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Over-wintering *Daphnia*: uncoupling the effects of temperature and food on offspring size and filtering screen morphology in *D. galeata*

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Field investigation of seasonal variation of filtering setae number (FSN) in the filtering screens of *D. galeata* showed a clear tendency toward low FSN in animals with low temperature and food levels (an over-wintering population and a deep-hypolimnion-inhabiting part of the population). Laboratory experiments were carried out to uncouple the effects of temperature and food level on the size of neonates and FSN in their filtering apparatus. Results of the first experiments indicated that temperature is the main factor inducing the changes in FSN and the induction takes place during embryogenesis. These results were confirmed in the second experiment. Eggs from the same clutch incubated *in vitro* at temperatures of 19, 10 and 6°C developed into neonates with smaller carapace length with decreasing temperature. Smaller neonates had lower FSN in their filtering screens. These results provide experimental evidence of temperature-mediated embryonic induction in *Daphnia galeata* and contribute to an understanding of the proximate causes of the morphological features observed in over-wintering populations in the field.

KEYWORDS: *Daphnia* size; filtering screens; phenotypic plasticity; embryonic induction; temperature effect

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INTRODUCTION

Planktonic crustaceans of the genus *Daphnia* are effective filter feeders, and are able to remove phytoplankton and other particles suspended in water and transform the utilizable part into their own growth and biomass. At the same time, they are favorable prey for most zooplanktivores, and are thus an important link in freshwater food webs. *Daphnia* species are widespread, especially in water bodies of the temperate zone, and they inhabit lakes within a broad range of altitude from lowlands up to high mountain lakes. This means that populations are exposed year-round to a broad range of temperatures, including temperatures close to zero in winter when reservoirs and lakes may be covered with ice for several months. There are two types of over-wintering strategy in *Daphnia*. One is to produce sexual resting eggs that are able to survive harsh winter conditions, and which hatch in spring when conditions get better. The second strategy is to survive winter as parthenogenetic females, with these females being founders of the new population in spring. Both of these strategies have been reported to occur naturally (Seda, 1989; Jankowski and Straile, 2004; Keller and Spaak, 2004).

Other low temperature habitats are the near-bottom regions of deep stratified lakes. In most deep temperate lakes, there is a typical pattern of thermal stratification in summer, with a warmest upper layer (epilimnion), a layer with an abrupt drop of temperature (metalimnion) and the deep part of the lake (hypolimnion) where the temperature may range between about 4 and 6°C year-round. *Daphnia* species, which perform diel vertical migrations (DVM), experience rather large but short time fluctuations in temperature, whose effect on *Daphnia* life histories has already been studied in detail (Lampert, 1989). *Daphnia galeata*, which is a common and widespread species, usually does not perform DVM, and during thermal stratification from spring to autumn most of the population stays permanently in the warmest upper layer. It has been discovered, however, that during summer a certain small part of the population may remain permanently in the deepest and thus coldest layers of the water column just above the bottom (Seda *et al.*, 2007).

Both over-wintering and deep-hypolimnion-dwelling daphnids are constantly exposed to low temperatures for several months. There is a clear and well-known relationship between water temperature and the duration of embryonic as well as postembryonic development in *Daphnia* (Hrbáčková-Esslová, 1962; Kořínek, 1970; Munro and White, 1975). In addition, the duration of postembryonic development and other life history traits are directly affected by feeding conditions (Weglenska, 1971; Orcutt and Porter, 1984; Lynch, 1989), and over-wintering and deep-dwelling daphnids also experience rather poor-feeding conditions (Seda and Macháček, 1998; Rellstab and Spaak, 2009; Lampert *et al.*, 2010). Feeding conditions are flexibly reflected in morphological parameters of the filtering screens on the third and fourth pairs of thoracic limbs in *Daphnia* (Koza and Kořínek, 1985; Pop, 1991). Pop (Pop, 1991) showed that the parameter filtering setae length is closely related to the filtering screen area (FSA). It can be quickly adapted to food amount, and thus exhibits a close relationship to feeding and ingestion rates (Stuchlík, 1991; Lampert and Brendelberger, 1996). On the other hand, Pop (Pop, 1991) found that the parameter filtering setae number (FSN) is constant for the whole life span of an individual and that its temporal variation within a population can thus be achieved only as a transgenerational response. Repka *et al.* (Repka *et al.*, 1999a) found no significant relationship of FSN to food amount, and on the other hand a significant clonal diversity in this parameter. Therefore, another hypothetical mechanism for the temporal variation of FSN may be clonal succession. These features suggest that changes in FSN could provide additional information on a population, such as the succession and sustainability of cohorts or clones, or the long-term strategy of a population in a parameter related to a qualitative aspect of filtration. Information that could be obtained from variations of FSN is supposed to be especially well expressed and changes easier to detect in low temperature environments when all processes are slow. The first aim of this study was to investigate seasonal variation of the number of setae in the filtering screens in one particular population of *D. galeata* with special attention to over-wintering and deep-dwelling animals. As a second step that followed the field investigation, we performed laboratory experiments to uncouple the effect of feeding conditions and the effect of temperature on the phenotypic plasticity in offspring size and FSN in the filtering screens of *D. galeata*.

METHODS

Field investigation of *D. galeata* filtering screen morphology

Field investigations were performed in the Římov Reservoir, a moderately eutrophic reservoir in the southern part of the Czech Republic. The reservoir is canyon-shaped, 13 km long, with a maximum depth of about 42 m near the dam. The average theoretical retention time is about 100 days. The reservoir is located in the temperate zone and is dimictic with two short periods of homothermy, one in spring (the end of March) and the

other in autumn (at the end of November to beginning of December). In summer, there is typical thermal stratification of the water column with an epilimnion (usually upper 5 m), metalimnion (from 5 to about 14 m) and hypolimnion (from 14 m down to the bottom). In winter, the reservoir is usually covered with ice (January through March) and thermal stratification is inverted, with temperatures close to zero below the ice and about 4°C in layers above the bottom.

In 2003–2005, we investigated the vertical distribution of *Daphnia galeata*, the dominant cladoceran species in the reservoir. We collected zooplankton samples at the deepest sampling station located near the dam (48°50′45″N; 14°29′21″E) from specific separate water layers using a closing plankton net. Samples were taken at 3- to 4-week intervals and preserved in 4% formalin solution for later processing in the laboratory. Detailed data on the *D. galeata* vertical distribution are presented elsewhere (Seda *et al.*, 2007); the general year round pattern, however, can be briefly summarized as follows. During the inverse thermal stratification under the ice cover in winter, most of the *D. galeata* population was in the warmest layer of the water column in the deep hypolimnion. In contrast, during summer stratification, most of the population was found in the epilimnion. However, even during summer stratification, a certain minority of the population was regularly found in the deepest layers of the hypolimnion. There were no signs of diurnal vertical migration behavior during summer stratification. To analyze yearly changes of *D. galeata* filtering screen morphology, we used samples from 2005, which represented the majority part of the *D. galeata* population within the vertical profile, i.e. samples from the deep hypolimnion (25–35 m) in winter (January, March), and samples from the epilimnion (0–5 m) in spring, summer and autumn (April–December). We also analyzed the minority part of the population established in the deep hypolimnion at the end of May and persisting there until December.

It had previously been found that adaptive changes of daphnid filtering screen parameters proceed in parallel in the third and fourth pairs of thoracic limbs, and that the difference between the two limbs in the pair is negligible due to bilateral symmetry (Kořínek *et al.*, 1986; Pop, 1991). Therefore, we only analyzed the morphology in one filtering screen of the third pair of thoracic limbs. In each individual examined, the filtering screen of one of the third pair of thoracic limbs was dissected and filtering setae were counted under the microscope.

The FSN is constant during the ontogeny of an individual, and any change of this parameter can only appear in the next generation (Pop, 1991; J. Macháček, unpublished data). To see these changes in a population, it is thus necessary to analyze FSN in different age (size) groups. Therefore, we investigated FSN in our field samples in three *D. galeata* age groups: (i) juveniles, i.e. individuals clearly of the first or preferably second juvenile instar, (ii) individuals of the pre-adult and apparently first adult instars, and (iii) large adult females, in which it is not possible to exactly determine their age. In every group, at least 10 individuals (if available) were analyzed. To compare means of FSN among these three age groups for each sampling date, we used one-way analysis of variance (ANOVA), and post hoc Tukey unequal *n* honestly significant difference (HSD) tests for multiple comparisons. All statistical computations were performed in Statistica 9.0.

Laboratory experiments

The main goal of our experiments was to uncouple the effects of food amount and temperature on the FSN in filtering screens of the third pair of thoracic limbs. For experiment 1, we collected zooplankton samples on 29 January 2010 from the deep layer (30–40 m) of the ice-covered reservoir, where the water temperature was about 4°C and the amount of seston determined as particulate organic carbon (POC) in the water filtered through 40 μm mesh size sieve was 0.2 mg L^{-1}. The zooplankton sample was transported in a thermo-insulated box to the laboratory, where large adult *D. galeata* females with fully developed embryos in their brood chambers were isolated. Then, their body and carapace lengths were measured and they were placed individually into 50 mL beakers with lake water collected from the same depth. There were three feeding treatments in the experiment: one was lake water filtered through a 40 μm mesh size sieve so that natural seston, amounting to about 0.2 mg L^{-1} POC, served as food for the experimental animals. POC in the lake water was determined once in 3 weeks during routine monitoring of the reservoir. For the second and third feeding treatments, natural seston was removed by filtration through GF/C glass fiber filters and a precise amount of a laboratory culture of *Scenedesmus subspicatus* (routinely determined turbidimetrically) was added so that resulting POC levels were 0.2 and 2 mg L^{-1}, for low and high food conditions, respectively. To adjust food levels, we used the relationship of turbidity and POC values previously determined for our *Scenedesmus* culture. Experimental animals in all three feeding treatments were cultured at two temperature levels. After isolation in the 50 mL beakers, animals in the low temperature condition were immediately placed into a temperature-controlled box at 5°C. Those at the high temperature were left to gradually reach room temperature, which was about 20°C. There were seven replicates in each of the six combinations of food and temperature; so altogether we had 42 pieces of 50 mL

beakers, each containing one female. All experimental cultures were carried out in complete darkness (except for the short period of media exchange). Thus the effect of light as an additional experimental variable was excluded and light conditions were similar to those in the deep hypolimnion where the animals were collected. In the high temperature treatment, fresh culture media was prepared and exchanged every day, and fresh lake water for the media was taken from the deep hypolimnion of the reservoir once every 3 days. In the low temperature treatment, the intervals for culture media exchange and fresh lake water collection were 3 and 6 days, respectively. Females isolated from the reservoir released their offspring (here referred to as the first clutch offspring) from the brood pouch within 12 h after isolation at high temperature and within 2–4 days at low temperature. After releasing the neonates, mothers cast off their old exoskeletons, and the new eggs that had been formed in the ovaries during the development of embryos in the brood pouches were laid into the emptied brood pouches. FSN in mothers was determined using the cast off exoskeletons, which is the method used by Pop (Pop, 1991). This makes it possible to dissect the filtering screen from the old exoskeleton and measure its parameters without killing the experimental animal, which can be further cultured. The same method was used to determine FSN in individuals of the offspring from the first, second and third clutches of the mothers. It was necessary to culture the neonates up to the second or even higher instars, because as Pop (Pop, 1991) discovered, it is often not possible to reliably determine FSN in first instar individuals. For statistical evaluations of FSN in the three clutches born in the laboratory after isolation of mother females from the reservoir, two-way ANOVA with food variant and clutch number as factors (Statistica 9.0) was performed separately for the two temperature levels. The effect of temperature on FSN in second and third clutch offspring was tested using one-way ANOVA with temperature as a factor (Statistica 9.0).

The results of these experiments suggested that water temperature was the principal factor inducing changes in FSN and that the relevant period for this induction was the period of embryonic development. There were also indications that concurrently with FSN, offspring size was affected. To test this hypothesis, experiment 2 with *in vitro* egg incubation was carried out. In these experiments, a laboratory stock culture of a *D. galeata* clone originally isolated from the Římov Reservoir and kept in the laboratory for about 7 years was used. The culture is routinely kept in aged tap water at about 20 ± 1°C with a surplus food supply of a laboratory culture of *Scenedesmus subspicatus*. Eggs from each clutch tested in the experiment were removed from the brood pouch 4–6 h after being laid and randomly divided into three groups. Each group containing three to five eggs was placed in one Petri dish with aged tap water (the same as used for *Daphnia* cultures) and incubated at different temperatures. Thus one part of the clutch was incubated at 19°C, the second at 10°C and the third at 6°C. Clutches from eight females were tested in these experiments so that representatives from each of the eight clutches were present in each of the three temperature groups. Carapace length of neonates hatched from these eggs was measured, and the animals then cultured up to the third juvenile instar at 19°C, when their FSN was determined. Mean values of the parameters investigated were compared in the three temperature groups using unequal *n* HSD tests for multiple comparisons (Statistica 9.0).

RESULTS

Field investigations

The yearly dynamics of FSN in 2005 (Fig. 1A) shows several clear trends. In winter, there was a tendency to produce offspring with significantly lower FSN ($F = 12.78$; df = 29; $P = 0.0001$; $F = 52.48$; df = 33; $P < 0.0001$; $F = 26.18$; df = 28; $P < 0.0001$ on 27 January, 04 March and 11 April, respectively). The high FSN in the group of adult females in January indicates that they were born in autumn of the previous year (November or even earlier), when average FSN values were high for all the three age groups (Fig. 1A). Due to low temperature and low food, they grew to adulthood in January the next year, and as indicated in Fig. 1A, adults with high FSN persisted in the population until March and April. The temperature in layers with the majority of the *D. galeata* population was in this period around 3.5°C and POC values around 0.2 mg L^{-1}. As the juveniles with low FSN grew into adults and the old individuals with high FSN died out, this low FSN gradually spread through the whole population and the differences among age groups became insignificant. This process was rather slow due to low temperatures, and it was completed as late as the end of April ($F = 2.23$; df = 30; $P = 0.125$ on 29 April). Then the situation reversed and juveniles had a higher FSN than their mothers, but due to fast growth and development at rapidly increasing temperatures, the FSN values of all age groups quickly leveled off and the differences were not statistically significant ($F = 2.27$; df = 26; $P = 0.124$ on 06 May). A picture similar to that seen in winter was found in the small proportion of the population inhabiting the deep hypolimnion in summer (Fig. 1B). These animals came to the deep hypolimnion from the upper strata, and the FSN in juveniles gradually became significantly lower than that of the other two age

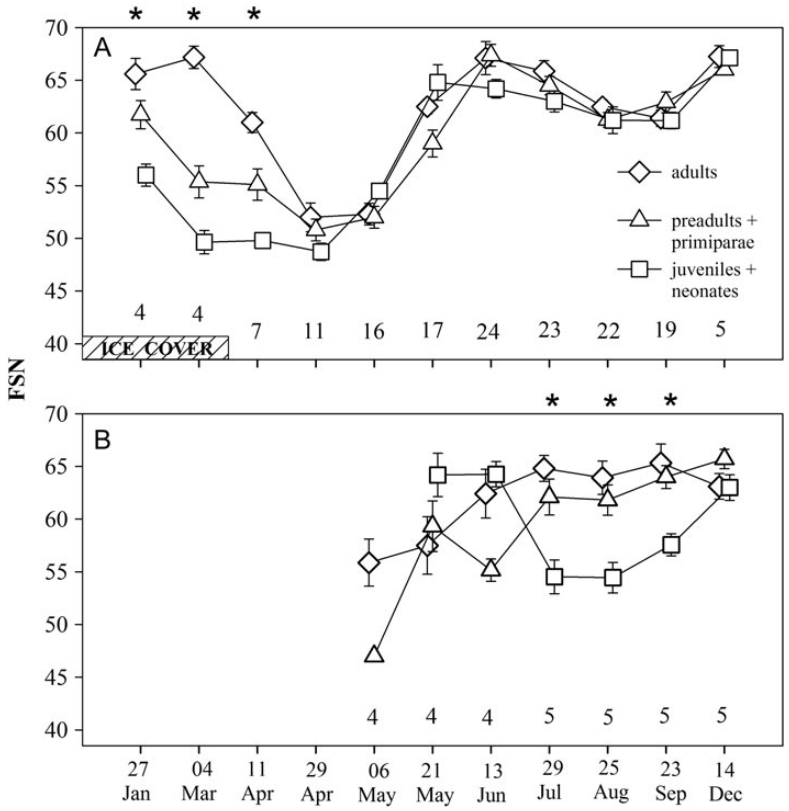

Fig. 1. Seasonal dynamics of FSN in three age groups of *Daphnia galeata* population in the Římov Reservoir in the year 2005. Each symbol represents the mean value of 10–15 individuals ± 1 SE. (**A**) Shows the major part of the population, (**B**) shows the minor part of the population usually found only in summer in the deepest layers above the bottom. Asterisks indicate the dates when significantly lower FSN were found in juveniles. The figures above the x-axis give the average temperatures in °C in the water layer sampled on the pertinent sampling dates.

groups ($F = 12.3$; df = 33; $P < 0.0001$; $F = 10.6$; df = 33; $P = 0.0003$; $F = 7.9$; df = 24; $P = 0.0023$ on 29 July, 25 August and 23 September, respectively). The temperature in the deep hypolimnion during summer and autumn was quite stable, only increasing slightly from 4°C in May and June up to 5°C in December. Food resources available in the deep hypolimnion ranged in terms of POC from 0.13 to 0.7 mg L^{-1}. In spite of these high POC values detected in July and August, the animals from the deep hypolimnion exhibited very low fecundity thus indicating strong food limitation. Seston particles in the deep layers were probably rich in organic carbon but poor in nutrients for daphnids. These results clearly indicated that under conditions of simultaneous low temperature and low food, there was a tendency to produce offspring with low FSN. The reason why the low FSN in juveniles was not gradually reflected in a decrease in FSN in older groups was most likely the severe food limitation, which together with low temperatures caused extremely slow postembryonic development and probably also high mortality in juveniles. The opposite trend to higher FSN in juveniles in December (Fig. 1B) was most likely the result of a gradual downward migration of the population from the epilimnion in autumn.

Laboratory experiments

In experiment 1, we used *D. galeata* isolated from the reservoir at the end of January 2010, when the reservoir was covered with ice and was inversely thermally stratified. The temperature in the layer about 10 m above the bottom, where the bulk of the population aggregated, was 4°C and the POC content was about 0.2 mg L^{-1}. Manipulation of temperature and food led to high mortality in the experimental animals, especially in the high temperature treatment combined with a high *Scenedesmus* level. In this experimental treatment, offspring mortality was 100 and 72% in the second and third clutch offspring, respectively. High mortality (86%) was also recorded in second clutch offspring in the high temperature–low *Scenedesmus* level treatment. In all other experimental variants, mortality did not exceed 43%. In spite of these gaps in the raw data, the results exhibited consistent trends and clearly showed several important findings.

The high FSN of mothers isolated from the reservoir in January corresponds to the situation illustrated in Fig. 1A, and indicates that these females survived in the reservoir from the last autumn when the FSN of all three age groups investigated were at this high level. The FSN of these mothers and that of their first clutch offspring in experiment 1 was different, with the offspring having a significantly lower FSN (Fig. 2, $F = 111.3$; df = 30; $P < 0.0001$). This demonstrates the trans-generational nature of the temporal variation in FSN. The offspring were released from the brood chambers of their mothers soon after transfer and isolation in the laboratory, which means that oogenesis and an overwhelming part of embryogenesis took place in the low temperature environment of the reservoir. In the following clutches, the picture was different between the two temperature treatments. In the variant where temperature was maintained at about the same low level as in the reservoir (5°C), the FSN in the second and third clutch offspring remained at the same low level as in the first clutch and there were no differences among the three feeding variants (Fig. 2A, Table I). In the high temperature treatment, there was already a clear trend to higher FSN in the second clutch offspring (the missing value for second clutch offspring FSN in the high food variant was due to the mortality of all individuals during the first juvenile instar), which continued in the third clutch offspring (Fig. 2B, Table I). There was also a significant effect of the feeding conditions in the high temperature treatment, which was consistent in all the three clutches and thus the interaction between clutch and food was not significant (Table I). The difference of FSN in second and third clutch offspring between high and low temperatures was highly significant ($F = 57.99$; df = 44; $P < 0.0001$). First clutch offspring were not included in the test because they were not affected by temperature manipulation. Results of experiment 1 clearly indicated that low temperature was the main factor inducing lower FSN in the *D. galeata* population investigated. The significant increase of FSN in the high temperature experiment was already found in the second clutch offspring. The eggs of this clutch were laid into the brood pouch of the mother immediately after releasing the first clutch neonates, which means that most of the oogenesis in the ovaries took place under the low temperature in the reservoir. This succession of ontogenetic phases and different temperatures suggests that

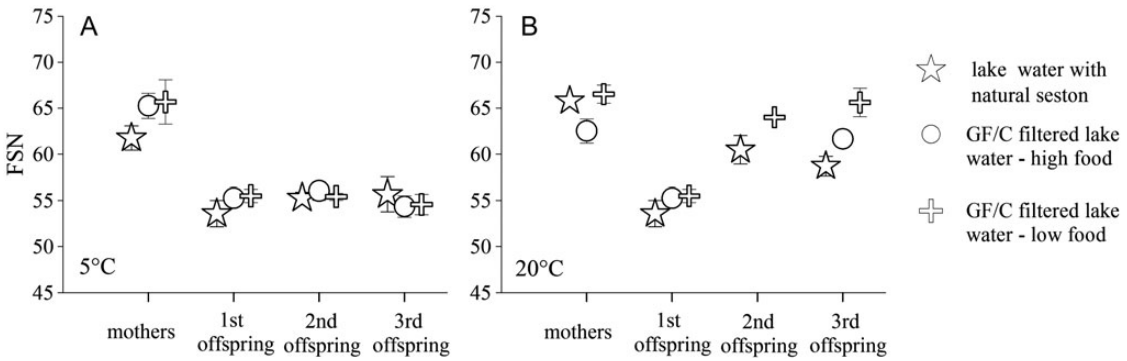

Fig. 2. FSN of *D. galeata* mothers isolated from the Římov Reservoir on 29 January with fully developed embryos in their brood pouches, and FSN of their offspring from the three consecutive clutches born after isolation and culture at two temperatures and three feeding treatments [(1) natural seston, (2 and 3) *Scenedesmus subspicatus* in the amount of 0.2 and 2 mg L^{-1} POC in low and high food conditions, respectively]. (**A**) Temperature similar to that in the reservoir, (**B**) room temperature in the laboratory. Mean values ± 1 SE are given.

Table I: Results of two-way ANOVA of FSN in Daphnia galeata *offspring in three consecutive clutches (factor CLUTCH) born immediately after isolation of mothers from the reservoir in winter (January) and maintained at two temperatures and three feeding treatments (factor FOOD)*

	Temperature 5°C				Temperature 20°C			
Factor	df	MS	*F*	*P*	df	MS	*F*	*P*
CLUTCH	2	2.8	0.58	0.565	1	298.3	36.10	**<0.001**
FOOD	2	0.5	0.11	0.898	1	76.9	9.31	**0.005**
CLUTCH × FOOD	4	3.5	0.71	0.588	3	10.8	1.31	0.294
Error	34	4.9			25	8.3		

Significant *P*-values are printed in bold.

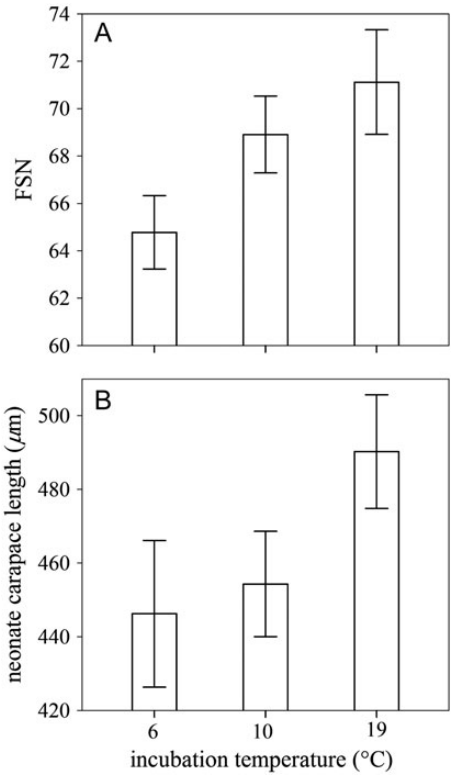

Fig. 3. (**A**) FSN and (**B**) carapace length of neonates of the *D. galeata* laboratory clone incubated during embryonic development *in vitro* at three different temperatures. Bars represent the mean values for the three temperature groups; the whiskers illustrate 95% CI. Means with non-overlapping CIs are significantly different.

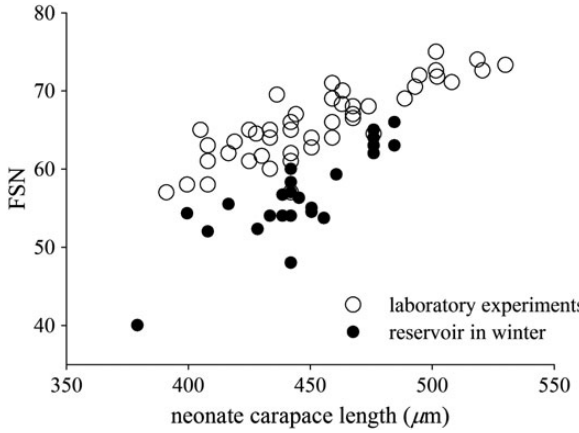

Fig. 4. Relationship between FSN and carapace length of neonates in *D. galeata*. Open symbols represent the individuals from the *in vitro* incubation of eggs at three different temperatures in the laboratory. Closed symbols represent neonates born to mothers within 6 h after isolation from the reservoir in winter (January, March).

the period in which low temperature may induce lower FSN is not during oogenesis but rather the period of embryonic development—embryogenesis. The results of experiment 2, which tested this hypothesis, as well as the effect of temperature on offspring size, are presented in Fig. 3. Eggs from the clutches of eight females, coming from a laboratory clone kept long term at 20°C, and which was thus also the temperature during oogenesis of these eggs, were incubated *in vitro* at three temperatures. The total number of eggs in *in vitro* incubations was 36, 37 and 37 at temperatures of 19, 10 and 6°C, respectively. Percentages of individuals that completed embryonic development were 86, 54 and 32% at the three temperatures, respectively. In Fig. 3A, there is a clear trend of decreasing FSN with decreasing temperature during embryogenesis. At the same time, there is a decrease in the size of neonates in terms of carapace length (Fig. 3B). These results suggest a relationship between the size of neonates and the FSN in their filtering screens. This relationship is illustrated in Fig. 4. There is, however, a difference between the individuals from laboratory experiments and the individuals hatched from females isolated from the reservoir in winter (ANCOVA with neonate carapace length as covariate, $F_{1,\ 49} = 96.499$; df = 1, 49; $P < 0.00001$). Possible causes of this difference are discussed below.

DISCUSSION

The interpretation of the field data shown in Fig. 1 had a critical influence on the direction of subsequent further experimental work. In this interpretation, two principles were taken into account: (i) the parameter FSN is constant for the whole postembryonic period of ontogenesis in the individual; (ii) the picture (FSN) we observed at a particular moment (the sampling date) was the result of environmental factors (temperature, food) during a certain decisive period (embryogenesis, oogenesis) in the past, and the lower the temperature, the further in the past this relevant period occurs. Similarly, the older the individuals analyzed (age group), the further in the past is this relevant period. At temperatures of 3–4°C and concurrently low food level, the ontogenetic development is extremely slow. It is likely due to the complications in performing such experiments that the only experimental information on postembryonic development time from neonata to the first clutch of eggs at 5°C and concurrently low food levels so far published is a small remark in Rellstab and Spaak (Rellstab and Spaak, 2009), 66 and 90 days, comparable with our own unpublished single observation (ca. 75 days). However, the period from neonata to the first clutch of eggs is a relatively small part of the total lifespan of *Daphnia*, and many adult instars follow if mortality is only due to senescence (Pietrzak, 2011). The embryonic development, and

hence roughly the duration of an adult instar, takes about 3 weeks at 4°C (Bottrell *et al.*, 1976). Therefore, we suppose that large adult females isolated from the reservoir at the end of January (analysis of field samples—Fig. 1A, and animals isolated from the reservoir for experiment 1—Fig. 2) were born not later than in the past November and very probably much earlier. At that time, as indicated in Fig. 1A, average FSN values were high for all the three age groups, because the temperature in the epilimnion was still "high" and only gradually decreasing (15, 12 and 9°C on the 6 October, 27 October and 17 November 2005, respectively). This means that high FSN was also prevalent in the group of juveniles which became adults in January the next year. As shown in Fig. 1A, the large adults with high FSN persisted in the population until March and April**,** which seems realistic due to prolonged lifespans at low temperature and especially due to the absence of size-selective fish predation in winter. The presence of these individuals kept the average FSN values high, and though a certain proportion of low FSN individuals was present in the group of adults throughout winter, their effect was too low to be reflected in the average values. They decreased only when the older individuals died out and younger low FSN adults dominated the population in early spring. In December the situation was reversed, and the FSN in juveniles from 14 December reflect the conditions ca. at the beginning of November when the effect of low temperature was not yet exhibited. In addition, as shown in Fig. 4, there is a relationship between the body size of the first juvenile instar (neonates) and their FSN, and it is necessary to take this relationship into consideration when interpreting Fig. 1. Neonate size is affected by a number of factors, especially food resources and the size of the mother (McKee and Ebert, 1996). These factors are most probably responsible for certain fluctuations of offspring size and thus FSN in periods when temperature is relatively stable, i.e. in the epilimnion during summer (end of May–September). Therefore, the increase in FSN in May is the result of both the increase in temperature and food limitation during the clear water phase when a small number of large-sized offspring are produced. Later, the feeding conditions again improve as the summer peak of phytoplankton appears, and *D. galeata* produce a higher number of smaller offspring with slightly lower FSN. In autumn, the resources again become limited and the offspring size and FSN increase, and the effect of low temperature on FSN is not yet apparent on 14 December.

The situation in the deep hypolimnion during summer illustrated in Fig. 1B is a specific case, because two extremely different microhabitats coexist: the warm and food-rich epilimnion and the cold and food-poor deep hypolimnion. Daphnids can theoretically freely move from one to the other, but as we do not have any detailed information on this potential migration the following description of the processes is based on our data of the vertical distribution dynamics of this *D. galeata* population (Seda *et al.*, 2007) and the FSN dynamics (Fig. 1B). After the spring period, when the whole *D. galeata* population is concentrated in the epilimnion and there are no daphnids in the deep hypolimnion, a certain number of individuals can be found in the deep hypolimnion starting around June; there were only a few individuals in the May samples, especially on 6 May, and no juveniles. We presume that these daphnids gradually moved down from the epilimnion. The picture that we observed in July, August and September was strikingly similar to what we observed in winter (January–March), namely a low FSN in the juveniles. First, this indicates that similar conditions (low temperature and low food) resulted in the production of offspring with low FSN, and secondly this is indirect evidence that the animals which moved to the deep hypolimnion stayed there for a period long enough for the effect of environmental factors on FSN to be manifested in their offspring. The reason why the reduction of FSN was not gradually reflected in the pre-adults and adults (like in winter) was that low temperature and extremely low resources (indicated by the dramatic decrease of adult fecundity in autumn) caused very slow development and probably also high juvenile mortality. The opposite trend to higher FSN in juveniles despite the constantly low temperature, especially noticeable in December (Fig. 1B), was most likely due to a gradual downward migration of the population from the epilimnion. The *D. galeata* population exhibits an inverse vertical distribution during winter and the tendency to this vertical pattern was observed already in the period of homothermy in December (Macháček and Seda, unpublished data).

The temperature driven plasticity of filtering screen morphology, namely the FSN in *Daphnia*, and the relation of FSN to body size of the first juvenile instar have not yet been published. Up to the present, plasticity in filtering screen morphology has been related almost exclusively to feeding conditions. Since the first studies on FSA plasticity (Kořínek and Macháček, 1980), many additional examples and details of this phenomenon have been reported (Koza and Kořínek, 1985; Lampert, 1994; Repka *et al.*, 1999a, b), and the ecological significance of FSA plasticity is generally acknowledged and a causal relationship to feeding conditions demonstrated. However, data on the parameter of FSN are less frequent and less unanimous. There are some results that suggest a relation of FSN to feeding conditions in *D. pulicaria* (Pop, 1991) and in *D. galeata* (Stuchlík, 1991). On the other hand,

Repka *et al.* (Repka *et al.*, 1999*a*) did not find any relationship between feeding conditions and FSN in *D. galeata*. In our experiment 1, there was no effect of food level of mothers on FSN in their progeny in three consecutive clutches in the low temperature treatment. In the high temperature condition, there was a consistent trend toward increasing FSN in all food treatments, but there were also differences attributable to food treatment and thus the role of food cannot be completely ruled out. Maternal feeding conditions are known to influence offspring size, and the effect of food on the FSN may be the result of the above-mentioned relationship of neonate size and FSN. Further experiments are needed to elucidate the interaction of food and temperature in their effects on FSN as well as on offspring size.

As is apparent from Fig. 4, there is a difference in the relationship between the size of neonates and the FSN in their filtering screens when data from the laboratory experiments and those measured in the over-wintering population in the reservoir are compared. The neonates from the reservoir have significantly lower FSN than similar sized ones from the laboratory experiments (ANCOVA; $F = 96.499$; df = 1, 49; $P < 0.00001$). There are several possible explanations for this difference. (i) In laboratory experiments, individuals of one single clone were used, whereas the field population encompasses a mixture of clones. Repka *et al.* (Repka *et al.*, 1999*a*) reported significant clonal diversity in FSN. This may have contributed to the difference between laboratory and field results, though the effect of low temperature in the field population seemed to be general, irrespective of clonal diversity. There might also be clonal diversity in the responsiveness of FSN to temperature. (ii) For the *in vitro* incubation, it was impossible to use eggs younger than 4–6 h after deposition into the female brood pouch (at 20°C). Freshly laid eggs are extremely sensitive to manipulation, and even in cases when they were not visibly damaged during transfer from the brood pouch into the incubation dish, their development was interrupted and they soon decayed. The effect of low temperature therefore did not include early embryogenesis, which may have affected the results of the experiments. (iii) Though our first experiments indicated that the period relevant for the induction of changes in FSN by low temperature is embryogenesis, more experimental results are needed to determine the effect of temperature during oogenesis. (iv) The lowest temperature in our experiments was 6°C, whereas in the reservoir the range of temperatures was from almost 0°C just below the ice to 4°C above the bottom. During this type of inverse stratification, the bulk of the over-wintering population is in the layers above the bottom; nevertheless, the mean temperature the population in the reservoir was exposed to was very probably somewhat lower than that in the experiment.

Another important finding of this study is that low temperature in the period of embryogenesis results in significantly smaller progeny. Even at a temperature of 10°C, there was a significant reduction in neonate size compared with 19°C. This is a very important phenomenon for understanding the structure and dynamics of *Daphnia* populations in general, as there is a broad range of temperatures daphnids are exposed to in lakes and reservoirs relative to season, geographic location and depth profile. It is also important with regard to general theories on the adaptive significance of the size of organisms living at different temperatures (Atkinson, 1994, 1995). To our knowledge, however, there are only two papers dealing with the effect of temperature during embryogenesis on the size of neonates, moreover with contradicting results. Esslová (Esslová, 1959) reported a smaller size of *Daphnia pulex* neonates incubated *in vitro* at 2–4 and 28°C compared with those incubated at 20°C, whereas Gulbrandsen and Johnsen (Gulbrandsen and Johnsen, 1990) found the largest neonates incubated at 5°C compared with 10 and 15°C. The two studies are not fully comparable, however, because of differences in reporting the size of animals (body length vs. body volume), and especially because in the latter study *D. pulex* from a Norwegian lake was clearly adapted long term to low temperatures and, as the authors note, the eggs failed to develop at temperatures higher than 15°C. Results from our experiments suggest that small offspring size, together with extremely slow postembryonic growth and development in cold and poor environments, are the main reasons for the shift towards smaller size classes in the size structure of the over-wintering *D. galeata* population regularly found in the Římov Reservoir (J. Seda, unpublished data). This is in sharp contrast with the overwhelming majority of studies reporting larger sizes for ectothermic organisms, including some cladocerans, in the cold (Atkinson, 1994, 1995). However, some data on planktonic crustaceans used in these analyses might be misleading, as they do not represent the full range of temperatures animals may encounter in their environments (Culver, 1980; Perrin, 1988). In addition, in field data the effect of temperature on offspring size may be masked by complex interactions with feeding conditions and maternal phenotype (McKee and Ebert, 1996), predation and predator-released infochemicals (Macháček, 1991; Lampert, 1993) and probably other environmental factors. The design of our experiment 2, when the effect of temperature was investigated within the period of embryonic development and eggs from the same clutch were compared, eliminated these interactions. The data of Esslová (Esslová, 1959), who reported a similar reduction in offspring size in extreme cold (2–4°C) as in extreme heat (28°C), may suggest higher expenditures to

complete this phase of development at both limits of the permissible temperature range. Therefore, a bell-shaped or "wigwam-shaped" (Atkinson, 1996) rather than simple inverse linear relationship may be expected. Higher metabolic costs at lower temperature, expressed as total oxygen consumption within the period of embryogenesis, are suggested by the data of Nielsen *et al.* (Nielsen *et al.*, 2007) for the marine copepod *Acartia tonsa*.

Our results from a field *D. galeata* population indicate that the early spring population is formed exclusively of individuals with low FSN (Fig. 1A), that is the ones that developed and matured at low temperature during winter. It is well known that early spring *Daphnia* populations usually exhibit rapid growth, predominantly due to their enormous fecundity (Brooks, 1946; Seda, 1989). This is usually attributed to the high quantity and quality of spring phytoplankton bloom (Sommer *et al.*, 1986). However, both morphological and physiological responses to low temperature in winter may hypothetically contribute to a temporary selective advantage by promoting rapid population growth once temperature and feeding conditions ameliorate.

The temperature-induced changes in filtering screen morphology as well as in neonate size are both new examples of phenotypic plasticity in *D. galeata*, with potentially important ecological consequences. They first came to our attention in the field, leading us to experimentally determine the proximate cue inducing the response. To what extent this phenomenon may be generalized to other *Daphnia* species and strains, and what really are the ultimate causes and ecological consequences, remain to be resolved.

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