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## REPRODUCTIVE FEATURES OF INDIGENOUS AND THE INVASIVE CHINESE FRESHWATER MUSSELS (MOLLUSCA, BIVALVIA, ANODONTINAE) IN UKRAINE

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**Reproductive Features of Indigenous and the Invasive Chinese Freshwater Mussels (Mollusca, Bivalvia, Anodontinae) in Ukraine.** Yanovych, L. M. — Propagation processes of indigenous mussels of water reservoirs and water currents of Ukraine proceed inadequately. In *Anodonta anatina* (Linnaeus, 1758), 100 % of female mussels reproduced in about 60 % of collection points, in the rest of collection points only 17–90 % of female mussels appeared to be “pregnant”, and in *A. cygnea* (Linnaeus, 1758) this number was about 50 %. In almost 70 % of collection points, *A. cygnea* and *Pseudanodonta complanata* Rossmässler, 1835 female mussels’ marsupia were not completely filled with reproductive products. On the other hand, invasive Chinese mussel, *Sinanodonta woodiana* (Lea, 1834), had multiple ovipositions during the whole year, its hemibranchs were completely filled with reproductive products.

**Key words:** mussels (Anodontinae), reproduction, “branchial pregnancy”, marsupia, Ukraine.

**Репродуктивные особенности коренных и инвазивных китайских пресноводных моллюсков (Mollusca, Bivalvia, Anodontinae) в Украине.** Янович Л. М. — Процессы размножения аборигенных видов беззубок из водоемов и водотоков Украины протекают неполноценно. Среди *Anodonta anatina* (Linnaeus, 1758) примерно только в 60 % пунктов сбора размножаются 100 % самок, в остальных пунктах «беременными» оказываются лишь 17–90 % самок, у *A. cygnea* (Linnaeus, 1758) — 50 % самок. Примерно в 70 % пунктов сбора самки *A. cygnea* и *Pseudanodonta complanata* Rossmässler, 1835 имеют не полностью (от ¼ до ¾) заполненные половыми продуктами marsупии. Зато вид-вселенец китайская беззубка, *Sinanodonta woodiana* (Lea, 1834), имеет многократные кладки в течение всего года. Полужабры половыми продуктами заполнены полностью.

**Ключевые слова:** беззубки (Anodontinae), размножение, «жаберная беременность», marsупии, Украина.

Studies on the reproductive processes of Unionidae, including Anodontinae, have become especially active in last decades in many countries (Finland, the USA, Turkey, and others) (Amyot, 1998; Başçınar, 2009; Hansten, 1997). This is due to the fact that the organization of environmental activities is impossible without the knowledge of the reproductive biology of species (particularly those in need of protection), because during the breeding season animals are most vulnerable. Abnormalities in reproduction of Unionidae species were reported in Europe (Pekkarinen, 1993). It was found, for instance, that marsupia were not completely filled with reproductive products, not all females had “branchial pregnancy”, hemibranchs were infected, and glochidia were abnormally developed.

Reproductive cycles of Unionidae from the European populations are clearly divided into two groups by reproductive features. Gametogenesis in representatives of the subfamily Unioninae takes place early in spring and, depending on the temperature, there can be 2–3 ovipositions. Embryogenesis lasts for several weeks and ends with formation of larvae (glochidia). In the Anodontinae, other direction of reproductive cycles is observed. Oviposition in marsupial branchial sacs happens only once in summer–early autumn. Glochidia develop until spring and then depart.

In Ukraine, reproduction of Unionidae has been thoroughly studied in water reservoirs and water currents of Central Polissia (Yanovych, 1998). As for the rest of the country, special researches of this subject have not been conducted before. The aim of this paper is to clarify the reproductive features of mussels of Anodontinae subfamily of the fauna of Ukraine.

## Material and methods

The material was collected in 2000–2012 in the river basins of Ukraine, including the Danube, Dniester, Western Buh, Prypiat, Dnipro, Desna, Siversky Donets, and in rivers of the Azov and Crimea regions. Mussels were gathered manually at the depth of 0.1 to 0.2 m within the year. The determination of mussel population density was carried out in places of their aggregation using the quadrant sampling method (Zhadin, 1952). The West European Mussel System (Glöer, 1998) was taken as the basis for the determination of the species origin, according to which three native species of Anodontinae subfamily (*Anodonta anatina* Linnaeus, 1758, *A. cygnea* Linnaeus, 1758, *Pseudanodonta complanata* Rossmässler, 1835) and invasive species (*Sinanodonta woodiana* Lea, 1834) live in Central Europe and in Ukraine. The gender of the studied mussels was determined by reproductive products received from gonads. “Branchial pregnancy” was deduced by the visual inspection or the temporary histological slides made from marsupial gills.

## Results and discussion

*A. anatina*, like other native freshwater mussels of Ukraine, is characterized by laying eggs in the gill cavity of hemibranchs at the end of summer. Glochidia develop during the cold period and then depart in spring. In the Dnipro basin upper (Lake Kamiane, Radomyshl, Zhytomyr Region) and middle (the Ros River, Bila Tserkva, Kyiv Region currents), in the Prypiat basin (the Horyn River, Horodets, Rivne Region; the Vydoloch River, Baranivka, Zhytomyr Region), in the Western Buh River (Pulmo Lake, Zalissia, Volyn Region) individuals laying eggs were recorded in the second decade of August. In the Southern Buh basin (the Southern Buh River, Lupolove, Kirovohrad Region) these females were recorded at the end of July, and in the Lower Danube (Vylkove, Odessa Region) in September. The percentage of “pregnant” individuals gradually increases. Within few weeks glochidia were observed in hemibranchs. At the same time, some females still had eggs in hemibranchs. It indicates that not all animals begin to reproduce at the same time. In particular, among *A. anatina* from the Huiva River (Kashperivka, Vinnytsia Region) collected on October 15, 2009, two specimens had glochidia, while the other one had eggs. In some cases, almost developed glochidia and developing eggs were observed in hemibranchs at the same time. It suggests that the female has multiple ovipositions. The release of glochidia in the basins of all rivers occurs gradually in April and May.

In total 14 *A. anatina* collection points were examined. In 8 of them, 100 % female “branchial pregnancy” was observed. In the rest of collection points 17–90 % of females were pregnant. In 4 collection points (the Southern Buh River, Lupolove, Kirovohrad Region; the Sukhyi Tashlyk River, Lypniashka, Kirovohrad Region; Pulmo Lake, Zalissia, Volyn Region; the Ros River, Bila Tserkva, Kyiv Region) there were only 25–33 % pregnant females collected at the end of July–August. The eggs were registered in their marsupia, this observation confirms the beginning of propagation processes. That is why, probably, the number of pregnant females would increase. In two other collection points (Kamiane Lake, Radomyshl, Zhytomyr Region; the Danube River, Vylkove, Odessa Region) examined late in autumn, winter or early in spring before departing, not all females reproduced. In marsupia of pregnant individuals, formed glochidia were observed. That meant that the spawning period was over. The histological examination of gonads of non-pregnant females showed the absence of ova in acini. These females did not reproduce at all or laid eggs but were not impregnated. On September 16, 2009 17 specimens were collected from Stone Lake (Radomyshl, Zhytomyr Region), 16 of them appeared to be females and only one was a male. Eight females had glochidia, the remaining 7 (44 %) were not “branchial pregnant”. At the same time, the gonads of 6 specimens that had not reproduced contained no ova and looked desolate and filled with small germinal cells. Parasitic castration was traced through the infection of almost all the gonad with trematodes, gills of one of the pregnant females were completely filled with glochidia, the rest were slightly more than half-filled. This phenomenon is sometimes observed in young specimens that reproduce for the first time. However, in this case mature puberal specimens had half-filled hemibranchs. Thus

reproduction of the following group of mussels is defective. The reason of such a phenomenon can be the low population density (less than 6 individuals/m<sup>2</sup>) that prevents the normal fertilization; the impact of aquatic environment pollutants: all germ cells are extremely sensitive even to the low concentration of toxicants and will experience destructions in the gonad acini (Yanovych, 2004). In addition, “abortion” of eggs of glochidia is a typical defense reaction of Unionidae in response to the negative influence of environmental factors (Stadnychenko, Yanovych, 2004; Aldridge, 2003). Only in some collection points (the Sluch and the Vydoloch Rivers, Baranivka, Zhytomyr Region; the Guiva River, Kashperivka, Vinnytsia Region; the Danube River, Vylkove, Odessa Region; Kamiane Lake, Radomyshl, Zhytomyr Region; the Teteriv River, Zhytomyr) mussels’ hemibranchs were filled completely or almost completely and 100 % of females had “branchial pregnancy”. Previously, this situation was common for the reproduction of *A. anatina* (Yanovych, 1998). The calculation of the number of glochidia in females’ hemibranchs of this species at two collection points (the Sluch River, Baranivka, Zhytomyr Region; the Kodenka River, Solotvyn, Zhytomyr Region) shows that the number of glochidia is 5,500–40,400 per female, while in the 90s in Zhytomyr Polissya individual female fecundity was 36,600–319,950 glochidia (Yanovych), and in the early XX century this number for freshwater mussels was from 288,000 to 694,000 (Troitskiy, 1939). The investigation of the vital activity of *A. cygnea*, including its reproductive cycles, is complicated due to its small frequency of occurrence (10 %) and small number of studied specimens. The “branchial pregnant” females in the Teteriv River were first observed on September 3. At the same time, eggs registered in marsupia and hemibranchs were filled completely. In the same collection point, *A. anatina* females had glochidia, i. e. they probably began to reproduce. Closer to the middle of the month one out of three females from this collection point had eggs in marsupia. In October, 50 % of females were noted to have glochidia and gills filled completely. In early October, only one *A. cygnea* was collected in the Huiva River (Kashperivka, Vinnytsia Region) and it had glochidia and its gills were filled completely. Females from Kamiane Lake studied on April 24 (Radomyshl, Zhytomyr Region) did not have glochidia. Females collected on May 28 from other collection point (the Sluch River, Myropil, Zhytomyr Region) were also non-pregnant. In June, pregnant *A. cygnea* were not found at all. Thus, reproduction processes of *A. cygnea* from the Teteriv River (Zhytomyr) are the most fully investigated, with about 50 % of females that participated in it. Our researches conducted in the 90s of the XX c. showed that 100 % of females of the Polissian population reproduced (Yanovych, 1998).

Therefore, the comparison of reproductive cycles of modern settlements with those described in literature (Stadnychenko, 1984) suggests that today the participation of only a part of females in reproduction processes considerably reduces the reproductive potential of the species (fig.1).



Fig. 1. *A. cygnea* female with the “branchial pregnancy”.

A very small number of *P. complanata* specimens (mainly single individuals) were collected from almost each of their habitats, therefore, the results concerning the reproduction of this species are rather fragmented.

Among other indigenous freshwater mussels *P. complanata* from watercourses of Ukraine begins laying eggs in marsupia the first — in early July, although a portion of last year glochidia generation occurs in spring — early in summer. Thus, carrying of “branchial pregnancy” by false freshwater mussels lasts the longest. Results obtained are consistent with the literature data. For instance, in British and Finnish populations *P. complanata* are free from pregnancy in May, in Austrian — in March and May (Mcvor 2007; Pekkarinen, 1993), in the European part of the former Soviet Union — in July and August (Zhadin, 1938). The maximum use by animals of warm season for spawning and glochidia development emphasizes *P. complanata* ability to exist in colder climate conditions and low water temperatures in contrast to other freshwater mussels and agrees with the type of habitat that covers the central and northern Europe.

In the waters of different river basins of Ukraine, false freshwater mussels start to reproduce at different times. For instance, in the Latorytsia River (Solomonove, Zakarpattia Region) developing glochidia were registered on July 2; in the Revna River (Semenivka, Chernihiv Region) the female with eggs was observed on July 16. At the same time, in the Murapha River (Bila, Vinnytsia Region) and the Stalineshti River (Mamalyha, Chernivtsi Region) the females with developing glochidia were observed in early August.

Mussels of this kind in populations of the Central Polissia (the Sluch River) and the Danube Delta begin to reproduce in mid-August and early September.

In British reservoirs *P. complanata* gradually come into spawn from June to August. Since only a small number of individuals of Ukrainian populations were studied, we could not follow the dynamics of spawning.

The terms of the beginning of the time when glochidia are released vary. In the populations of Polissia from the Ubort River (Kyshyn, Zhytomyr Region) females release glochidia in mid-March – early April (Mel'nichenko, Yanovich, 2000), from the Sluch River and its tributaries (the Sluch River, Baranivka, Zhytomyr Region; the Tnia River, Molodizhne, Zhytomyr Region) — at the end of May. In the Danube River (Vylkove, Odessa Region) females without the “branchial pregnancy” were registered on June 6.

We believe that the differences in the beginning and in the end of the spawning period, releasing of glochidia are caused by temperature conditions of specific habitats. The quantity of glochidia that are carried by *P. complanata* females is the smallest among freshwater mussels. According to our data (Mel'nichenko, Yanovich, 2000), animals of Polissia population carry 21,600–38,100 glochidia, animals of British population carry 5,000–50,000 (Mcvor, 2007). Some researchers report even higher numbers, up to 310,000 (Stadnichenko, 1984).

In addition, not all hemibranchs of these females are often filled with reproductive products, as indicated by other researchers (Pekkarinen, 1993). It is necessary to emphasize that among other native freshwater mussels *P. complanata* becomes sexually mature the latest: according to our data, in 3–4 years (Mel'nichenko, Yanovich, 2000), according to the literature — in 4–5 years (Stadnichenko, 1984).

Thus, analyzing the reproductive biology of *P. complanata*, we can conclude that false freshwater mussels of ponds and streams of Ukraine not fully realize their reproductive potential because of the low population density, little fecundity, etc., which in turn can be the cause of the low occurrence frequency and settlement density.

In order to study the reproductive cycle of the invasive mussel *S. woodiana* a year-round examination of its individuals collected within the Ukrainian part of the Danube Delta (Vilkovo, Odessa Region), analysis of samples taken in the Latorytsia river and its system (Zakarpattia Region) were held. The reproduction of *S. woodiana* has substantial differences from indigenous species. Females with the “branchial pregnancy” are traced



Fig. 2. *S. woodiana*'s hemibranchs filled with glochidia.

during the whole year, whilst some of them contain glochidia in marsupia; others have egg cells on different levels of segmentation. The population from the Danube estuary witnessed 100 % of females in March, May, June, July and October while in other months — from 33 to 89 %. It proves that egg laying occurs frequently during the year. Hemibranchs are filled with reproductive products completely and look turgid, the amount of glochidia is 220–680 thousand (fig. 2).

Consequently, the detailed research of reproductive processes of indigenous species (*A. anatina*, *A. cygnea*, *P. complanata*) from Ukrainian populations has proved that their reproductive potential is realized not entirely. In *A. anatina*, 100 % of females propagate in approximately 60 % collection points, whereas other collection points witness 17–90 % of “pregnant” females. Being the well-explored population, only 50 % of *A. cygnea* females carry eggs. *A. cygnea* and *P. complanata* do not have marsupia fully filled with reproductive products (from  $\frac{1}{4}$  to  $\frac{3}{4}$ ) in nearly 70 % collection points. In comparison with indigenous species, the invasive mussel *S. woodiana* has multifold egg layings during the year; from 33 to 100 % of females gestate the pregnancy. Hemibranchs in *S. woodiana* are filled with reproductive products entirely.

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