

Temporal Changes in Gametogenesis of the Invasive Chinese Pond Mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) from the Konin Lakes System (Central Poland)*

Piotr HLIWA, Bogusław ZDANOWSKI, Grzegorz J. DIETRICH, Aneta ANDRONOWSKA,
Jarosław KRÓL, and Andrzej CIERESZKO

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Gametogenesis and the temporal changes occurring in the ovaries and testes throughout the reproductive cycle in the invasive alien bivalve, Chinese pond mussel *Sinanodonta woodiana* (Lea), from the heated Konin lakes system (central Poland) were studied using histological techniques. *S. woodiana* was confirmed to be a gonochoristic species with overall sex ratio of 1:1. The examined morphological parameters of Chinese pond mussel spermatozoa, i.e. 42 μm mean total length; 4.3 μm mean head length and the maximum size of previtellogenic (34-43 μm) and vitellogenic oocytes (75-83 μm) are consistent with values established for closely related members of the Unionidae family. Our results suggest that *S. woodiana* in the Konin lakes system are able to spawn throughout March to October, with a season of higher reproductive activity in females extending from March to April. This type of reproductive biology may contribute to the Chinese pond mussel's success in thriving in freshwater ecosystems.

Key words: Gametogenesis, size of gametes, Konin lakes system, invasive mussel, *Sinanodonta woodiana*.

Piotr Hliwa, Jarosław Król, Department of Ichthyology, University of Warmia and Mazury in Olsztyn, Warszawska 117A, 10-957 Olsztyn, Poland.

E-mail: phliwa@uwm.edu.pl

Bogusław Zdanowski, The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Ocza-powskiego 10, 10-719 Olsztyn, Poland.

Grzegorz J. Dietrich, Andrzej Ciereszko, Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Science, Bydgoska 7, 10-243 Olsztyn, Poland.

Aneta Andronowska, Department of Hormonal Action Mechanisms, Institute of Animal Reproduction and Food Research, Polish Academy of Science, Bydgoska 7, 10-243 Olsztyn, Poland.

The Chinese pond mussel, *Sinanodonta woodiana* (Lea, 1834), is one of the largest unionid species native to the Amur-Yangtze river basin (WATERS 1997). *S. woodiana* arrived in Poland from Hungary with herbivorous cyprinid fish fry in the late 1980's and since then a large population has become established in the Konin lakes system, in specific heated waters (AFANASJEV *et al.* 2001; KRASZEWSKI & ZDANOWSKI 2001). Initially the presence of *S. woodiana* was related only to the heated water of Konin-Pątnów and Dolna Odra power plants but at present there are about ten con-

firmed locations of this species in different parts of Poland (URBAŃSKA *et al.* 2012).

Like many invasive species, *S. woodiana* constitutes a significant threat to its new environment. It is established that *S. woodiana* may compete for food with the local native population of molluscs in the Konin lakes system similarly to competition between native and invasive unionids in North America (DOUDA *et al.* 2012). The Chinese pond mussel is likely to compete for hosts with native European unionids which release their glochidia at the same time (BLAZEK & GELNAR 2006).

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S. woodiana might also be responsible for a change in the bottom fauna composition and considerably affect the ecological stability, similarly to such phenomena repeatedly recorded among freshwater molluscs and other invertebrates (HEBERT *et al.* 1989; STRAYER 1991; SPIDLE *et al.* 1994; LEE 1999). Finally, contact with glochidia can trigger an adaptive immune response by hosts, immunizing them against repeated infections and significantly decreasing the number of available for native mussels (ROGERS & DIMOCK 2003).

Two basic brooding patterns have been recognized in freshwater unionids (HEARD & GUCKERT 1970). Some species retain their glochidia for a relatively short-term (i.e. tachytictic brooders); bivalves become gravid in the late winter or spring and retain and release larvae only during summer. Other bivalves (bradytictic brooders) become gravid in late Nearctic summer or fall and retain their glochidia in the brood chambers all winter until release in early to late spring. Quantitative assessments of unionid reproductive biology suggest that the duration of gametogenesis, spawning, and brooding periods may be related to the seasonal timing of these reproductive activities (GARNER *et al.* 1999; HAGGERTY & GARNER 2000).

Effectiveness of reproduction is one of the critical factors responsible for colonization success. Unfortunately detailed descriptions of temporal changes in gametogenesis of *S. woodiana* outside their natural range are very limited. DUDGEON and MORTON (1983) established that this species is dioecious with one peak of reproductive activity each year in Plover Cove Reservoir, Hong Kong. Recruitment occurs during the summer soon after the females attain gonadal maturity. Males contain mature spermatozoa throughout the year. Regarding natural conditions, the gonads of *S. woodiana* were only estimated by reference to five indices, from primordial to spent.

Effective invasion of mussels in new freshwater reservoirs is possible through the establishment of a breeding population, however published data concerning *S. woodiana* reproductive biology in the Konin lakes system are unavailable. Moreover data on basic reproductive characteristics such as oogenesis and spermatogenesis are also missing for *S. woodiana* populations living outside of their natural range in different geographical zones. Furthermore, there are no data about either morphology of mature gametes or histological changes of the gonads during the annual cycle. This study is intended to provide basic information concerning *S. woodiana* reproduction in a heated lake system as a basis for future comparative analyses between bivalves inhabiting thermally diversified reservoirs. Such data are a prerequisite for a better understanding of the successful spread of Chinese pond mussel into new areas.

The aim of the study was to investigate the annual cycle of gonads (ovaries and testis) and to determine morphological characteristics of germ cells of *S. woodiana* in the heated Konin lakes system during the reproductive season.

Material and Methods

Origin of material

Chinese pond mussels were collected by scuba diving (1-2 m depth) from a canal and its adjacent initial cooling reservoir – representative habitats with a special thermal regime of the Konin and Pałnów power plants. The hydro-ecological characteristics and disturbances in heated Konin lakes and the water cooling system were described by ZDANOWSKI (1994). This power plant cooling system network consists of 26 km of discharge channels and 6 km of water intake channels. Water exchange in the reservoir occurs every three to four days (KRASZEWSKI & ZDANOWSKI 2001).

Altogether 91 individuals of Chinese pond mussels were collected at monthly intervals (7-8 per month) from March to October 2009 and 2010 from the area of the power plant intake and discharge canal. Mussels were kept in water and transported to the laboratory at the Department of Ichthyology of the University of Warmia and Mazury in Olsztyn, where they were measured (total length, total width to the nearest 1 mm) and weighed (total mussel weight, total soft tissue weight to the nearest 1 g). Data concerning temperature (means \pm SEM) and photoperiod during experiment were adapted from measurements made by Pałnów-Adamów-Konin Power Plant Company S.A. (Fig. 1).

Histological analysis

The mussels were dissected and the visceral mass of each specimen was evaluated to locate gonadal tissue. Two different sections of the central part of each mussel's visceral mass were cut for histological examination. These wedges were fixed for 24 hours in Bouin's fluid and then preserved in 70% ethanol. Dehydration and embedding protocols followed ZAWISTOWSKI (1986). Slices 4-5 μ m thick were cut using a rotational microtome model RM 2155 (LEICA Microsystems, Wetzlar, Germany), stained with haematoxyline (Aqua-Med ZPAM – Kolasa Ltd. Łódź, Poland) and counterstained with eosin (POCH S.A. Gliwice, Poland). Histological analyses of cross-sections for the shape, the type of germ cells and size (range; mean \pm SEM) present in gonads were made using a LEICA DM 3000 transmission light

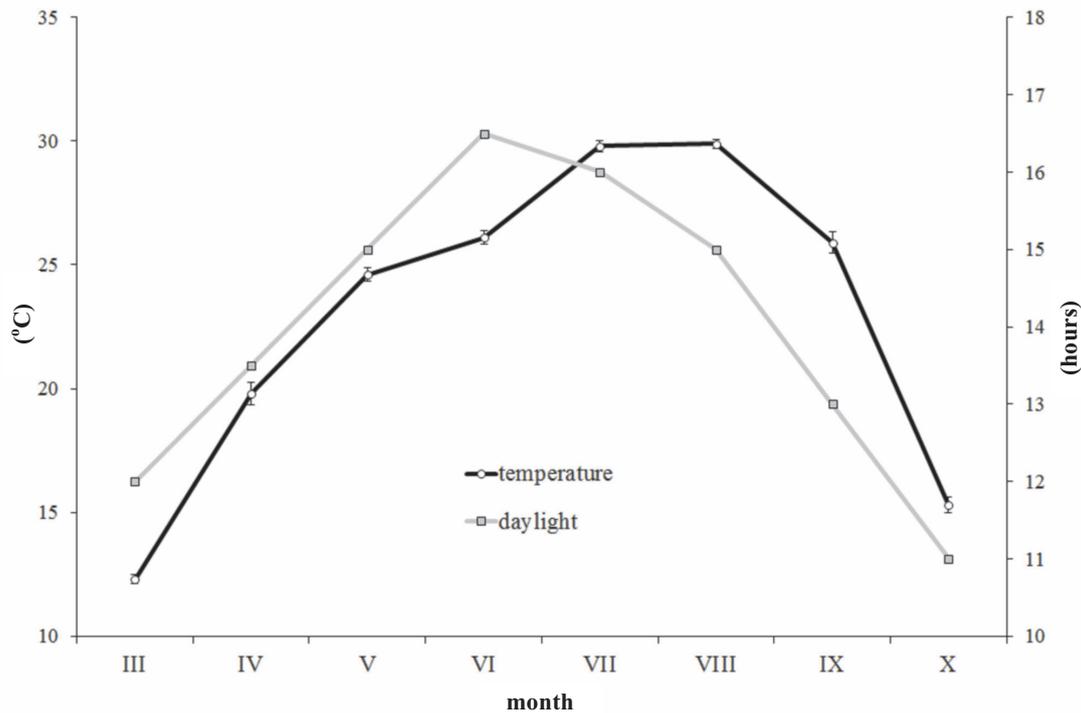


Fig. 1. Seasonal changes of temperature (means \pm SEM) and daylight in Konin lakes system during 2009-2010 years.

microscope and micro image computer analysis software LEICA QWin Pro (LEICA Microsystems AG, Heerbrugg, Switzerland). In order to determine the monthly changes in the number of oocytes at different developmental phases, oocytes were counted using the method of KOPIEJEWSKA (2003). The nomenclature of germ cells and cellular structures was used according to PARK and CHUNG (2004) and CHATCHAVALVANICH *et al.* (2006).

Scanning electron microscopy

Suspensions of fixed spermatozoa (in Karnowsky's fixative) were allowed to settle on glass coverslips coated with poly-L-lysine. Then they were post-fixed in 1% OsO₄, dehydrated and dried in a critical point drier and mounted on aluminum stubs. Samples were sputter coated with gold, and examined under a scanning electron microscope (JSM 5200 model). Morphological spermatozoa parameters were measured using MultiScanBase v. 8.08 (Computer Scanning System Ltd., Warsaw, Poland) (KOWALSKI *et al.* 2006).

Results

Size of mussels and sex ratio

All of the analyzed Chinese pond mussels were mature with a mean total shell length of 15.3 (\pm 0.2 SEM) cm and shell width of 9.4 (\pm 0.1 SEM) cm, respec-

tively. The average total weight of mussels was 336 (\pm 12 SEM) g and soft tissue weight was 96 (\pm 4 SEM) g. The relationship between shell length and shell width showed that males and females shared a similar mode with no evidence of a wider shell shape in females. No evidence of sexual growth dimorphism of *S. woodiana* was found (Fig. 2).

The majority of examined Chinese pond mussels in the Konin lakes system were gonochoristic – 44 males and 46 females were found. Only one (1.1%) specimen was a hermaphrodite.

Spermatogenesis and annual testis cycle

Spermatogenesis in the Chinese pond mussel was characterized by the presence of the following cells: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (Fig. 3A-H). Spermatid morulae, specific for mussel germ cells, were also observed in their testes (Fig. 3C-H). Additionally, food elements (unicellular and multicellular algae) from the gastrointestinal tract were visible in some histological cross-sections of gonads (Fig. 3A). During the annual testes cycle, from March to October, three to five stages of sex cells were found (Fig. 3). Primary and secondary spermatocytes, spermatid morulae, and spermatids were embedded in poorly marked, quite irregular seminal follicles with walls separated by a thin layer of stroma. Except for spermatozoa, spermatogonia and all other germ cells of Chinese pond mussel were round.

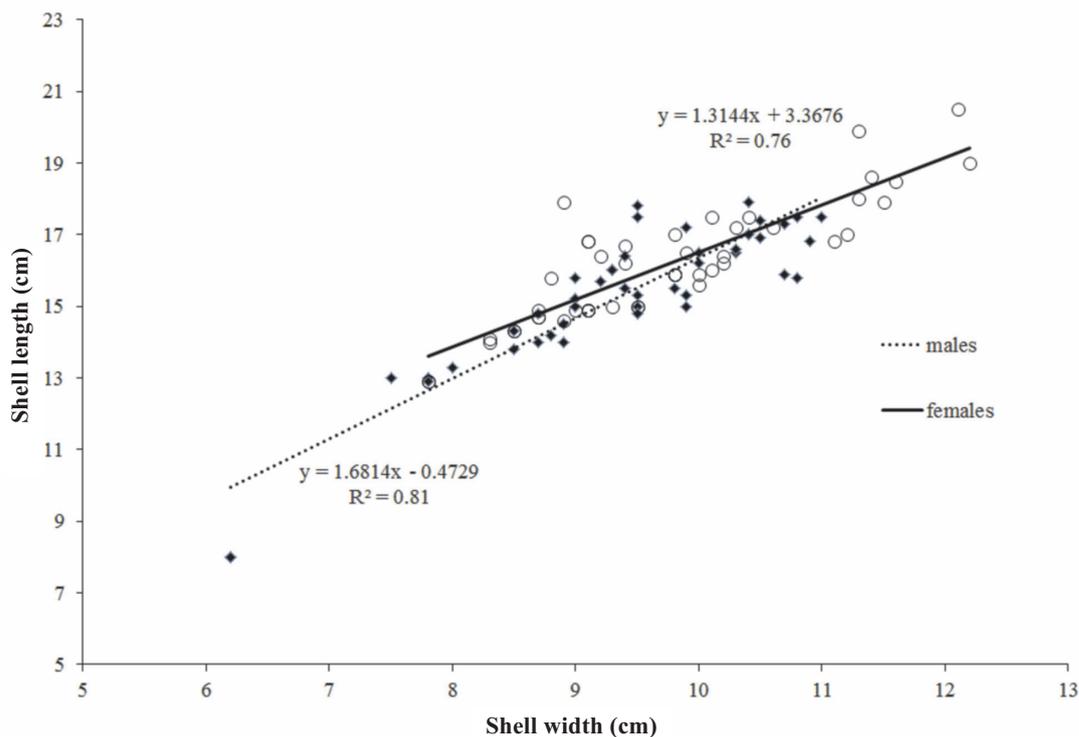


Fig. 2. Relationships between shell length and width of Chinese pond mussel from the Konin lakes population. ○ – females, ◆ – males.

Spermatogonia, germ cells with the largest diameter, contained relatively little cytoplasmic material were deposited near the seminal vesicle walls (Table 1, Fig. 3H). In March when the water temperature exceeded 12°C (Fig. 1), secondary spermatocytes quantitatively dominated in the testes. Spermatids and a few primary spermatocytes were also visible. The walls of seminal follicles were irregular and separated by wide splits (Fig. 3A). In April, the seminal vesicles contained primary and secondary spermatocytes, cells with a large heterogeneous nucleus stained intensively with haematoxyline. Spermatocytes did not differ in size (Table 1), however both types of these cells could be distinguished by the staining intensity of their nuclei. Secondary spermatocytes were clearly marked and located usually in the external part of seminal vesicles. In April, the central part of the seminal vesicles of testes contained spermatids and spermatozoa which were characterized by a smaller nuclear diameter (Table 1, Fig. 3B). In May and June, the round seminal vesicles were filled by spermatids, primary and secondary spermatocytes. Single spermatogonia and spermatid morulae were also observed on the edges of these structures. It should be mentioned that mature spermatozoa were evident during this period (Fig. 3C). Seminal vesicles of testes collected in June (Fig. 3D) and August (Fig. 3E) had an irregular shape and their walls were thinner than in previous months. In August with the highest daily water temperature

(Fig. 1), most germ cells were classified as spermatids, but in the histological cross-sections of *S. woodiana* testes, primary and secondary spermatocytes, single spermatogonia and spermatid morulae were also observed. Furthermore, spermatids were present on gonoducts, whose walls were lined with epithelial ciliated cells directed to the lumen of canals (Fig. 3E). A similar microscopic structure of testes was noted in September (Fig. 3F). In October, the germ cells in testes were diffuse and the seminal vesicle walls were fragmentary and very thin. In October, spermatogonia and spermatid morulae – the most differentiated cells in size (Table 2), were placed mainly in the lumen of seminal vesicles (Fig. 3G-H).

Morphology and morphometric parameters of spermatozoa

S. woodiana spermatozoa had a bullet-shaped head and long flagellum (Fig. 4A-B). The morphometric parameters of spermatozoa are shown in Table 2.

Oogenesis and annual cycle of ovaries

Ovaries of *S. woodiana* contained oogonia (og), previtellogenic oocytes (po), early (ov1) and late vitellogenic oocytes (ov2) and mature oocytes (mo) (Fig. 5A-F). Oogenesis occurred in follicles that varied in thickness of walls during the repro-

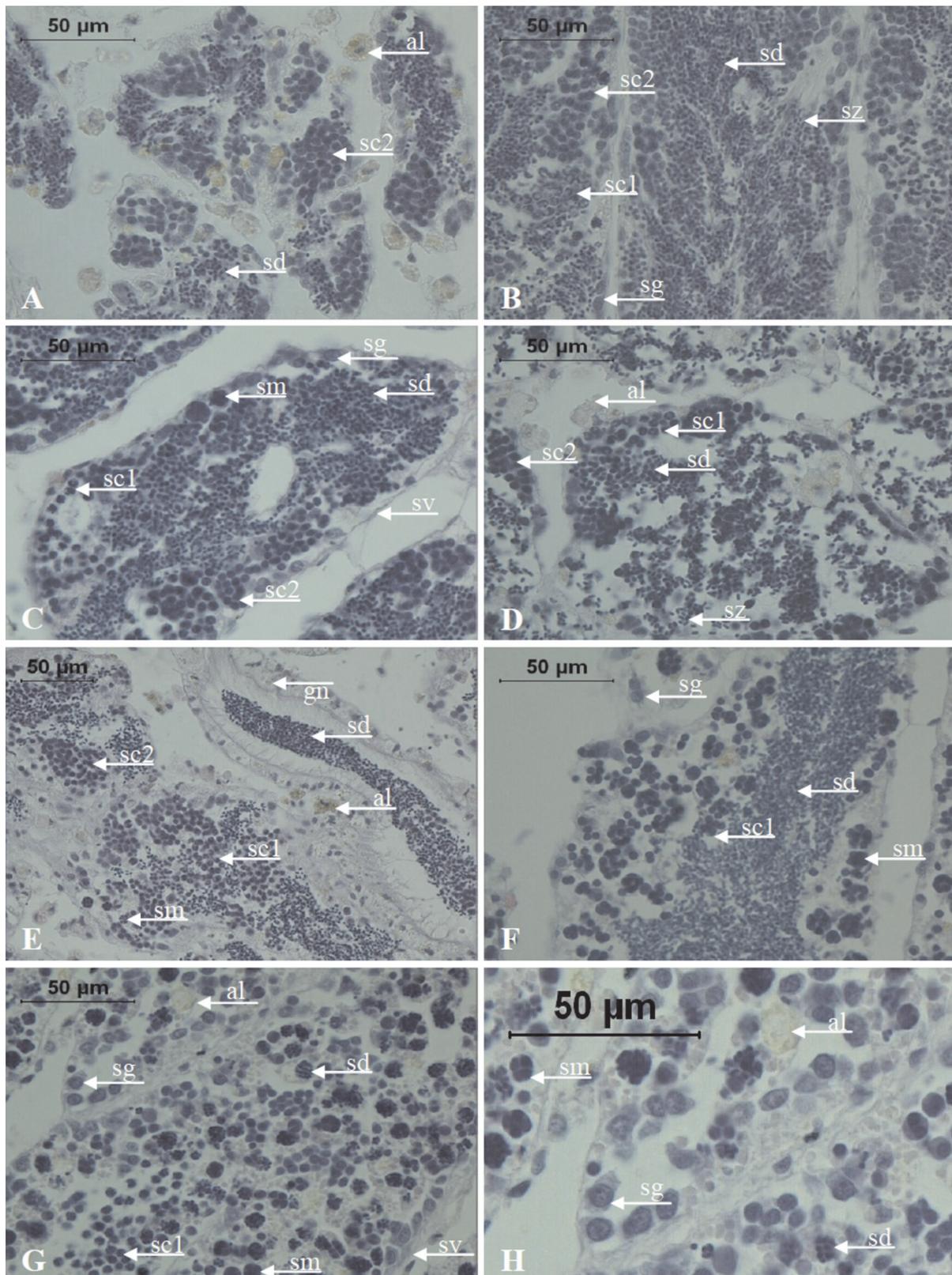


Fig. 3. Histological cross-sections of Chinese pond mussel testes during the annual cycle. A – March; B – April; C – May; D – June; E – August; F – September; G,H – October. Descriptions: al – algae; gn – gonoduct; sd – spermatids; sg – spermatogonia; sc1 – primary spermatocytes; sc2 – secondary spermatocytes; sm – spermatid morulae; sv – seminal follicle; sz – spermatozoa.

Table 1

The size of Chinese pond mussel germ cells from Konin lakes system (n = 50 for each type of cell)

Type of cell	Range (μm)	Mean \pm SEM (μm)
spermatogonia	5.9 – 7.5	6.7 \pm 0.08
primary spermatocytes	3.6 – 4.7	4.3 \pm 0.04
secondary spermatocytes	3.3 – 3.9	3.5 \pm 0.04
spermatid morulae (2 cells)	4.6 – 6.7	5.4 \pm 0.09
spermatid morulae (4 cells)	5.9 – 7.6	6.7 \pm 0.07
spermatid morulae (6 cells)	8.1 – 9.6	8.8 \pm 0.06
spermatid morulae (8 cells)	9.9 – 14.5	11.8 \pm 0.23
spermatids	1.1 – 1.5	1.2 \pm 0.01
oogonia	14.9 – 17.1	16.1 \pm 0.11
previtellogenic oocytes	34.0 – 43.1	37.0 \pm 0.47
late vitellogenic oocytes	74.6 – 82.9	78.7 \pm 0.54
mature oocytes	84.1 – 105.5	89.2 \pm 1.13

Table 2

Parameters of Chinese pond mussel spermatozoa (n = 50 cells)

Name of structure	Range	Mean \pm SEM
total length of spermatozoa (μm)	40.0 – 43.9	42.0 \pm 0.18
length of flagellum (μm)	34.5 – 38.3	37.2 \pm 0.27
length of the head (μm)	4.1 – 4.6	4.3 \pm 0.03
width of head (μm)	1.9 – 2.2	2.1 \pm 0.03

SEM – standard error mean

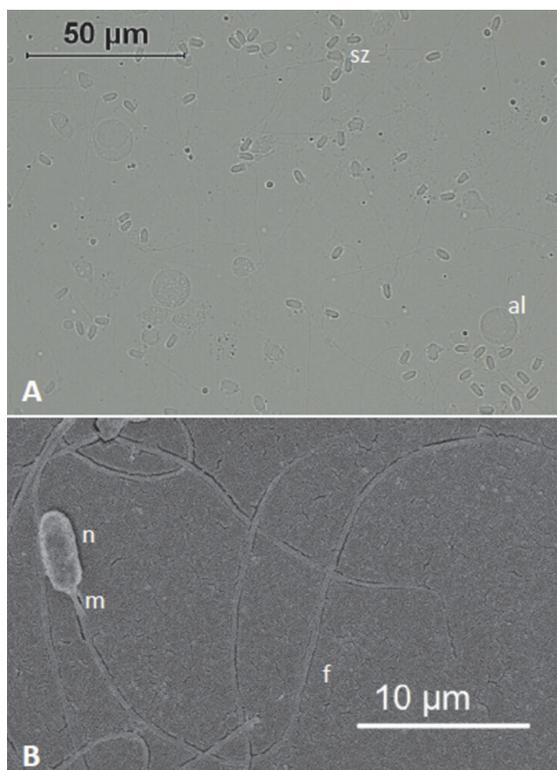


Fig. 4. Morphology of Chinese pond mussel spermatozoa. A – light microscopy image; B – scanning electron micrograph. Descriptions: al – algae, sz – spermatozoa, n – nucleus, m – midpiece, f – flagellum.

ductive season. In March, almost 70% of the germ cells present in gonadal tissue were mature oocytes with nuclei situated eccentrically (Table 1, Fig. 6). Additionally late vitellogenic oocytes and a few previtellogenic oocytes attached to the follicle walls of the ovaries by an egg stalk were visible (Fig. 5A). All oocytes were surrounded by stroma, and follicles reached their maximum size and the interfollicle space was minimal (Fig. 5A). Late vitellogenic oocytes dominated in April (37% of quantitative share of germ cells present in gonads). Mature oocytes and early vitellogenic oocytes were also found (Fig. 5B and Fig. 6).

In May, previtellogenic and early vitellogenic oocytes located in the lumen of follicles were dominant, constituting 37 and 27% of the quantitative share, respectively (Fig. 6). Spherical oogonia with diameters in the range of 14.9–17.1 μm (Table 1) were dispersed with chromatin among the stroma mainly close to or in the walls of follicles (Fig. 5C). They had large nuclei and light basophilic cytoplasm. However, previtellogenic oocytes had larger nuclei with multiple nucleoli and were generally arranged on the periphery of the germinal vesicle.

The follicles collected in June were mostly cytologically differentiated and filled with single oo-

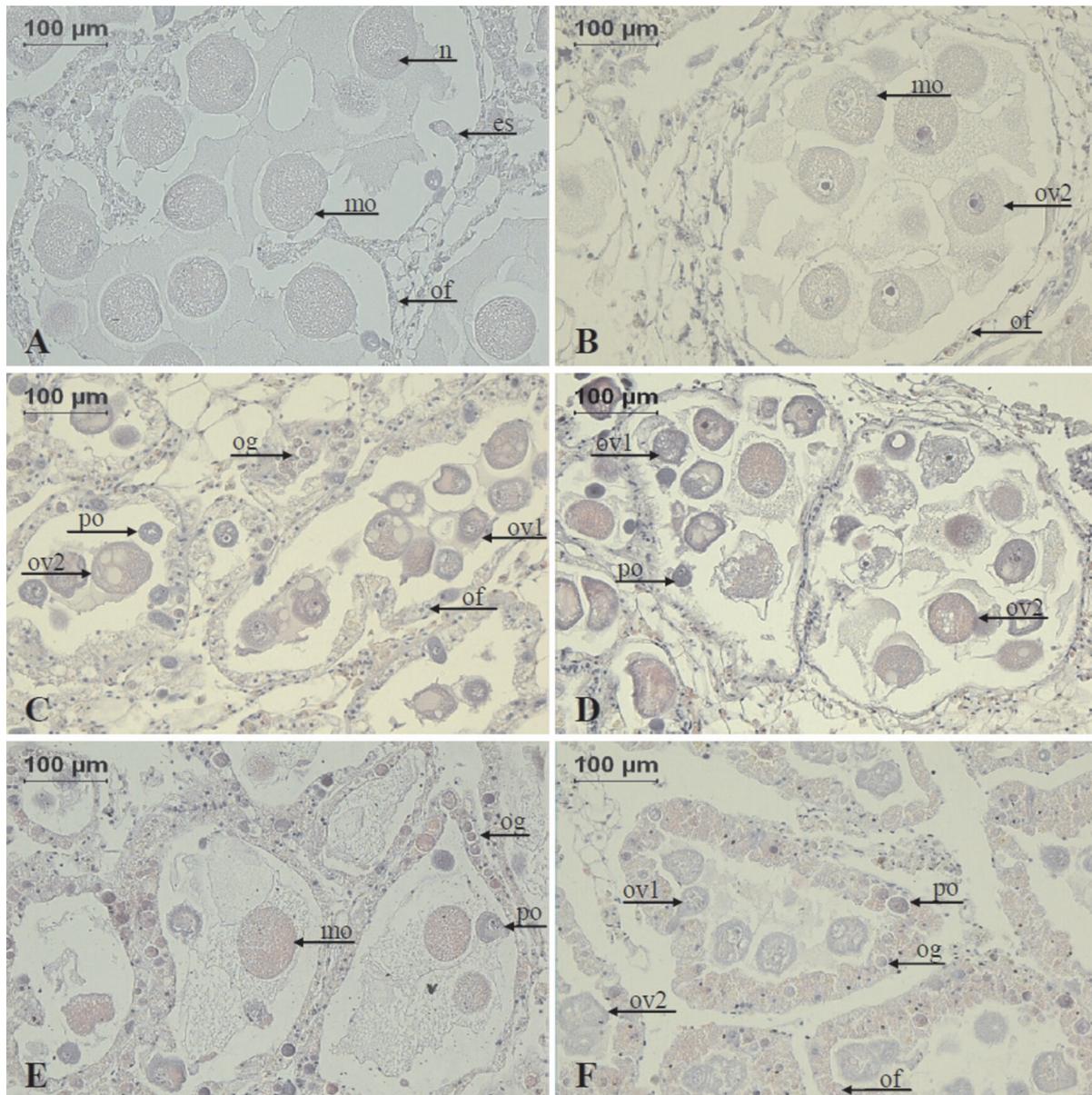


Fig. 5. Histological cross-sections of Chinese pond mussel ovaries during the annual cycle. A – March; B – April; C – May; D – June; E – August; F – October. Descriptions: es – egg stalk; n – nucleus of oocyte; of – ovarian follicles; og – oogonia; ov1 – early vitellogenic oocytes; ov2 – late vitellogenic oocytes; po – previtellogenic oocytes.

gonia, previtellogenic oocytes, early and late vitellogenic oocytes (Fig. 5D). Fibrillar elements of walls were widely distributed among follicles. In August, the follicles of ovaries were extended and emptied. They contained over 27% of residual mature oocytes in the center and numerous previtellogenic oocytes (38% of the quantitative share) located in the peripheral space of the gonads (Figs 5E and Fig. 6). Follicle walls were thickest in October and contained oogonia, previtellogenic and early vitellogenic oocytes situated mainly eccentrically in the follicle walls (Fig. 5F). The nuclei of vitellogenic oocytes consisted of many vacuoles and were irregularly shaped. At the beginning of

cytoplasmic growth, each oocyte had an egg stalk (Fig. 5A) and was attached to the follicle walls. Most of these oocytes remained attached to the germinal epithelium by this basal stalk until an advanced stage of development and then moved to the lumen of ovarian follicles.

Discussion

In the present study, we described the histology of oogenesis and spermatogenesis in Chinese pond mussels from the Konin lakes system, an alien invasive species for the Polish malacofauna. Various

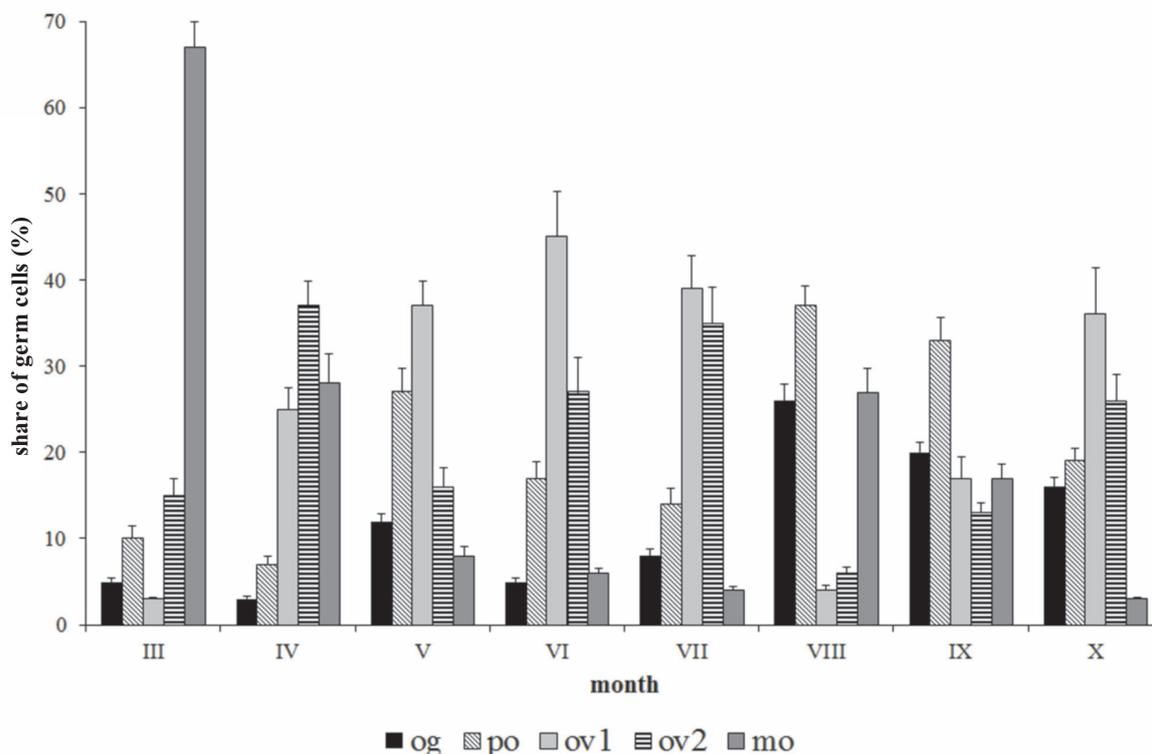


Fig. 6. Quantitative share of germ cells (means \pm SEM) in ovaries of Chinese pond mussel during the 2009-2010 seasons. Descriptions: og – oogonia; po – previtellogenic oocytes; ov1 – early vitellogenic oocytes; ov2 – late vitellogenic oocytes; mo – mature oocytes.

stages of development of the female and male germ cells were characterized. The basic morphological parameters of spermatozoa and spermatid (sperm) morulae, specific cells for atypical spermatogenesis, were established. We have documented that *S. woodiana* is a gonochoristic species and in the Konin lakes system shows considerable variability regarding the stages of the reproductive cycle. Our results suggest that *S. woodiana* in the Konin lakes system are able to spawn throughout March to October, with a season of higher reproductive activity extending from March to April. On the other hand, variation in the timing of spawning among females is also possible. In such case there is only one time (peak) of spawning in early spring.

The bivalves are characterized by gonochoristic and/or hermaphroditic breeding. Variability in sex ratio was also recorded among populations (PARK & CHUNG 2004; ŞEREFLİŞAN *et al.* 2009). Histological analyses indicated that one sex does not dominate in the Chinese pond mussel population of the Konin lakes system: 48.9 and 51.1% of the population was female and male, respectively. KISS and PETRO (1992) estimated the sex ratio for *S. woodiana* as 45.6% (males) to 54.4% (females)

in Hungary. Although sex ratios did not significantly differ from the expected 1:1 ratio ($P > 0.05$), a slightly female-biased sex ratio was recorded. DUDGEON and MORTON (1983) found that the Plover Cove Reservoir (Hong Kong) population living in the natural range had a sex ratio of 60:40 with female predominance. These results are slightly different from the data of SOROKA (2000), who investigated microscopically 54 *S. woodiana* individuals from the three sampling sites in the Konin reservoirs and recorded an overall sex ratio of 1:1. However in one group collected from Lake Licheńskie, the sex ratio was significantly biased towards females (84%). Perhaps these differences in sex ratio reflect environmental changes, especially temperature, which is highly variable in the Konin lake system. In sum, it seems that the general sex ratio 1:1 is characteristic for *S. woodiana*, however deviations towards higher proportions of females are possible (see also ŁABĘCKA 2009).

S. woodiana is much larger than indigenous European unionids species and is characterized by significant individual size (120-250 mm) as well as highly variable shell morphology (KRASZEWSKI & ZDANOWSKI 2007; LAJTNER & CRNČAN 2011). Results of the relationship between shell length

and shell width in the population from Konin lakes system showed that males and females shared a similar mode with no evidence of a wider shell shape in females. Regression lines for these relationships were similar for both sexes and there was no evidence of sexual dimorphism in terms of shell sizes.

Hermaphroditism in the Chinese pond mussel is relatively rare. DUDGEON and MORTON (1983) reported only 0.3% of hermaphroditic specimens in the population from Hong Kong. KISS (1995) did not observe hermaphroditic *S. woodiana* in Hungary. We found, and confirmed histologically, only a single hermaphroditic specimen from the Konin lake population (sampled in March 2009) which possessed distinctly separated female and male tissues filled with oocytes or spermatozoa. Based on these observations, the Chinese pond mussel can be classified as an accidental or abnormal hermaphrodite (typical for gonochoristic species).

Contrary to data in DUDGEON and MORTON (1983), variation in gonadal development from March to October was noted in the population from the Konin lakes system. Mature oocytes were most numerous in ovaries in springtime (March and April) and August. These data are different for Chinese pond mussel living in their natural range, where the peak of maturity occurs in the summer from May through July (period of embryonic development in the gills of females and incidence of glochidia on fish hosts) (DUDGEON & MORTON 1983). However, we found that in October oögonia, previtellogenic and early vitellogenic oocytes appeared, indicating that the reproductive cycle is starting over. Development likely continued through the winter months and resulted in the mature oocytes found in March. This mode of development was already observed by DUDGEON and MORTON (1983) for a native population of Chinese pond mussel and was also confirmed by our results.

The morphometric parameters of *S. woodiana* female and male germ cells were characterized for the population from the Konin lakes system. These are the first published complete sets of data concerning this species. The only available information was that of DUDGEON and MORTON (1983) who estimated the size of mature oocytes to be close to 75 μm for Chinese pond mussels from Hong Kong. In a comprehensive review on the maximum diameter of bivalves oocytes, BENINGER and LE PENNEC (1997) provided only one record for unionids *Cucumerunio novaehollandiae* for which 40 μm was reported as the maximum size (JONES *et al.* 1986). Recently ÇEK and ŞEREFLİŞAN (2006) presented the range of diameters in early vitellogenic oocytes (50-70 μm) and vitellogenic oocytes (80-100 μm) for the freshwater unionid *Unio terminalis delicatus* (LEA 1863) inhabiting

Gölbasi Lake in Turkey. For another unionid species, *Leguminaia whaetleyi* from the same reservoir, the values oscillated between 35-50 μm for previtellogenic and 75-90 μm for vitellogenic oocytes, respectively (ÇEK & ŞEREFLİŞAN 2011). Our results documented that the maximum size of previtellogenic (34-43 μm) and vitellogenic oocytes (75-83 μm) of *S. woodiana* are consistent with values established for closely related members of the Unionidae family. These data could be important for comparative studies within unionids or to elucidate phylogenetic relationships with other taxa.

The morphology of the Chinese pond mussel spermatozoa seem typical for freshwater Unionidae characterized by structural simplicity (MATOS *et al.* 1998). Characteristic features of unionid spermatozoa include a bullet-shaped head, uniflagellated tail, and the presence of a very reduced acrosome (ROCHA & AZEVEDO 1990). The acrosome is usually only visible by transmission electron microscopy (SHEPARDSON *et al.* 2012) therefore it could not be identified in this study. Unionidae spermatozoa are small, for example the head length is 2.8 μm for *Truncilla trincata* (WALLER & LASEE 1997), 4.0 μm for *Anodonta grandis* (LYNN 1994) and 4.2 for *Prisodon alatus* (MATOS *et al.* 1998). These values agree with 4.3 recorded in this study for *S. woodiana*, although it seems that spermatozoa of this species can be among the longest within the Unionidae.

Histological analysis of *S. woodiana* spermatogenesis revealed the presence of spermatid morulae, especially in August and October. The role of these structures is still not completely known, but MATOS *et al.* (1998) linked them with an alternative mode of spermatogenesis and reported them as cells initially containing spermatids. SHEPARDSON *et al.* (2012) described two spermatogenetic pathways (typical and atypical) in *Vemustaconcha ellipsiformis* (Unionidae) for an entire year. The spermatozoa produced by both pathways were essentially morphologically indistinguishable, but atypical spermatozoa appeared to be produced in a pathway that included spermatozoa morulae and spermatids derived from spermatogonial cells located in Sertoli cells. Spermatid morulae in Chinese pond mussel may constitute an atypical mode of spermatogenesis. On the other hand, we did not notice any Sertoli cells in the histological cross-sections of *S. woodiana* testis which had been proposed by SHEPARDSON *et al.* (2012) as the origin of the morulae. Therefore further studies, preferably with the use of scanning electron microscopy, are necessary to provide information concerning developmental stages of sperm morulae in *S. woodiana*.

In summary, the gametogenesis of *S. woodiana* from the Konin lakes system was characterized.

This process is distinguished by considerable variation of gonadal development stages. The increase in the number of mature oocytes in samples collected between March-April and then in August suggests that a breeding season from spring to late summer is a characteristic feature for this population of Chinese pond mussels. Moreover, the presence of spermatid morulae may constitute an atypical mode of spermatogenesis. In the analysed population, the sex ratio was close to 1:1, which suggests that the Chinese pond mussel could be classified as an accidental or abnormal hermaphrodite (typical for gonochoristic species). The sizes of *S. woodiana* previtellogenic and vitellogenic oocytes are consistent with those of closely related members of the Unionidae family, while spermatozoa of this species can be among the longest within unionids.

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