Arch. Ryb. Pol.	Archives of Polish Fisheries	Vol. 6	Fasc. 1	115 - 122	1998

NEW DATA ON BREEDING AND EARLY ONTOGENESIS OF THREE-SPINED STICKLEBACK (*Gasterosteus aculeatus* L.)

Aleksander Winnicki, Agata Korzelecka, Adam Tański

Academy of Agriculture in Szczecin, Poland

A B S T R A C T. Studies were carried out on the content of stickleback (*Gasterosteus aculeatus* L.) nests, spatial (in vertical and horizontal light beam) distribution of developing embryo, size and shape of perivitelline space.

Number of eggs in one nest was up to 1810, laid by several females for several days.

Germinal disk, thus also the embryo, was always placed laterally in the equatorial zone. Oil drops, which cause superior position of the germinal disk in other freshwater fishes, do not play such a role in the stickleback because they are not integrated with the disk.

Key words: *Gasterosteus aculeatus*, EGGS, PERIVITELLINE SPACE, GERMINAL DISK, OIL

INTRODUCTION

Three-spined stickleback is a cosmopolitan fish, occurring in coastal waters of northern seas (Nikolskij 1954). It also occurs in estuarine and inland waters (Berg 1949, Staff 1950). Systematic status of stickleback (Baruš, Oliva 1995), and general biology (Poepke 1983) are well known. Spawning behaviour, including nest building by a male to attract females, is very interesting. Incubation of fertilised eggs takes place in the nests. The nest is protected by a male which defends the eggs and sways the tail to create water movement. After hatching, the male still guards the larvae, until yolk-sac resorption. If they attempt to leave the nest, he brings them back in his mouth (Baruš, Oliva 1995). Due to this behaviour, the stickleback has become a textbook example of parental care in fish, and much attention is paid to its spawning behaviour.

Nests themselves, however, numbers, distribution and morphology of eggs, and ebryogenesis have not been thoroughly studied. Egg morphology and development have been assumed to be similar as in other fish.

Absolute fertility of stickleback females is rather low and ranges from several dozen (Staff 1950) to several hundred (Fries 1965) eggs. A female may lay up to 400 (Staff 1950), 500 (Deckert 1973), or up to 567 (Fries 1965) eggs. Number of eggs laid in one nest is highly variable and ranges from 400 (Staff 1950) to 800 (Poepke 1983), or even 1283 (Nikolskij 1954). All authors, however, agree that embryos at various age develop at the same time in a nest because eggs are laid over some time by various females. Nest structure and distribution of stickleback eggs are quite well known, while little is known about embryonic development.

Average diameter of eggs is equal to 1.7 mm (Staff 1950) or 1.65 mm and ranges from 1.49 to 1.76 mm. Scarce data on development time indicate that it depends on water temperature. According to Nikolskij (1954), stickleback eggs develop 4-27 days at 8-27^{o}C, and according to Baruš & Oliva (1995) – 4.28-46 days at 5.6-27^{o}C. After conversion, this makes 108 and 120 degree-days at high temperature, and 216 to 262 degree-days at low temperature respectively. Despite some discrepancies, it seems that the development is about twice extended at low temperature. This was also confirmed by Radziej (1986) who observed that stickleback eggs developed 84 hours at 21^{o}C, and 144 hours at 18^{o}C.

Almost nothing is known about stickleback egg structure over its development, particularly about dimensions of the germinal disk and its movement, and about ebryogenesis itself. The only paper on stickleback egg structure (Thomopoulos 1953) reported that the germinal disk was placed in superior position, as in other fish. The author, however, noticed and drew distribution of oil drops and lateral position of germinal disk different than in other fish eggs, but he ignored that.

Taking this into consideration, a detailed study of stiskleback nests and embryonic development was undertaken using a fine light microscope.

MATERIAL AND METHODS

The study was performed in June and July 1997 in Henryk Bak's private fish farm in Izdebno, in a field laboratory at Krzemień Lake hatchery.

Stickleback eggs were obtained from the nests situated in the lake littoral, on sandy bottom, at 40-60 cm depth. The nests were built from plant debris and sticks. The eggs were covered with filamentous algae and leaves. The nests were firm and durable, easily distinguishable from the substrate as small piles with one to three holes.

The nests were removed together with the substrate. Then the sand was washed out and the nest was cautiously torn to collect eggs which were not very sticky and easily separable. Then the eggs were placed in glass vessels filled with lake water to provide natural chemical and physical conditions.

The eggs were separated, counted and grouped according to development stage. Individual eggs were filmed during the development.

Water temperature was 21±2^{o}C and changed in a daily pattern corresponding to that in the lake littoral.

Observations were done using two microscopes with 2x Nikon objective connected with a digital camera CDD of high resolution, and with a video. Microscopic images were recorded on a video tape. One microscope camera was placed vertically and produced a view from above, and the other – horizontally, which allowed to observe eggs from the side. In the latter case, the eggs were placed in one row in special chambers 2-4 mm wide.

Embryogenesis was observed with special attention paid to spatial distribution of embryonic structures within the egg. Egg dimensions, yolk diameter, and perivitelline space were measured. Video recording improved considerably the accuracy of measurements.

RESULTS

In the nests collected from the lake the eggs were agglutinated with gelatinous matter and quite easily split into layers. Individual eggs were easily separable. Total number of eggs in each of the 5 nests was equal to: 1- 344, 2 – 418, 3 – 1210, 4 – 948, and 5 – 1810. Detailed microscopic observation revealed that eggs of each layer were at different stage of development, from cleavage or morula to embryo with developed brains, eye vesicles and body segmentation (Fig. 1a, b, c). Almost all eggs were fertilised (over 99%). Average egg diameter was 1.61 mm (1.40-1.85 mm), average egg volume – 2.18 mm^3, and surface – 8.12 mm^2. Thus, surface to volume ratio was 3.73. Yolk comprised 72.9% of total egg volume, and the remaining 27.1% of the space was filled with perivitelline liquid. Perivitelline opening diameter (at the top) was 0.16 mm, making 10% of egg diameter.

Animal pole with the germinal disk did not overlap (contrary to other fish eggs) the apex of the egg, and it was placed laterally, in equatorial position. Convex germinal disk contacted the egg shell (Fig. 2). Developing embryo took the same position.

During early ontogenesis, slow contractions of the periblast and ectoplasm surface were observed. Only several oil drops occurred in the yolk, sometimes several small drops fused into one. They were always placed at the top of the egg (Fig. 3a). If the egg was turned up-side-down, oil drops slowly moved to the top, which indicated that they were not connected to the germinal disk. When the egg was turned with the germinal disk up, oil drops floated underneath, and after another turning of the

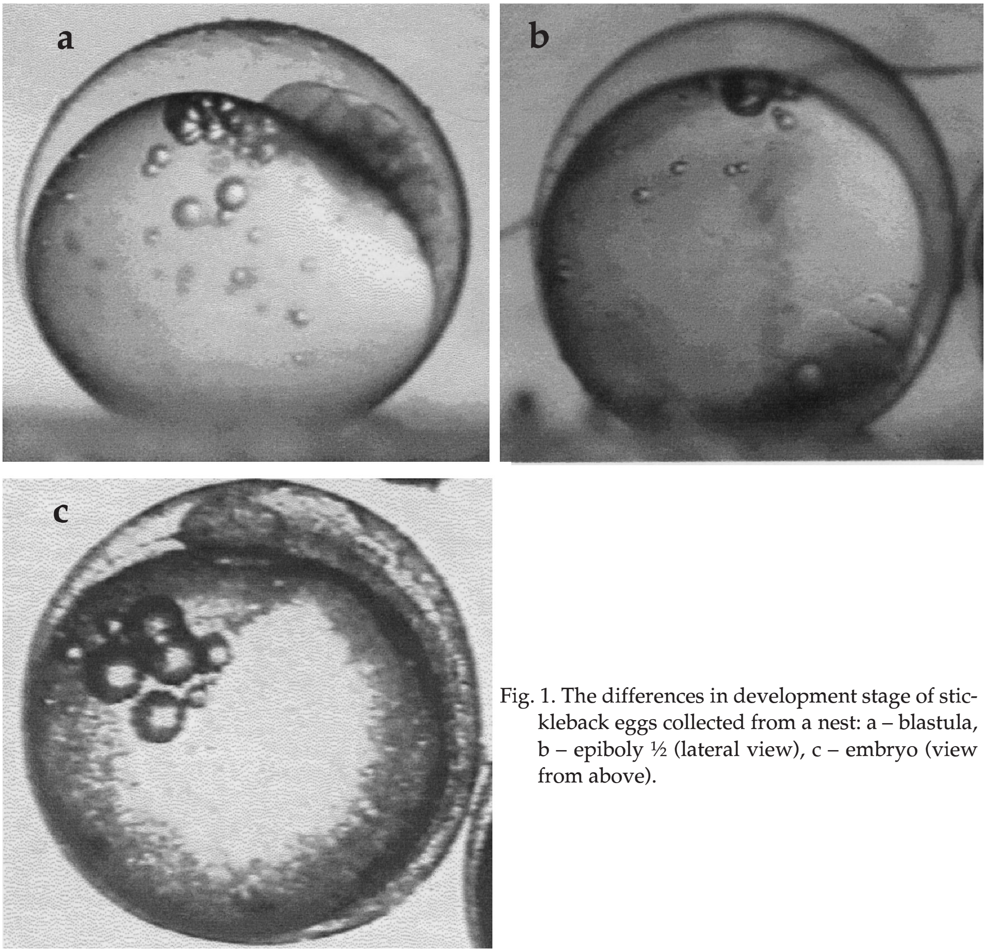

Fig. 1. The differences in development stage of stickleback eggs collected from a nest: a – blastula, b – epiboly ½ (lateral view), c – embryo (view from above).

egg – they drifted freely towards the yolk top (Fig. 3c).

DISCUSSION AND CONCLUSIONS

Observations confirmed earlier data on stickleback nest structure, content, egg arrangement, and male behaviour. The only difference concerned the number of eggs in a nest, which was over 500 eggs higher than reported by Nikolskij (1954), and seemed not to be a maximum number. Taking into consideration high number of eggs and only slight differences in their development, it was difficult to evaluate number of batches. Assuming about 100 eggs per one batch (estimated number of upper layer eggs at the stage of blastula formation), and provided that 1810 eggs found in one nest

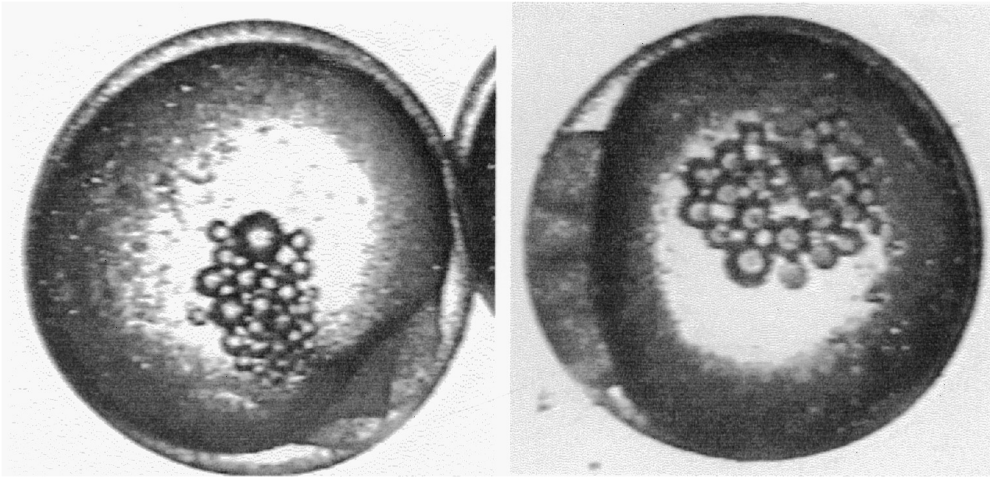

Fig. 2. Lateral position of the germinal disk in an egg. The eggs at stage 2 and 8 blastomers (view from above).

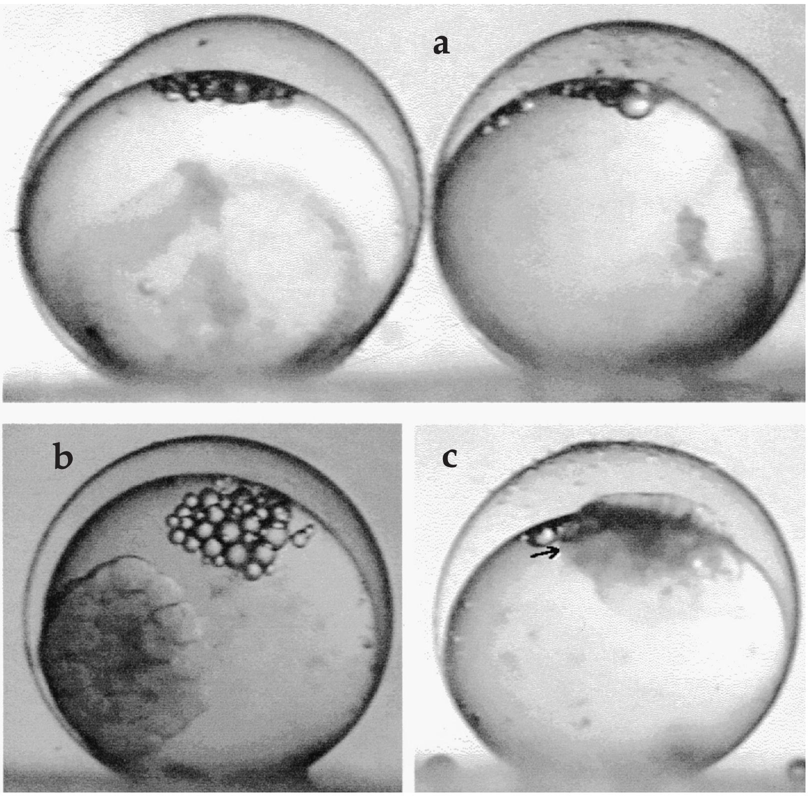

Fig. 3. Movements of oil drops non-connected with the germinal disk (details in the text) (view from above).

exceeded maximum fertility of one female (Fries 1965), it seems that the nests were used by several females; some of them might have visited a nest repeatedly.

Adaptive meaning of laying the eggs in layers, apart from providing protection, is not clear. Older eggs remain at the bottom covered with younger ones (up to several dozen degree-hours), which may cause oxygen depletion in deeper layers, favouring younger eggs. Such stratification may affect development rate: extend development time of older (bottom) eggs and shorten – in surface layer eggs. This, in turn, may cause shortening of the development time of the whole batch of eggs.

If the above hypothesis was a true one, the differences of the incubation time observed by various authors (Nikolskij 1954, Barus, Oliva 1995) would not result from temperature differences alone. Extension of incubation time caused by oxygen depletion was noted by Mourisier 1918, Kulmatycki 1925, and Winnicki 1967a.

It is interesting that in stickleback eggs the germinal disk takes lateral position, and tends to move down until stopped by a narrowing perivitelline opening. This seems to result from the lack of structural and spatial connection between oil drops and the germinal disk. Oil drops were observed to move freely at the yolk surface, always upward. In other teleost fishes, oil drops act as *sui generis* "fat raft" which pushes the germinal disk up towards the widest part of perivitelline space (Pasteels 1958, Winnicki 1967, Grodziński 1981). This may be explained by the fact that salinity-tolerant three-spined stickleback occurring in all types of waters was originally a marine fish. As other teleosts breeding in saline (thus – heavier) water, it supplied its eggs with oil drops (fusing into one) which pushed the yolk up, creating perivitelline space at the bottom of the egg, thus leaving plenty of space for the germinal disk to develop. Similar situation is observed in typically marine fish such as tuna (Joseph et al. 1988) and hake (Porębski 1981).

In freshwater three-spined stickleback this mechanism is useless and is a relic of a remote past, in which the fish ancestors had inhabited the seas. Other studies are planned to check this hypothesis.

The results and observations of the present study lead to the following conclusions:

1. Number of fertilised eggs in stickleback nests is about 30% higher than it was supposed. They come from several egg batches laid over several days.
2. The germinal disk, and then morula, blastula, and the developing embryo, always take lateral position in the equatorial zone of the egg.

3. Oil drops playing an important role in positioning the germinal disk in other freshwater fishes, do not play such a role in three-spined stickleback since they are not connected with the disk.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Committee for Scientific Research for financial support (grant P06D 001-12), to Mr. Joachim Skowronek for his help in research post equipment, and to Mr. Henryk Bak, the owner of the specialist fish farm, for fishery equipment and facilities.

REFERENCES

Baruš V., O. Oliva,1995 – Michulovci (*Petromyzontes*) a ryby (*Osteichthyes*) – Vol. 2, Academia, Praha

Berg L.S., I949 – Ryby presnych vod SSSR i sopredelnych stran – Leningrad.

Deckert K., l973 – *Acrania*, *Chondrichthyes*, *Osteichthyes* – In: Urania Tierreich. Bd 4.3.Leipzig Jena-Berlin.

Fries G., 1965 – Langen-Gewichts und Eiverhaltnise beim dreistichligen Stichling (*Gasterosteus aculeautus* L.) – Z. Fisch. (N.F.),13 (3-4):171-180

Kulmatycki W., 1925 – Notatki o próbach wylęgu ikry ryb łososiowatych bez przepływu wody – Arch. Ryb. Pol.,1:1-30

Mourisier P., 1918 – L'incubation de oeufs de truite en milieu stérile et pouvre en oxygène – Extrait des proces-verbaux de la Societé Vendoise des Sciences Naturelles.

Nikolskij F.,1954 – Častnaja ichtiologija – Moskva.

Pasteels J.,1958 – Development des Osteichthyes – In: Traité de Zoologie (P. Grassé ed.).13, 2:1711-1754

Poepke H.-J., 1983 – Die Stichlinge. Neue Brehm Bucherei – Wittenberg Lunherstadt.

Porębski J., I981 – Morfologia ikry i larv oraz rozmieszczenie tarlisk morszczuków Południowego Atlantyku (*Merluccius capensis* Cast., *M. paradoxus* F., *M. huhbsi* M., *M. polylepis* Ginsb.) – Rozprawa doktorska, Akademia Rolnicza w Szczecinie

Radziej J.,1986 – Ciernik (*Gasterosteus aculeatus* L.) – In: Ryby słodkowodne Polski [M. Brylińska ed.). PWN, Warszawa.

Staff F.,1950 – Ryby słodkowodne Polski i krajów ościennych – Warszawa.

Thomopoulos A.,1953 – Sur 1'oeuf de l'Épinoche (*Gasterosteus aculeatus* L.) – Bull. Soc. Zool. France, 78:142-148

Winnicki A.,1967a – Embryonic development and growth of *Salmo trutta* L. and *Salmo gairdneri* Rich. in conditions unfavourable to respiration – Zool. Pol.,17 (1-2): 45-58.

Winnicki A., 1967b – Plasticity and hardening of the rainbow trout egg membranes – Bull. Acad. Pol. Sci., Cl. II,15 (12): 779-783

STRESZCZENIE

NOWE DANE DOTYCZĄCE ROZRODU I WCZESNEJ ONTOGENEZY CIERNIKA (*Gasterosteus aculeatus* L.)

Badano skład skupisk ikry wewnątrz gniazd ciernika, a także, w pionowej i poziomej wiązce światła, za pomocą kamery cyfrowej przy powiększeniu l00x przestrzenne rozmieszczenie struktur rozwijającego się w jaju zarodka oraz wielkość i kształt przestrzeni okołożółtkowej.

Stwierdzono, że liczebność jaj w gniazdach ciemika jest o ok. 30% większa niż dotychczas sądzono i może dochodzić do 1810 szt. Na tę liczbę składają się jaja kilkunastu miotów dokonywanych przez różne samice w ciągu kilku dni, w wyniku czego w poszczególnych porcjach ikry znajdują się zarodki w różnych stadiach rozwoju.

Tarczki zarodkowe, a następnie zarodki zajmują u ciemika zawsze pozycję boczną w pasie równikowym jaja.

Krople tłuszczu początkowo dość liczne (do kilkunastu), w miarę upływu czasu zlewają się tworząc w połowie rozwoju zarodkowego zaledwie kilka, a nawet jeden pęcherzyk znacznych rożmiarów. Pęcherzyki tłuszczowe nie są z tarczką zarodkową zintegrowane, a zatem w przeciwieństwie do innych słodkowodnych ryb kostnoszkieletowych nie mają żadnego wpływu na góme usytuowanie tarczki zarodkowej

Przestrzeń okołożółtkowa niewielka, a objętość płynu okołożółtkowego stanowi do 23% objętości całego jaja.

ADRESY AUTORÓW:

Prof. dr hab. Aleksander Winnicki
Mgr Agata Korzelecka
Adam Tański
Zakład Anatomii i Embriologii Ryb
Akademia Rolnicza w Szczecinie
71-550 Szczecin, ul. Kazimierza Królewicza 3