Annual reproductive cycle in two free living populations of three-spined stickleback (*Gasterosteus aculeatus* L.): patterns of ovarian and testicular development

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Abstract

The annual reproductive cycle in two wild populations of three-spined stickleback was studied. Sticklebacks from the Dead Vistula River (Martwa Wisła) (brackish water) and the Oliva Stream (Potok Oliwski) (freshwater) were exposed to annual environmental changes in their natural habitats. Ovaries and livers (females), and testes and kidneys (males) were collected during 1–2 years. The gonadosomatic I_G, hepatosomatic I_H, nephrosomatic I_N indices, kidney epithelium height (KEH) and size of oocytes were calculated. The number of mature oocytes and percentage of ovulating females were determined during the spawning season. Histological changes in the ovaries and testes were described throughout a year. Annual reproductive cycles were similar in both populations of sticklebacks. This is the first histological and morphological study carried out throughout a year, simultaneously in two wild populations of three-spined sticklebacks inhabiting different environments. An improved scale of gonadal development in conjunction with the determined indices and fecundity give a comprehensive description of the reproductive cycle. These new observations, in combination with previously reported features, provide a universal scale that can be successfully used to distinguish all phases of gametogenesis in sticklebacks in different habitats.

1. Introduction

The three-spined stickleback (*Gasterosteus aculeatus* L.) is a species widely distributed in the northern hemisphere. There are morphological differences between marine and freshwater stickleback populations as a consequence of their adaptation to various salinity conditions (Wootton 1976). All sticklebacks, irrespective of habitat, are typical long-day breeders, in which the lengthening day and increasing water temperature in spring stimulate spawning (Baggerman 1990). Changes in appearance and behaviour indicate that sexual maturity has been attained. Breeding males defend their nests in a fixed territory and care for the eggs and offspring. The kidney of the male secretes a protein glue that is used in nest building (De Ruiter & Mein 1982, Borg et al. 1993). Females produce the eggs and spawn into the nests of selected breeding partners. The advanced reproductive strategy of sticklebacks compensates for the low fecundity of this species and leads to a high offspring survival.

The stickleback, one of the most ubiquitous and adaptable species with a short life-cycle and specific reproductive behaviour, is a widely-accepted model in many physiological and behavioural studies. Recently, the importance of the stickleback has risen significantly, since it is used as a biomarker for endocrine disruptions (Handy et al. 2002, Katsiadaki et al. 2002, Hahlbeck et al. 2004). Therefore, a well-defined gonadal cycle in the stickleback is essential in many aspects of its studies. Gametogenesis in sticklebacks has been documented, but previous investigations have focused mainly on freshwater fish (Craig-Bennett 1931, Ahsan & Hoar 1963) or fish kept under laboratory conditions (Borg 1982, Borg & Van Veen 1982). Moreover, observations have been limited to selected seasons (Van den Eeckhoudt 1946, Tromp-Blom 1959, Wallace & Selman 1979). The aim of the present work is to provide a morphological and histological description of the reproductive cycle in two wild populations of sticklebacks inhabiting different environments – the Dead Vistula River (Martwa Wisła) and the Oliva Stream (Potok Oliwski).

2. Material and method

Adult three-spined sticklebacks of both sexes were caught with a handnet in the Dead Vistula River at Górki Wschodnie near Gdańsk (salinity 5–6 PSU) and in the Oliva Stream in Gdańsk (freshwater). The fish were sampled monthly, from March 1998 (Dead Vistula) or March 1999 (Oliva) to February 2000.

In females, ovaries and livers, and in males, testes and kidneys were dissected. The fish body (W) and organs were weighed to the

nearest 0.001 g. In females, the gonadosomatic I_G and the hepatosomatic I_H indices were calculated as follows: (gonad weight $\times$ W^{-1}) $\times$ 100 and (liver weight $\times$ W^{-1}) $\times$ 100. The diameter of the largest oocytes [μm] was measured. The percentage of ovulating females, the numbers of females with overripe eggs and the numbers of mature oocytes were determined during the spawning season from April to August. The entire ovaries were fixed in Bouin's fluid, embedded in paraffin, sectioned at 6 μm, and stained with Mayer's haematoxylin and eosin. Three well-separated sections from each ovary were examined under a light microscope to determine the developmental state. An extended and improved 8-degree scale of ovary development is proposed. This scale combines the present data, acquired in two wild populations of sticklebacks throughout a year, with the features reported by Tromp-Blom (1959) (6-degree scale), Borg & Van Veen (1982) (7-degree scale) and Braekevelt & McMillan (1967). Ovaries were classified on the basis of the most mature oocytes in the gonads.

In males, the gonadosomatic index I_G and the nephrosomatic index I_N were calculated as follows: (gonad weight $\times$ W^{-1}) $\times$ 100 and (weight of kidneys $\times$ W^{-1}) $\times$ 100. The kidney epithelium height (KEH [μm]) was also measured. The entire testes and kidneys were fixed in Bouin's fluid, embedded in paraffin, sectioned at 5 μm, and stained with Mayer's haematoxylin and eosin. Three well-separated sections from each organ were examined. Testes were classified according to Borg's (1982) 9-degree scale, but present observations and the complementary information on spermatogenesis in sticklebacks by Craig-Bennett (1931), Ahsan & Hoar (1963) and Ruby & McMillan (1970) were also taken into consideration. The annual changes in the interstitial tissue and in the activity of phagocytes in the testes were added to the scale. Photographs of the various stages of gametogenesis in both sexes were taken with a Nikon FDX-35 camera attached to a Nikon Eclipse 400 microscope.

Values of I_G, I_H, I_N, KEH and the number of mature oocytes were presented as means $\pm$ standard error of the mean (S.E.M.). The data were compared statistically using one-way analysis of variance (ANOVA) followed by Student's t-test. Differences were considered to be significant if $p < 0.05$.

3. Results

3.1. Females

Histological features of the ovaries, I_G and I_H indices, the diameter of the largest oocytes, the number of ovulating females and females with overripe eggs, and the number of mature oocytes were examined during the annual cycle in female sticklebacks. The following stages of ovarian development

were distinguished during oogenesis in the three-spined stickleback from both populations:

Stage 1: There is previtellogenesis and initial differentiation of the oogonia. Follicles proliferate from the germinal epithelium. They are round, very small and macroscopically undistinguished. The nucleus is large and occupies most of the oogonium. The nucleoli are located along the peripheral part of the nucleus. The cytoplasm of the oogonium is stained violet by haematoxylin and the nucleus is stained pink by eosin. The follicular epithelium of the oogonium is visible (Figs 1a, a').

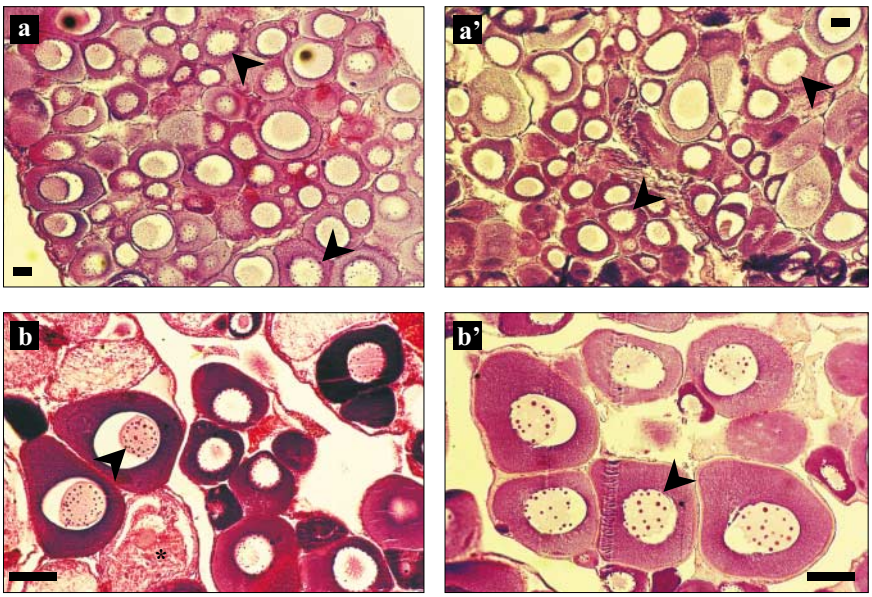

Fig. 1. Previtellogenesis of the oocytes: stage 1 (a, a'), stage 2 (b, b'). Bars indicate 100 μm, arrow – nucleoli, star – remains of regressed oocytes

Stage 2: The numbers of nucleoli increase – they are visible on the surface of the whole nucleus. The follicular and thecal layers are well defined around the oocytes. The oolemma (stained red) is formed between the cytoplasm and the follicular epithelium (Figs 1b, b').

Stage 3: Vitellogenesis is just beginning. There is initial (light) vacuolisation of the cytoplasm. Single small vacuoles (primary yolk) appear peripherally in the cytoplasm. The vitellogenic oocytes are opaque and are enlarging by the addition of cytoplasm (Fig. 2a).

Stage 4: Vacuolisation of the cytoplasm has reached an intermediate stage. Vacuoles are gradually increasing in size and number in the peripheral and central zones of the oocyte (Fig. 2b).

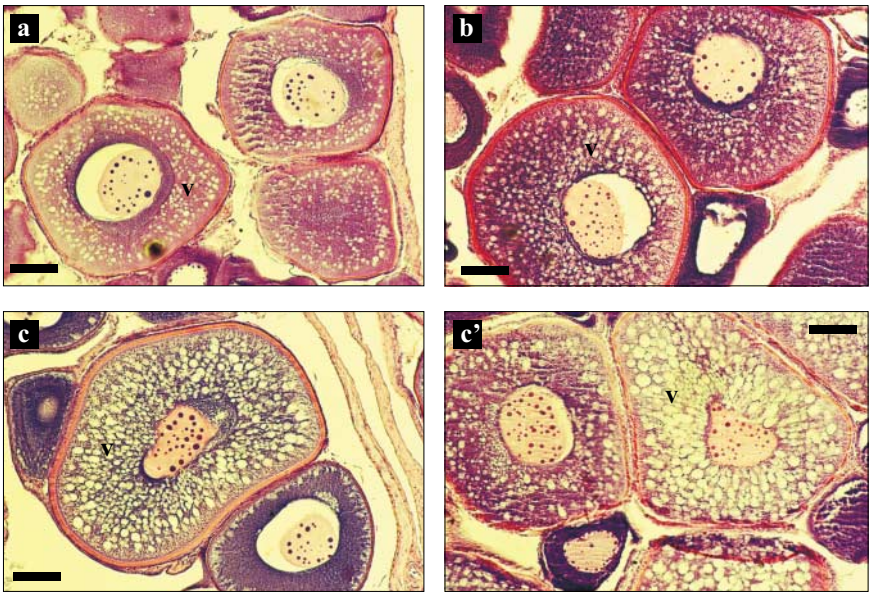

Fig. 2. Vacuolisation of the oocytes: initial-stage 3 (a), intermediate-stage 4 (b), complete-stage 5 (c, c'). Bars indicate 100 μm, v – vacuoles

Stage 5: Vacuolisation of the cytoplasm is complete (heavy). The whole cytoplasm is densely filled with vacuoles. Oolemma and theca are conspicuous (Figs 2c, c').

Stage 6: Secondary yolk is beginning to form. Yolk vesicles are initially accumulated in the periphery of the oocyte. Deposited yolk inclusions are stained pink/red by eosin. Maturing oocytes are enlarging (Figs 3a, a').

Stage 7: Most of the cytoplasm is filled with secondary yolk. Yolk is visible as densely packed globules or a homogeneous red mass in the cytoplasm (Figs 3b, b').

Stage 8: The ovulating oocytes, full of yolk, are spent during ovulation. Mature (fully transparent) and immature (opaque) oocytes are arranged in layers. Mature ova are immersed in bathing fluid and form an upper layer in the ovarian cavity near the oviduct. Postovulatory follicles consisting of follicular and thecal layers are present (Figs 3c, c').

Regression stage (R): The whole ovary is in an advanced stage of regression, with granulation and disintegration of the cytoplasm and the surrounding layers of all oocytes. Folded and ruptured remains of oocytes in different stages are visible (Figs 4a, a').

Since the same stages of ovarian development and similar distributions of the indices under study were observed in both fish populations, the

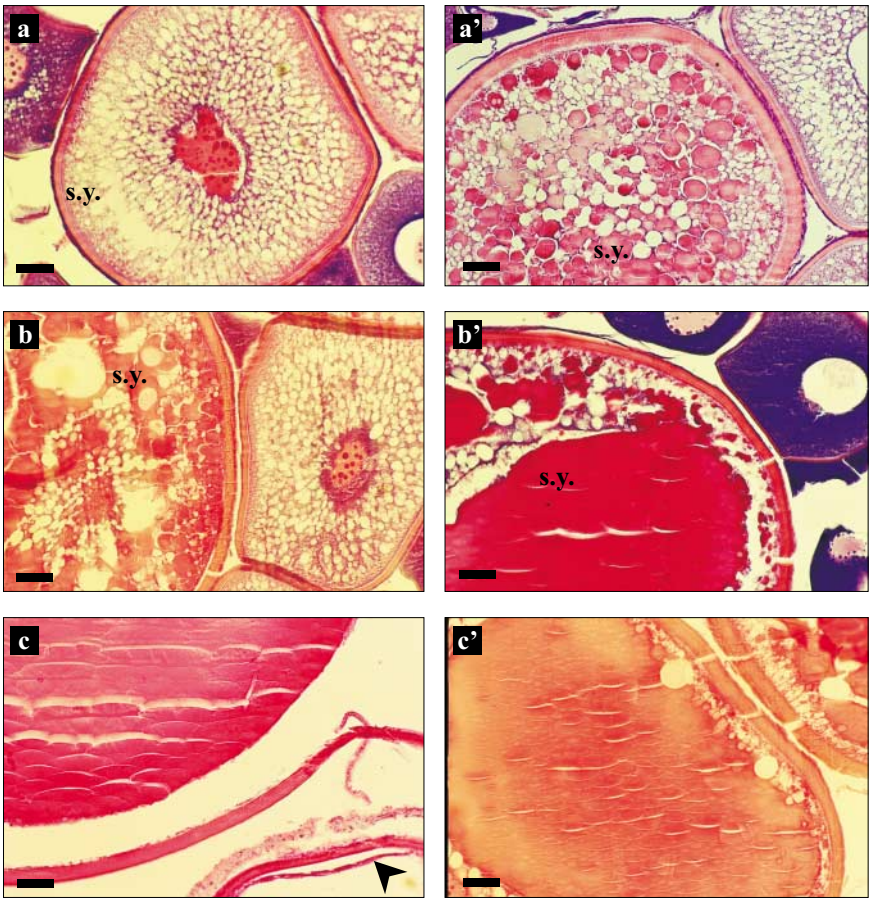

Fig. 3. Accumulation of secondary yolk (s.y.): stage 6 (a, a'), stage 7 (b, b'); ovulation: stage 8 (c, c'). Bars indicate 100 μm, arrow – postovulatory layers

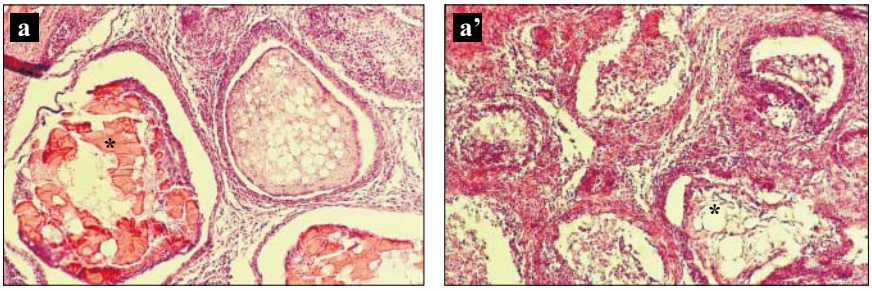

Fig. 4. Regression (R) of the ovaries (a, a'). Star – remains of regressed oocytes

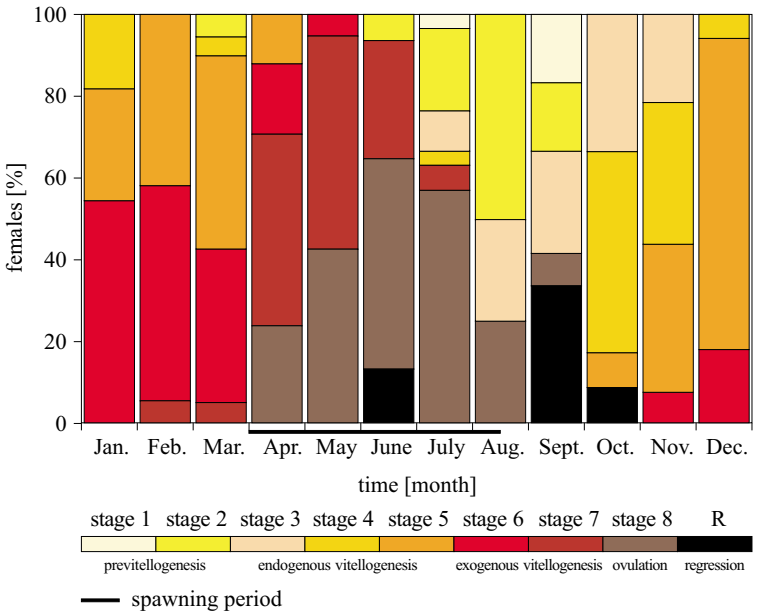

Fig. 5a. Seasonal changes in ovarian maturity in females of the three-spined stickleback. 205 fish were examined

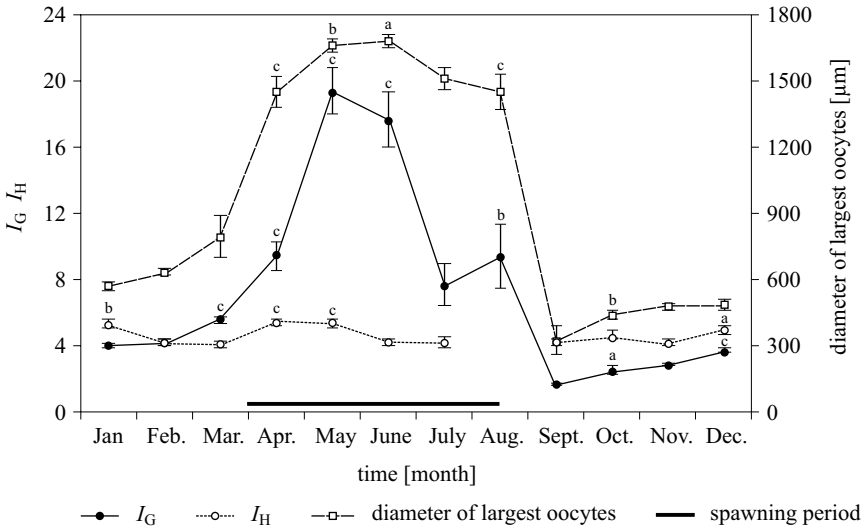

Fig. 5b. Seasonal changes in gonadosomatic I_G and hepatosomatic I_H indices, and oocyte diameter in females of the three-spined stickleback. 417 (I_G), 262 (I_H) and 129 (oocyte diameter) fish were examined. Values are presented as means ± S.E.M. a: $p < 0.05$ vs I_G September value, I_H November value, oocyte diameter–July value; b: $p < 0.01$ vs I_G September value, I_H February value, oocyte diameter–April and September values; c: $p < 0.001$ vs I_G February, March, April, July and November values, I_H March and June values, oocyte diameters–March and September values

combined values are presented in Figs 5a and 5b. As a result of asynchronous maturation, the ovaries contained oocytes in various stages of development throughout the year; their proportions varied seasonally. The stage of oogenesis was determined on the basis of the most mature oocytes present in the gonads. Earlier stages were always present in the ovaries, the oogonia being reservoirs for the next breeding season. Three phases – post-spawning, preparatory, and spawning – could be distinguished in the annual ovarian cycle. The post-spawning phase began in June–July, when part of the population had completed spawning and oogenesis was starting. Oogenesis was initiated by mitotic division and the protoplasmatic growth of the oogonia (Fig. 1). The decrease in I_G after spawning ($p < 0.001$) was caused by the appearance of oogonia 100–300 μm in diameter, which reduced gonad weight (Fig. 5b). The high diameter index in July and August ($p < 0.001$) was due to our measuring large ovulating oocytes, including overripe ones. Some females displayed initial vacuolisation of the cytoplasm already in July.

Vacuolisation continued in the second, preparatory phase in the autumn –winter period, from the light to the fully vacuolated stage (Fig. 2). Vacuolisation caused a slight but statistically significant increase in I_G ($p < 0.05$) and oocyte size ($p < 0.01$) (Fig. 5b). Oocyte membrane formation was completed by the end of the trophoplasmatic growth. The first granules of secondary eosinophilic yolk appeared already in November (Figs 3a, a' and 5a). Accumulation of this yolk continued in spring.

The sticklebacks became sexually active and started to breed during the third phase of the reproductive cycle. This was initiated by a rapid increase in I_G in March ($p < 0.001$) and indicated the transition of the fish to the advanced vitellogenous stage (Figs 5a, 5b). Oocyte diameters increased significantly in April ($p < 0.001$) as a result of the deposition of a large amount of secondary yolk (Figs 3b, b'). The significant increase in I_H in this period ($p < 0.001$) was indicative of advanced activity of the liver, producing the components of this yolk. Both the preovulatory (stage 7) and the ovulatory phases (stage 8) extended over several months.

Ovulating females were observed from April to August. The numbers of mature oocytes ranged from 26 to 870 in females from the Dead Vistula. There was a simultaneous increase in the number of gravid females and the number of mature oocytes in the Dead Vistula population in contrast with that in the Oliva Stream (Fig. 6). Ovulated oocytes reached a diameter of at least 1400 μm (apart from the overripe ones). The numbers of mature oocytes decreased gradually to the end of breeding (Fig. 6) as a consequence of multiple spawning, a process distinguished by the simultaneous presence of vitellogenic oocytes in 5–7 maturity stages, ovulated eggs and fresh postovulatory follicles. The first females with a lower weight and smaller

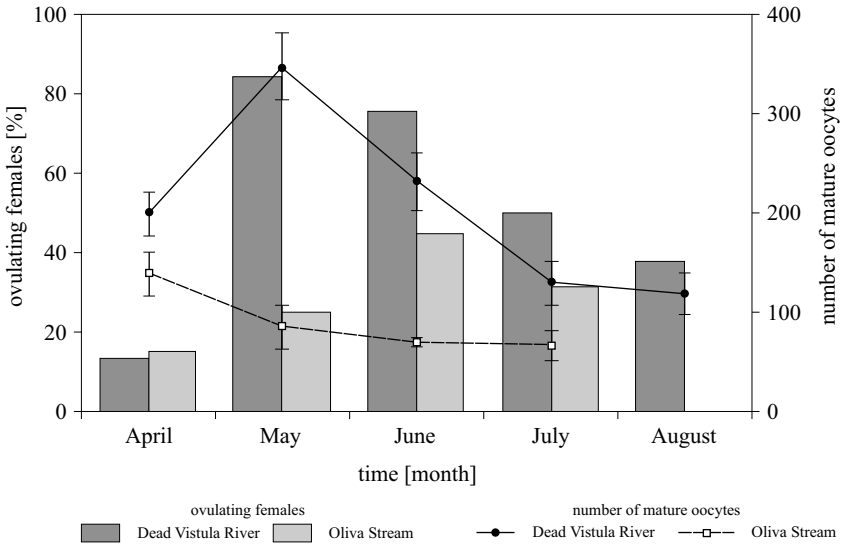

Fig. 6. Changes in the percentage of ovulating females and the numbers of mature eggs in fish in the Dead Vistula River (Martwa Wisła) and the Oliva Stream (Potok Oliwski) during the spawning season. 161 fish from the Dead Vistula and 59 from the Oliva Stream were examined. The number of mature eggs is presented as the mean ± S.E.M. for each month

gonad size were noted in May, which indicated that at least one spawning had been accomplished. The 1.5–1.9 mm-long offspring appeared in the shallow inshore zone of the Dead Vistula at the beginning of June. The majority of sticklebacks had completed ovulation in August, but in some fish a post-breeding regeneration of gonads was observed already in June. Stage 8, which was noted in September, indicated the presence of fresh postovulatory follicles after the August breeding, but no actual ovulation (Fig. 5a). The fresh postovulatory follicles and some of the vitellogenic oocytes were resorbed in the ovaries towards the end of the breeding season. Remnants of single atretic eggs were always present in the ovaries. Apart from normally ovulating females, overripe ovulating females were also found in both populations throughout the breeding season. They were most numerous in the Dead Vistula in May and June. Overripe oocytes either dominated or occurred as single cells in the ovaries.

Ovaries in the stage of advanced regression (R) were found in several females in June, September and October (Fig. 5a). This process differed from the typical regression of vitellogenic oocytes and empty follicles mentioned above. Oocytes of all stages underwent retardation, which was determined by the granulation and disintegration of the whole cytoplasm, and by the fibrosis and separation of the surrounding membranes in

the ovaries (Figs 4a, a'). The reproductive functions of sticklebacks were inhibited through the degradation of germinal epithelium in the ovaries.

3.2. Males

Histological analysis of the testes and measurement of I_G, I_N and KEH values were carried out in male sticklebacks during the annual cycle. The following stages of testicular development were distinguished during spermatogenesis in three-spined sticklebacks from both populations:

Intermediate spermatogenesis

Stage 1: Most of the seminiferous tubules are filled with spermatocytes and spermatids. Their concentrations are stained pink. Spermatozoa are

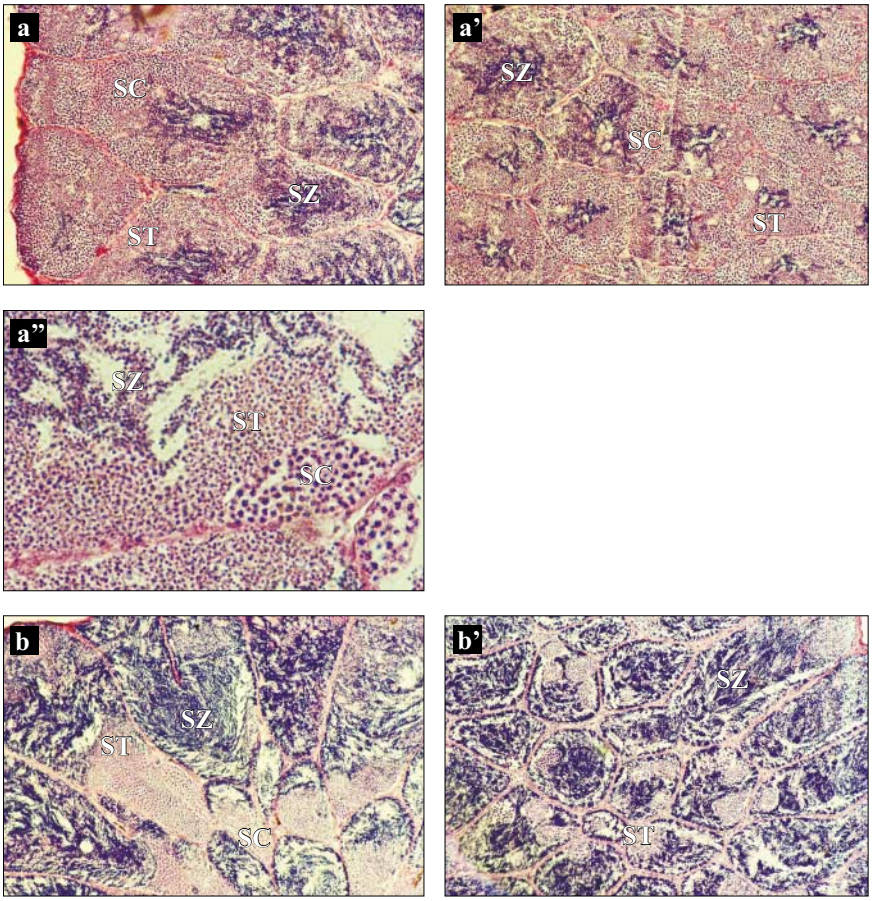

Fig. 7. Intermediate spermatogenesis: stage 1 (a, a', a"), stage 2 (b, b'); SC – spermatocytes, SG – spermatogonia, ST – spermatids, SZ – spermatozoa, $\times 200, \times 500$

stained violet and are visible in the central part of the tubules. Single spermatogonia and single interstitial cells are present (Figs 7a, a', a").

Stage 2: There are large numbers of spermatozoa, and characteristic 'cysts' in the peripheral zone of the tubules filled with spermatocytes and spermatids. Spermatocytes and spermatids are less common than in stage 1 (Figs 7b, b').

Completed spermatogenesis

Stage 3: Spermatozoa are dominant. Dense spermatozoa fill the lateral seminiferous tubules and do not pass to the central duct. Single spermatocytes, spermatids and spermatogonia are present. There are numerous interstitial cells. The first phagocytes appear (Figs 8a, a').

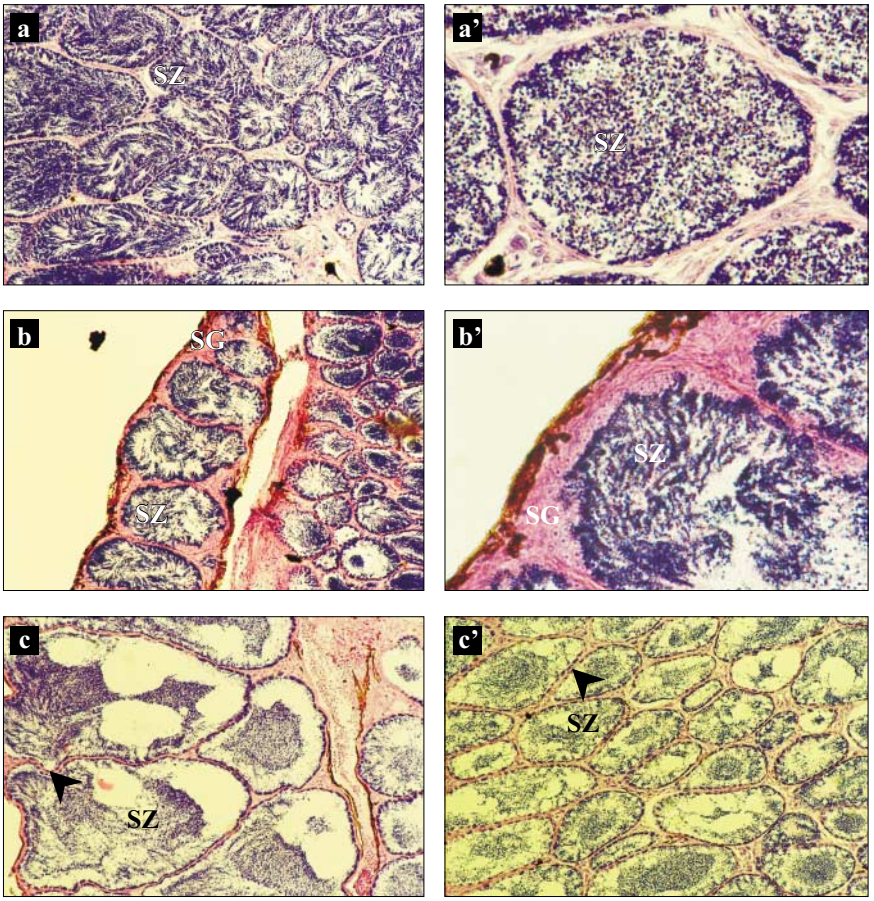

Fig. 8. Completed spermatogenesis: stage 3 (a, a'), stage 4 (b, b'). Release of sperm: stage 5 (c, c'). Arrow – phagocytes, SG – spermatogonia, SZ – spermatozoa, $\times 200$, $\times 500$

Stage 4: Spermatozoa are dominant. Primary spermatogonia form layers around the periphery of some tubules. The interstitial tissue is well developed. Phagocytes are present (Figs 8b, b').

Spawning; release of sperm

Stage 5: Spermatozoa are released during the sexual phase. The seminiferous tubules are filled with diluted spermatozoa, or tubules are partly or completely empty according to the male's sexual activity. Viable spermatozoa are present in the central tubule and in the spermatic ducts. Only a few spermatogonia and/or spermatocytes are observed. The interstitial tissue is very well developed and wider than in stage 4. Phagocytes are numerous (Figs 8c, c').

Beginning of spermatogenesis

Stage 6: Testes are in the quiescent phase. Seminiferous tubules become filled with large numbers of spermatogonia. Residual spermatozoa are resorbed after breeding. Primary and secondary spermatocytes appear. The interstitial tissue is reduced. Phagocytes are absent (Figs 9a, a').

Stage 7: Seminiferous tubules are completely filled with spermatogonia and spermatocytes. Spermatids are less common. Due to the presence of

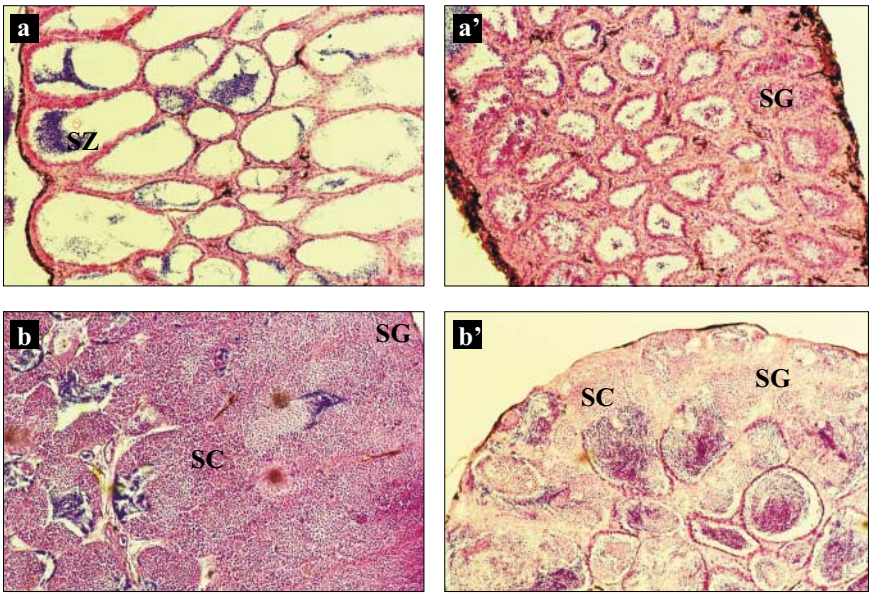

Fig. 9. Beginning of spermatogenesis: stage 6 (a, a'), stage 7 (b, b'). Star – residual spermatozoa, SC – spermatocytes, SG – spermatogonia, SZ – spermatozoa, × 200

early developmental stages the whole section is stained a characteristic pink colour. The interstitial tissue is reduced (Figs 9b, b').

Intensive spermatogenesis

Stage 8: Spermatogonia, spermatocytes and spermatids are dominant. Only a few spermatozoa are present (Figs 10a, a').

Stage 9: Spermatocytes and spermatids are dominant. There are a small number of spermatozoa. Single spermatogonia are present (Figs 10b, b', b").

Some numbers of spermatogonia and spermatozoa were always present in the seminiferous tubules of the testes, the spermatogonia constituting a reservoir for the next breeding season. The same stages of testicular maturity and a similar distribution of the indices under study were

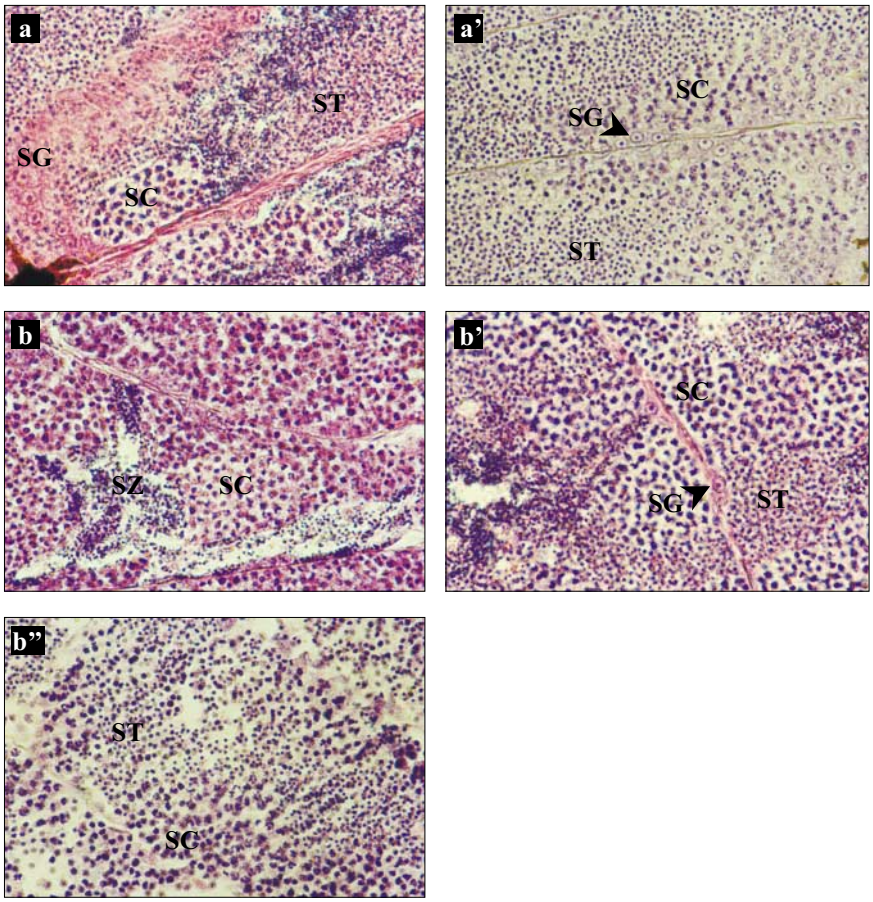

Fig. 10. Intensive spermatogenesis: stage 8 (a, a'), stage 9 (b, b', b"). SC – spermatocytes, SG – spermatogonia, ST – spermatids, SZ – spermatozoa, × 500

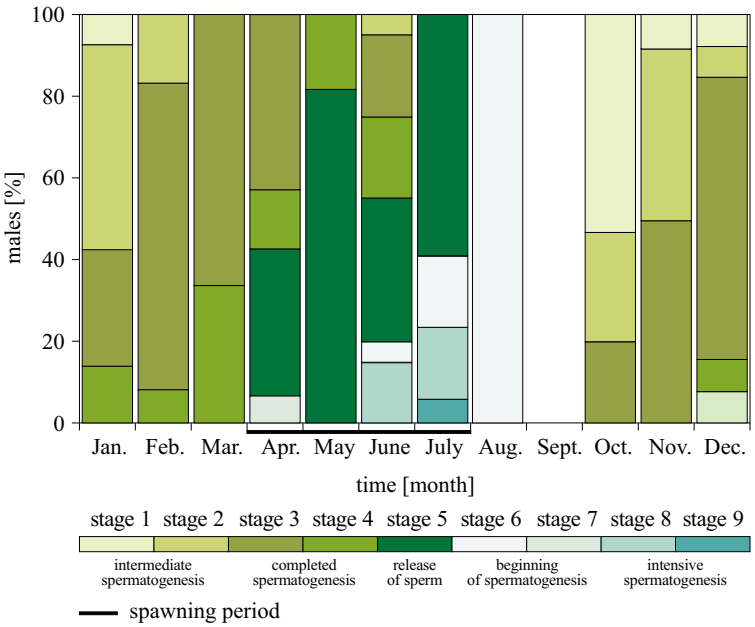

Fig. 11a. Seasonal changes in testicular maturity in males of the three-spined stickleback. 146 fish were examined

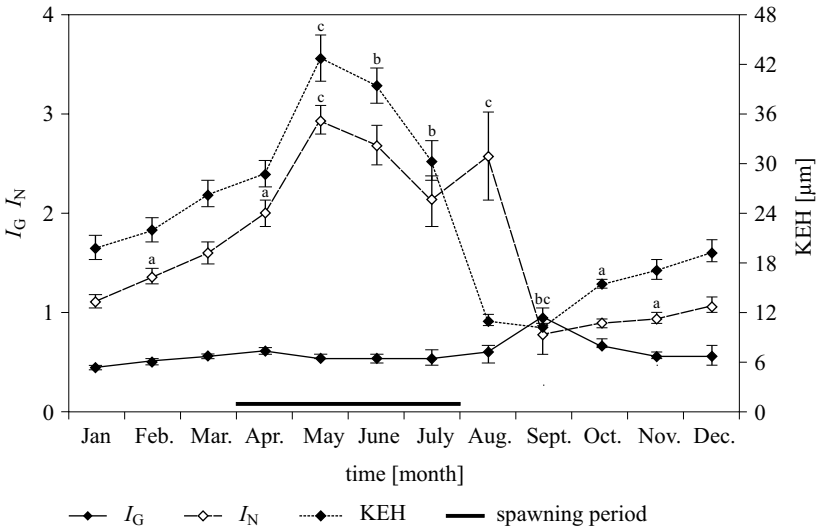

Fig. 11b. Seasonal changes in gonadosomatic I_G and nephrosomatic I_N indices, and kidney epithelium height (KEH) in males of the three-spined stickleback. 330 (I_G), 329 (I_N) and 146 (KEH) fish were examined. Values are presented as means $\pm$ S.E.M. a: $p < 0.05$ vs I_G November value, I_N January, March and September values, KEH September value; b: $p < 0.01$ vs I_G August value, KEH July and August values; c: $p < 0.001$ vs I_G October value, I_N April and September values, KEH April value

observed in both fish populations; hence, the combined values are presented in Figs 11a and 11b.

As in the females, three phases were observed in the annual testicular cycle: post-spawning, preparatory and spawning. The first, short phase began with the post-spawning regeneration of the gonads, when the breeding season was over. This process was in progress already in June in part of the male population (Figs 9a, a', 10a, a', 11a). A significant decrease in KEH ($p < 0.01$) and I_N ($p < 0.001$) in July–August and September, respectively, confirmed the end of the breeding season (Fig. 11b).

Intensive spermatogenesis after breeding represented the second, preparatory phase of the reproductive cycle. Vigorous development of reproductive cells by their division, growth and maturation was observed. Spermatogonia were the largest cells with a distinct nucleus (Fig. 10). The increase in I_G in September ($p < 0.01$) (Fig. 11b) was due to the large size and weight of spermatogonia. At the same time, I_N and KEH increased progressively ($p < 0.05$) as the kidney tubules were transformed to the secretory function. The spermatogonia divided within thin-walled cysts to form primary spermatocytes that quickly ripened into smaller secondary spermatocytes (Figs 9, 10). Once the cysts had broken down, the spermatids were released. Their further development to form spermatozoa occurred in the lumen of the tubule (Fig. 7). The high rate of this process slowed down in autumn. Differentiation of the reproductive cells during the 6–9 stages of maturation progressed relatively quickly during the year (Fig. 11a). I_G values decreased in the autumn-winter period ($p < 0.001$) (Fig. 11b) because of the predominance of lightweight reproductive cells, i.e. the smallest spermatozoa. In most of the males, spermatogenesis was complete in December, but the spermatozoa were not yet ready to be released. The spermatozoa were thickly packed in the lateral seminiferous tubules (Figs 8a, a', 8b, b'). Spermatozoa were dominant in the testes throughout most of the year (from October to the next breeding period) (Fig. 11a). The first, small, elongated phagocytes appeared when spermatogenesis was completed: they occurred around the periphery of the tubules and contained a few sperm nuclei. Cyclic changes in the shape of the interstitial tissue in the testes were also observed during the year. The number of interstitial cells increased markedly in autumn–winter.

The pre-spawning and spawning periods occurred during the third phase of the reproductive cycle. The sticklebacks bred from April to July, but the majority of them were sexually active from May onwards. Breeders displayed a well-developed nuptial coloration and aggressive behaviour. At the onset of spawning the seminal fluid diluted the thickly packed spermatozoa in the testicular tubules. Sperm could move easily to the central seminiferous

tubule and then to the spermatic ducts. Spawning was manifested by the presence of diluted sperm, the remains of spermatozoa in the tubules, or empty tubules following sperm release (Figs 8c, c'). The interstitial tissue was very well developed and formed a distinct, wide layer between the testicular tubules. The invasion of the periphery of the tubules by a large number of phagocytes was recorded during the breeding period. The numbers of phagocytes decreased steadily and the interstitial tissue was almost completely reduced after the breeding season and during the early stages of spermatogenesis.

The lowest kidney epithelium (10 μm on average) was observed immediately after spawning in August–September (Figs 11b, 12a, a'). Hypertrophy of the kidneys developed concurrently with the sticklebacks' sexual activity.

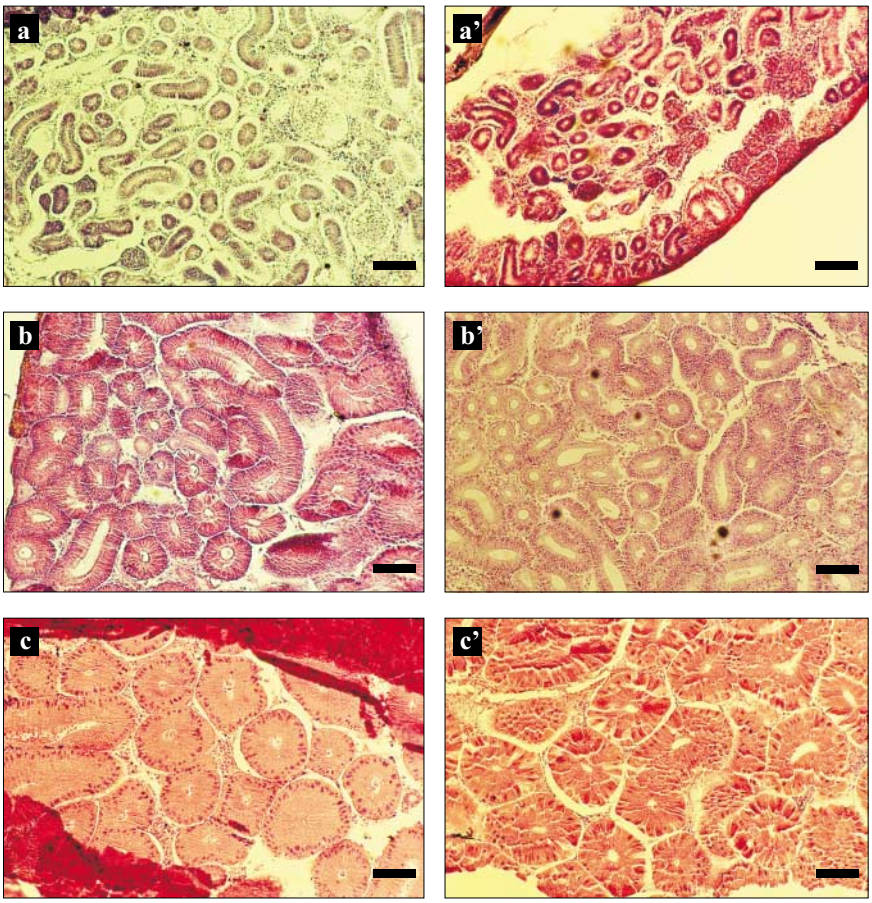

Fig. 12. Height of kidney epithelium cells in August–September (a, a'), in March –April (b, b') and in May–June (c, c'). Bars indicate 50 μm

KEH reached nearly 50 μm in May–June, and I_N increased accordingly ($p < 0.001$) (Figs 11b, 12c, c').

4. Discussion

This is the first histological and morphological study to have been carried out simultaneously in two populations in the field throughout a year. The result is a comprehensive description of the reproductive cycle and an improved scale of gonadal development in sticklebacks. Several scales of gonadal maturation have been proposed for females (Tromp-Blom 1959, Borg & Van Veen 1982) and males (Craig-Bennett 1931, Ahsan & Hoar 1963, Borg 1982), but some of them are based on features observed mainly in freshwater sticklebacks and/or in laboratory fish. The same timing of the reproductive cycle and stages of gonadal development were recorded in sticklebacks from the Dead Vistula River and Oliva Stream. Water temperature and photoperiod are known to be the main environmental cues, which determine the reproductive cycle in sticklebacks (Baggerman 1957, 1989). The combination of these factors in different seasons regulates sexual maturation during the year (Merriman & Schedl 1941). Three-spined sticklebacks are typical long-day breeders, and increasing water temperature and the lengthening days in spring stimulate spawning in males and females (Borg 1982, Borg & Van Veen 1982, Borg et al. 1987). The duration of the breeding period recorded in the present study is in agreement with previous findings by Baggerman (1989) for anadromous sticklebacks and is similar to that observed in stickleback populations inhabiting different environments (Greenbank & Nelson 1959, Muchomebiarov 1966, Nellbring 1985, Foster 1995). However, the exact time of the same phase of gametogenesis may vary in two distant populations, because of the differences in temperature and day length. Accordingly, in the present study, the males hypertrophied and started intensive spermatogenesis one month earlier than those observed by Borg (1982) at higher latitudes.

In this study, three main phases of gonadal development were distinguished in sticklebacks: post-spawning, preparatory and pre-spawning /spawning. The post-spawning phase corresponded to previtellogenesis, when protoplasmatic growth of young follicles took place and only immature nonyolky oocytes were present in the ovaries. In the preparatory phase in the autumn-winter period, trophoplasmatic growth of the oocytes entailed synthesis of the yolk substances in the oocyte. Synthesis of endogenous, primary yolk was expressed as endogenous vitellogenesis (Khoo 1979, Baggerman 1990). The secondary yolk accumulated in the oocytes during the third, pre-spawning/spawning phase. The components of secondary yolk were synthesised in the liver; the process is therefore referred to

as exogenous vitellogenesis (Khoo 1979, Baggerman 1990). Baggerman (1989, 1990) has postulated the two-phased reproductive cycle separated by winter as the period of quiescence. In our opinion, winter should be considered as a separate phase, because of the important progress in gametogenesis at that time, i.e. the completion of spermatogenesis and the beginning of secondary yolk accumulation that take place during this season. Low winter temperatures and short days are essential for sticklebacks to complete the normal process of gametogenesis and attain full sexual maturity in spring. The low water temperature in the preparatory phase is the stimulus for final maturation in four-spined sticklebacks *Apeltes quadracus* (Merriman & Schedl 1941). In addition, exposure to cold water prior to spawning advances and synchronises ovulation and spawning, and improves egg survival in salmonids (Nakari et al. 1987, Taranger et al. 2000). Rainbow trout (*Oncorhynchus mykiss*) exposed to high temperatures in the preparatory phase demonstrate dysfunction of ovaries and disturbances of ovulation (Pankhurst et al. 1996, Davies & Bromage 2002). The results obtained in our study corresponded to the four-phased scale of gonadal development, proposed earlier by Baggerman (1957).

Vitellogenin is a precursor and main component of the secondary yolk, which is synthesised in the liver and incorporated into the oocytes before breeding (Khoo 1979, Baggerman 1990, Bieniarz & Epler 1991). In this study, the increase in I_{H} observed in April indicated the temperature-stimulatory character of vitellogenin production. A temperature of 10–16°C affects both the synthesis and the concentration of vitellogenin in oocytes in other species as well, e.g. tench (*Tinca tinca*), salmon (*Salmo salar*) and rainbow trout (*O. mykiss*) (Breton et al. 1980, Korsgaard et al. 1986, Olin & Von Der Decken 1989, Mackay & Lazier 1993). In our study, some sticklebacks completed spawning and started a new gametogenetic cycle in June–July. Temperatures as high as 20°C probably limited breeding in the populations examined and a further increase of temperature could have been the reason why the initial phase of gametogenesis in both sexes was disturbed. Wallace & Selman (1979) have also suggested that environmental temperatures experienced by sticklebacks at the end of June may effectively inhibit reproductive activity in this species. Obviously, there is a temperature limit for sexual activity and breeding in fish (Chmilevsky 2000, Davies & Bromage 2002). A high temperature is known to reduce the volume of sperm, inhibit ovulation and decrease fecundity and egg survival in salmonids (Taranger & Hansen 1993, Pankhurst et al. 1996, Pankhurst & Thomas 1998, Davies & Bromage 2002). High temperatures have also been shown to damage previtellogenic oocytes, to cause atresia in mature oocytes in tilapia (*Oreochromis mossambicus*), and to arrest

gonadal development in young rainbow trout (*O. mykiss*) (Chmilevsky 2000). According to Blahm & Snyder (1975) and Jordan & Garside (1972), temperatures over 22°C may be lethal for sticklebacks.

In the present study, females with ovaries in an advanced stage of regression were probably the oldest, as their body size demonstrated. Their reproductive ability was naturally reduced. Dissected ovaries showed morphological symptoms of decay. The advanced and irreversible degeneration of gonads in old females was probably preceded by long-term resorption of overripe eggs. This process differed from the reversible regression of post-ovulatory follicles and ovarian epithelium commonly found following oviposition in sexually active females. In addition, behavioural aberrations were noted in the fish in their natural habitats, e.g. isolation from the shoal, lack of courtship responses to male, and apathy.

The prolonged breeding activity of sticklebacks, for as long as five months, enabled the fish to reproduce in the most favourable environmental conditions. This is in agreement with the conclusions of Bornestaf & Borg (2000), who found that sticklebacks living in cool temperate regions tend to start breeding as soon as external conditions allow. In our study, post-spawning spermatogenesis was completed in the autumn-winter period. The short days and low temperatures, however, naturally delay the final maturation of males until the spring, when they are again exposed to a favourable temperature and photoperiod.

The present studies, performed simultaneously in two wild populations of sticklebacks in their natural environment throughout a year, have yielded a detailed description of the reproductive cycle in this species. The proposed scale of gonadal development may be useful in the histological identification of the stage of gametogenesis in sticklebacks inhabiting different environments.

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