Reproductive features of *Pisidium casertanum* (Poli, 1791) (Bivalvia: Sphaeriidae) in relict lakes of Bolshezemelskaya Tundra

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Abstract

The reproductive features of the pea clam *Pisidium casertanum* in relict lakes of Bolshezemelskaya Tundra were studied. The *P. casertanum* population in the Vashutkiny Lakes of Bolshezemelskaya Tundra has a single period of summer reproduction. We suppose that the release of embryos mainly occurs between July and August and the breeding season could probably begin in May-June. The positive relationship between brood size and parent shell length was detected. We did not find a specific brooding mechanism, accompanied by asynchronous development and release of embryos by the parent in the population of *P. casertanum* in the Vashutkiny Lakes. The reason for this is probably related with the environmentally more stable freshwater habitats of the relict Vashutkiny Lakes in comparison with the lakes of the High Arctic.

Keywords

brooding, Bolshezemelskaya Tundra, embryonic growth, *Pisidium* species, reproduction, relict lakes

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Introduction

The Vashutkiny Lakes represent the largest water system in the East of the Bolshezemelskaya Tundra and they have attracted the attention of hydrobiologists for many years (Zvereva et al. 1964, 1966; Leshko 2002; Sidorov 2002). The study of freshwater fauna of the Vashutkiny Lakes was started by A.V. Zhuravsky (1904). More than half a decade later, complex studies were carried out by the researchers of the Komi Branch of the USSR Academy of Sciences in European northeast Russia (Zvereva et al. 1964, 1966; Popova 1966; Zvereva 1966). These lakes are comparable to northern boreal lakes in terms of mollusk species diversity and density. The hypotheses proposed by Zvereva (1966) on the relict origin of the ecosystem of these lakes and on the leading role of intrazonal factors in the formation of their abnormally high level of production have been analyzed by Bolotov et al. (2014). Recently, the species diversity of freshwater mollusks has been studied and the influence of environmental factors on their distribution in lakes has been assessed (Bolotov et al. 2014).

According to previous data, 24 species of mollusks have been identified and the *Pisidium casertanum* (Poli, 1791) was considered dominant in the lakes (Bolotov et al. 2014, our unpublished data). It is well known that the *P. casertanum* is the most common *Pisidium* species and it is truly cosmopolitan, being distributed worldwide (Korniushin and Glaubrecht 2006; Guralnick 2004). Their widespread distribution in the Arctic and on nearly every continent is likely facilitated by a broad variation in life-history traits, such as age at first reproduction, time of egg-laying, time of embryo release, brood size and the number of generations per season. However, these studies of sphaeriid life histories in the Arctic are limited (Kuiper et al. 1989; Bespalaya 2015; Bespalaya et al. 2015). The reproduction of the *P. casertanum* in three types of Arctic lakes (glacial-tectonic, thermokarstic lakes, and glacial lakes) is currently under study. It was established that the average number of embryos was positively correlated with only the Mg$^{2+}$ content (Bespalaya et al. 2019). Patterns of clam reproduction were influenced by differences in freezing times of the water body. The brooding mechanism involves asynchronous development and embryo release by the parent (Bespalaya et al. 2015).

The aim of our study is to describe the reproduction of *P. casertanum* in the relict Vashutkiny Lakes System.

Material and methods

Study area

This study was conducted in the Bolshezemelskaya Tundra. This territory is bounded by the Pechora River from the West and the Usa River from the South, as well as the Polar Urals and Pai-Khoy Mountain from the East (Goldina 1972). In August 2016, mollusks were collected and water parameters were measured from nine lakes of the Vashutkiny Lakes System in the Eastern part of the Bolshezemelskaya Tundra (68°04’N, 61°35’E) (Fig. 1). The Vashutkiny Lakes represent the largest lake system of the Bolshezemelskaya Tundra, which belongs to the Adzva River basin (a tributary of the Usa River) with a total area of about 85 km$^2$ (Zvereva et al. 1966). The system includes nine lakes: Vanukty, Diyaty, Bolshoy Starik, Maliy Starik, Pernaty, Makty, Bolbanty, Seito, and Yurto. The research area is located within the tundra zone (Khokhlova 2002) and is characterized by a long cold winter with a stable snow cover, a short growing season, and a large amount of precipitation (Goldina 1972). The natural conditions of the area are characterized by the presence of permafrost (Khokhlova 2002). The average temperature of the warmest month (July) varies from +11.6 to +12.3 °C and of the coldest month (February) varies from −18.5 to −19.6 °C (Goldina 1972). The start of ice formation on the Vashutkiny Lakes System has been recorded in October–November. The beginning of the spring ice drift has been observed of from the end of June onwards (Goldina 1972).

Sampling and laboratory analysis

To characterize the sizes and reproductive features of *P. casertanum*, 197 benthic samples were collected at 47 stations in the 9 lakes of the system. For each station, 2–7 replicates were gathered with a
Fig. 1. Map of the study area. A – general view of the map revealing the geographic position of the Vashutkins Lakes, (red point); B – general view of the Vashutkins Lakes System. Sampling locations marked in orange circles; C – view on the Seito Lake from a helicopter; D – field works on the Seito Lake

rectangular hand net (dimensions 0.28 m × 0.5 m, mesh size 200 μm), covering a total sampling area of 0.14 m², or with a Peterson dredge (0.024 m²) in deeper waters. Samples were washed using a hydrobiological sieve (mesh size 0.56 mm) and fixed in 96% ethanol. A total of 412 *P. casertanum* specimens of all size classes were collected, measured and dissected. The specimens of *P. casertanum* were examined in the laboratory using a Leica M165C stereomicroscope (Fig. 2). The estimation of age at sexual maturity was undertaken in accordance with Bespalaya et al. (2015). Shell length (SL) was measured for all *Pisidium* specimens. After dissection, the number of brooding specimens in each size class was recorded. For this, each left and right marsupial sac was dissected, and embryos were counted and measured (maximum anteroposterior dimension) using a microscope with a stage micrometer. The early-stage embryos (≤0.05 mm) were not measured. The ontogenetic stages of embryos were recorded in accordance with Meier-Brook (1977) and Heard (1977), and as detailed by Bespalaya et al. (2015).

Shell length measurements were used to estimate the cohort structure of *P. casertanum* in the nine lakes. Photographs of the shell, and anatomy of *P. casertanum* were obtained with a Leica M165 C stereomicroscope and a Leica DFC 425 digital camera.

The differences in the shell length, number and length of embryos of *P. casertanum* between lakes were estimated using Kruskal-Wallis (multiple comparisons) tests in the program package PAST (Hammer et al. 2001). Relationships between number of embryos and the parent shell length were analysed by linear regression.
Fig. 2. The external morphology of the shell, anatomy and hinge of the right and left valves of *P. casertanum* from the Vashutkiny Lakes. **A** – external morphology of the right valve; **B** – internal anatomy of the dissected *P. casertanum*, **C** – teeth of the right valve; **D** – teeth of the left valve; **E** – general view of the male and female gonad of *P. casertanum*: o – oocyte, n – nucleus, sp – spermatocytes and spermatids aggregations.

Hydrochemical characteristics of water from lake's surface (pH, conductivity, dissolved O$_2$, Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, HCO$_3^-$, SO$_4^{2-}$, Cl$^-$ concentrations) were taken according to the methodology (Shevchenko et al. 2016).

**Histological examination**

For histological examination were taken 5 randomly selected specimens of *P. casertanum*. Tissues were dehydrated through a graded alcohol series and embedded in paraffin. Histological sections with a thickness of 6 μm were made using a rotary microtome (HM 325, Thermo Scientific). The sections were stained with Mayer’s hematoxylin-eosin double stain for examination under a light microscope (Axio Lab.A1, Carl Zeiss, Germany).

The materials are stored in the collection of the Russian Museum of the Biodiversity Hotspots of the N. Laverov Federal Center for Integrated Arctic Research of Russian Academy of Sciences, Arkhangelsk, Russia.

**Results**

**Field sampling**

The hydrochemical characteristics of the Vashutkiny Lakes are presented in Table 1. In general, the lakes are characterized by low mineralization, pH varies from neutral to weakly alkaline, and the differences between the lakes with respect to hydrochemical indicators within the system are low (Goldina 1972; Shevchenko et al. 2016; Table 1).

**Size structure and reproductive features of Pisidium casertanum**

The size frequency structure of the *P. casertanum* population is presented in Fig. 3. In general, populations of this mollusk had similar size-frequency across lakes. In the studied populations of *P. casertanum* the clams with shell length in the range 2.2–2.9 mm (63.5% of the total sample, *N* = 412) prevail. The maximum shell length of *P. casertanum* in the Vashutkiny Lakes was 3.8 mm. The bivalves from Vanukty Lake, Bolshoy Starik Lake, and Yurto Lake had significantly larger shells compared with those from any other lake (Kruskal-Wallis test: *H* (df = 8, *N* = 412) = 37.8, *P* < 0.0001) (Table 2).

The average shell length of juveniles at birth was 0.96 mm ± 0.2 (0.5–1.3 mm) (*N* = 73). The bivalve populations in all nine lakes were characterized by having a higher number of nongravid than gravid individuals. The percentage of gravid mollusks varied from 3 to 15%. According to our data, the brood sacs with embryos in the examined population are formed when the shell length of mollusks is at least 2.2 mm. The proportion of juvenile (pre-reproductive) to mature bivalves was from 31.6 to 68.4%, respectively, in our total sample.

The mean number and length of embryos by class are shown in Table 2. The number of embryos...
brooded by P. casertanum was not significantly different across the nine studied lakes (Kruskal-Wallis test: H (df = 8, N = 185) = 7.6, P = 0.4). The length of embryos in Classes 1, 2, and 3 also were not significantly different among thee studied lakes (Kruskal-Wallis test: H (df = 8, N = 185) = 5.6, P>0.05). The brooding individuals from lakes Vanukty, Bolshoy Starik, Maliy Starik, Makty, Bolbanta, Seito, and Yurto, have a fully developed extramarusial larvae (Class 4); and clams from the other two lakes showed a higher proportion of larvae corresponding to Classes 1 to 3 (Fig. 4).

The presence of mature eggs and sperm in the gonads, as well as developed oogonic and spermatogenic areas, may indicate that the mollusks are at the stage of active reproduction (Fig. 2).
According to our results, the number of embryos is correlated with the parent shell length, i.e. the greater shell length of the parent individuals, the higher the number of embryos (Fig. 5). Variations in embryo size within individuals were observed (Table 2). At the same time, we did not find clams that contained embryos within a single brood sac at different stages of development.

**Discussion**

We studied the size distribution and some reproductive features of *P. casertanum* from the Vashutkiny Lakes. The gonads conditions indicate that the mollusks are at the stage of active reproduction (Fig. 2). In early August, the individuals of medium size classes 2.2–2.9 mm (63.5%) prevailed in the population of *P. casertanum* in the Vashutkiny Lakes (Fig. 3). The proportion of juvenile individuals was 31.6.0%. Further, the bivalve population is characterized by having a higher number of nongravid mature individuals. The brooding specimens mainly have fully developed extramarsupial larvae (Fig. 4). This suggests that birth mainly occurs between July and August and the breeding season can probably begin in May – June (Fig. 2E). Our results are in accordance with observations of *P. casertanum* populations from Europe and North America as presented by Holopainen and Hanski (1986) and Bespalaya et al. (2015, 2019).

The shell length of adult individuals, the size of juveniles at birth, the length of embryos and their general number, correspond to the size parameters of *P. casertanum* in other parts of its distribution range (Holopainen and Jónasson 1983, Holopainen and Hanski 1986).
Fig. 4. Proportion of embryos in each class in *P. casertanum* collected from the nine Vashutkiny Lakes. Embryos in classes 1–3 are present in brood pouches; those in class 4 are extramarsupial.

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y = 16.172x - 3.2016 \\
R^2 = 0.6, P=0.002
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Fig. 5. Mean number of embryos versus shell length of *P. casertanum* from the Vashutkiny Lakes.
According to a number of studies the shell length of a *Pisidium* species being positively correlated to high calcium, conductivity, pH, alkalinity values and adult shell length (Hornbach and Cox 1987; Kilgour and Mackie 1991). Despite the generally low mineralization of the Vashutkiny Lakes, some of their areas have increased levels of dissolved salts as a result of local influences of mineralized subterranean waters (Zvereva et al. 1966, 1970; Goldina 1972). Probably, an increase the conductivity in Vanukty Lake, Bolshoy Starik Lake, and Yurto Lake could explain the larger length of the shell compared with those from any other lake. Although this question requires additional research.

Our previous research shows that the freshwater mollusk *P. casertanum* in the Arctic lakes of Vaigach Island and Yamal Peninsula has a specific process of breeding, accompanied by asynchronous development and spawning of embryos (Bespalaya et al. 2015, 2019). This reproductive strategy aims to improve the breeding success of the population within this extreme environment (Bespalaya et al. 2015, 2019). In general, variation in embryo size within a clutch was discovered in a number of works devoted to the study of the reproduction of sphaeriid species (Thut 1969, Meier-Brook 1977, Mackie 1979, Guralnick 2004). However, we have not found such adaptive strategies in populations of *P. casertanum* from the Vashutkiny Lakes. This variation in the developmental stage of embryos is not typical in populations of *Pisidium* species in temperate water bodies (Heard 1965, Mitropol’skii 1969, Araujo et al. 1999).

It can be assumed that the habitat conditions in the Vashutkiny Lakes of the Bolshezemelskaya Tundra were not stressful for the population of *P. casertanum*. Overall, the Vashutkiny Lakes are comparable to northern boreal lakes regarding mollusk species diversity and population density levels (Bolotov et al. 2014). The primary reason for the abnormal production of lakes is their flow regime, which offers intense convective heat exchange between water masses and supra-permafrost taliks under the lakes and leads to the accumulation of summer heat in friable Quaternary sediments, which are widespread in lake depressions. Intense water exchange together with strong wind-induced mixing hinders the stratification of water masses (Zvereva 1966, Zvereva et al. 1964, 1966, Bolotov et al. 2014).

The positive relationship between brood size and parent shell length was detected. This agrees with previous observations (Araujo et al. 1999, Guralnick 2004, Holopainen and Hanski 1986, Beekey and Hornbach 2004). It was established that there is a trade-off between offspring number and size, with larger embryos being found in smaller clutches when controlling for adult body size (Guralnick 2004). Probably, this fact may be related with the environmentally more stable habitat conditions of the Vashutkiny Lakes in comparison with the lakes of the High Arctic.

**Conclusion**

The *P. casertanum* population in the Vashutkiny Lakes probably has a single period of summer reproduction. We suggest that birth mainly occurs between July and August and the breeding season can probably begin in May – June. The positive relationship between brood size and parent shell length was detected. Hence, with respect to the freshwater mollusk *P. casertanum* in the Vashutkiny Lakes, there is no specific process of breeding, accompanied by asynchronous development and spawning of embryos.

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