature individuals, but with significantly higher amplitude. The difference of the ΔS_{max} value between the periods of large and small shifts equaled 1.1 ± 0.3 cm/min, i.e. approximately the same as among the adults in the control and by 40.2% higher than the adults in the gradient.
zone which corresponds to the optimum zone) was twice as large (18.5–28.5 ºC) compared to the adults (22.5–27.0 ºC). It enlarged towards low temperatures by 4.0 ºC, and by 1.5 ºC towards high. The juveniles also had a larger range of ΔS<sub>ave</sub> values compared to adult females. The mean values of standard deviations of the juvenile Daphnia equaled 0.9, and 0.7 of the adults. Higher activity of the young was manifested also in the fact that they were found at significantly lower temperatures than the adults (12.5 and 21.0 ºC respectively), demonstrating maximum values of ΔS<sub>ave</sub> at these temperatures (4.1–4.7 cm/min). After the transitional process and as the FST level was reached, the range of independence of the ΔS<sub>ave</sub> level from the selected temperature (ST) significantly enlarged in both age groups of Daphnia (Fig. 6b). Thus, with the young the upper border zone of the plateau remained at the same level as in the transitional period (28.5 ºC and the lower border shifted from 18.5 to 13.5 ºC). As a result the plateau zone extended by 1/3. For the adult Daphnia, the upper border of the plateau zone declined from 27 to 25 ºC, and the lower — from 22.5 to 8.5 ºC, i.e., the plateau zone enlarged almost 4 times. The motor activity of the adult Daphnia decreased compared to the transitional period (from 0.7 to 0.6 cm/min), and that of the young increased from 0.7 to 1.0 cm/min. The range of the ΔS<sub>ave</sub> values, determined using the value of standard deviation, increased from 0.9 to 1.2 among the young, and remained at the level of 0.7 among the adult females.

![Fig. 5. Mean minimum and maximum relative shift of the D. magna individuals in the experiment: 1 – ΔS<sub>ave</sub>, 2 – ΔS<sub>max</sub> 3 – ΔS<sub>min</sub>; vertical lines – standard deviations of ΔS<sub>ave</sub>](image)

For checking the symmetry of behavioural selective reactions of the Daphnia in the control and in the experiment, we analyzed the dynamic of percentage ratio of the number of shifts, when the Daphnia moved towards the higher temperatures and towards the decreasing temperatures (in the control, this movement corresponded to the right and to the left along the chamber respectively). In the control, the dynamic of the number of shifts of adult females to the right or to the left had a symmetrical fluctuating pattern with a constant period of 7.5 days with an amplitude which increased from period to period (5–10%) (Fig. 7a). The calculation of the coefficients of Spearman rank correlation indicated the absence of reliable differences between the sequences of data in the replications (R<sup>2</sup> = 0.81 at P < 0.001). In the gradient, the adult females maintained a fluctuating regime for the first 10 days, but beginning from day 13, the symmetry was disrupted and day 23, the shifting towards the decreased temperature reliably dominated by 2–18% (R<sup>2</sup> = 0.40 at P = 0.20) (Fig. 7b). At the same time, the amplitude of fluctuations during the first 10 days was close to the values in the control (~7%), and after day 13, it increased to 10%. Initially the young Daphnia in the thermogradient had no cyclicity in the number of shifts (Fig. 7c). Beginning from day 15, the shifts towards the increased temperatures predominated by 7–10%, and from day 17 to 24 – towards the decreased temperature. However, this difference was not statistically reliable (R<sup>2</sup> = 0.69 at P = 0.003).

Discussion

As we know, the level of motor activity of ectotherms is one of the most significant components of their food procuring efficiency and their capacities of adapting to habitat in general (Wang & Greenfield, 1994; Gorskà-Andrzejak & Wojtusiak, 2003).

The lower activity determined for adult individuals of D. magna in the gradient compared to the control could have been caused by one of two factors. The first cause — in the conditions of temperature gradient, the Daphnia were "seized" in the central favourable zone by the non-favourable temperatures from the edges of the trays, while those in the control trays could travel along all the entire length of trays. The second cause could be the abovementioned (Lagerspetz & Vainio, 2006) assumption that the decrease in the motor activity is a general behavioural mechanism which occurs during thermal selection.

The first cause is supported by the fact that the Daphnia in the control were distributed relatively uniformly all along the trays (Fig. 6a), whereas in the conditions of the temperature gradient, the distribution showed a clear pattern of preference (Fig. 6b). However, the presence of a fully avoided zone was observed only over the first four days of the experiment in the transitional period, when the crustaceans selected higher temperatures and totally avoided 1/3 of the length of the trays (0-60 cm) with low temperatures. Throughout the following period, along with clearly manifested preference in the range of 30–110 cm (74% of records), they were found along the entire length of the trays.

The first assumption is contradictory to the data on the young, the activity of which was higher not only compared to the adults in the gradient, but also compared to the control. Therefore, the extent of the activity of the Daphnia directly depended on the value of the tray length available for movement only over the initial, transitional period, after they were placed in the conditions of the temperature gradient.

![Fig. 6. Mean relative shifts (ΔS<sub>ave</sub>) of a single D. magna individual depending on the temperature of the environment over the transitional period (a) and over the period after reaching the level of FST (b): 1 – juveniles, 2 – parthenogenetic females, 3 – plateau zone of the juveniles, 4 – plateau zone of adult females; each variant in 3 replications; vertical lines – standard deviations](image)

It seems that the reason for the decreased activity of the adult Daphnia in the conditions of the temperature gradient was most likely a well known phenomenon, when in the preferred temperature range, many animals demonstrate minimum motor activity. Therefore, on the example of different species of crustaceans, it has been demonstrated that crustaceans acclimated to selected temperatures demonstrate lower motor activity (decrease in orthokinesis) (Fraenkel & Gunn, 1961). Similar results were obtained for D. magna at 25 ºC.
tion among the adult females in the control remained at the same high and the general physiological state of their organism, of relationship between the behavioural selective reaction of juvenile high motor activity and selection of higher temperatures indicates the heated water, which influenced both the increase in the total level of activity decreases in the zone of selected temperatures. Having values juveniles also proved the abovementioned assumption that motor which one of the parameters is motor activity. At the same time, the variability in the selec-
tion of ΔS^26.0 to 43.5 cm, from 32.0 to 53.5 cm among the young individuals, t. e. the values of variability of the young were higher by 6–10 cm compared to the adults. At the same time, the variability in the selection among the adult females in the control remained at the same high level of 46 cm throughout the experiment.

The high motor activity of the young individuals in the conditions of the temperature gradient could be caused by their presence in more heated water, which influenced both the increase in the total level of metabolism and motor activity. At the same time, a combination of high motor activity and selection of higher temperatures indicates the relationship between the behavioural selective reaction of juvenile *Daphnia* and the general physiological state of their organism, of which one of the parameters is motor activity. At the same time, the juveniles also proved the abovementioned assumption that motor activity decreases in the zone of selected temperatures. Having values of ΔS^26.0 to 43.5 cm, from 32.0 to 53.5 cm among the young individuals, t. e. the values of variability of the young were higher by 6–10 cm compared to the adults. At the same time, the variability in the selection among the adult females in the control remained at the same high level of 46 cm throughout the experiment.

The obtained data that the upper border of the juveniles’ range of ΔS^26.0 to 43.5 cm, from 32.0 to 53.5 cm among the young individuals, t. e. the values of variability of the young were higher by 6–10 cm compared to the adults. At the same time, the variability in the selection among the adult females in the control remained at the same high level of 46 cm throughout the experiment.

The earlier suggested assumption (Lagerspetz & Vainio, 2006) that the decrease in the motor activity of ectotherms within the selec-
ted temperature range is a behavioural mechanism typical for tempera-
ture selection was proved experimentally. Parthenogenetic *Daphnia* females kept for a long period in the conditions of a temperature gradient demonstrated motor activity 43.4% lower than when kept in water at constant temperature. The juveniles, whose total level of mean relative shifting was higher compared to the adults, had mini-

Conclusions

The determined combination of higher motor activity of the juvenile *Daphnia* and reaction of selecting higher temperatures demonstrates the relationship between behavioural preference reaction and motor activity which characterizes the general condition of the organ-
ism. In the absence of the temperature gradient, the *Daphnia* showed symmetry of motor activity, which was manifested in the fluctuating cyclic pattern of sequence of predomination in the number of shifts to the right and to the left along the length of the tray. In the conditions of thermogradient, we observed asymmetry of motor activity, which was manifested among the adult females in the dominance of the number of shifts towards lower temperatures, and towards higher temperatures among the juveniles. Such behaviour indicates a clearer search reaction of the *Daphnia* in the conditions of a non-uniform environment.

The author expresses gratitude to V. D. Snirnov for technical servicing of the experimental setup and for his many-sided assistance in conducting the exper-
iment.

**Fig. 7.** Dynamic of the number of counts of relative shifts to the right (1) and to the left (2) (in % of the total), a - adult females in the control, b - adult females in the gradient, c - juveniles in the gradient

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