Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC
JOURNAL
OF POLISH
AGRICULTURAL
UNIVERSITIES

2001
Volume 4
Issue 2
Series
FISHERIES

RECHULICZ J. 2001. INCUBATION TEMPERATURE EFFECTS ON THE DEVELOPMENT OF HATCHING GLAND CELLS IN IDE, *Leuciscus idus* (L.) **Electronic Journal of Polish Agricultural Universities**, Fisheries, Volume 4, Issue 2.
Available Online http://www.ejpau.media.pl

INCUBATION TEMPERATURE EFFECTS ON THE DEVELOPMENT OF HATCHING GLAND CELLS IN IDE, *Leuciscus idus* (L.)

Jacek Rechulicz
Department of Biological Bases of Animal Production, Agricultural University of Lublin, Poland

ABSTRACT

The ide eggs were incubated in the laboratory in three temperature ranges: 11–13, 15–16, and 18–20°C. Throughout the entire period of incubation, the live eggs were observed and samples were collected for histological assays. Microscopic slides were made to record the timing of appearance of hatching gland cells. The distribution, number, and size of the hatching gland cells, relative to incubation temperature, were determined. The cells appeared at the earliest (after 75 h of incubation) in the eggs incubated at 18–20°C and took the longest to appear (145 h) in those eggs incubated at the lowest temperature. The hatching gland cells

were observed to be present only in the anterior part of the ide embryo and were lacking in the caudal part. Depending on the temperature, an embryo showed the presence of 200 to 390 hatching gland cells. The largest cells, having 29.30 µm mean length, 24.54 µm mean height, and 9694.58 μm^3 mean volume, were typical of those embryos incubated at 15–16°C.

Key words: ide, egg incubation, hatching gland cells (HGCs)

INTRODUCTION

Water temperature is one of the major environmental factors influencing life processes of fish as aquatic organisms. Water temperature controls not only such basic processes as feeding and reproduction, but also the duration of egg incubation, and hence egg survival. Fish hatching depends on two mechanisms. The first involves the action of the enzyme chorionase on the egg membrane, while the second relies on mechanical movements of an embryo [6, 20]. Chorionase is an enzyme which decomposes internal layers of the egg membrane and is produced by epidermal glandular cells called the hatching gland cells (HGCs) [4, 7, 8, 16, 17]. Distribution, abundance, and size of HGCs have been studied in a number of fish species. Experiments showed the parameters to be species–specific [17, 18, 21, 22]. In addition to the problem of HGCs localisation in an embryo, already discussed in the literature, this work strives to determine the abundance and size of HGCs as well as effects of incubation temperature on the timing of appearance and size of HGCs in the eggs of ide *Leuciscus idus* (L.). The parameters mentioned may be decisive for the hatching success rate and for the suitability of the newly hatched larvae for further culture. Information on temperature effects on the ide HGCs, and – in consequence – on egg survival, will contribute to enlarging the knowledge on reproduction physiology of the species.

MATERIALS AND METHODS

Incubation temperature effects on the ide HGCs development were studied in eggs obtained during spawning conducted in 1997 and 1998 at the Fisheries Experimental Station at Łąki Jaktorowskie. The eggs, collected from several pond–farmed ide females, were separated using Woynarowicz's technique and incubated.

The eggs were incubated in experimental aquaria filled with water kept at constant temperatures of 11–13, 15–16 (the temperature regarded optimal for ide reproduction), and 18–20°C. The hatchery water temperature at which the eggs had been fertilised was 14.5 and 11.8°C in 1997 and 1998, respectively. In both years, the eggs were subjected to gradual thermal adaptation, following the study reported by Backiel and Horoszewicz [1]. Both the temperature reduction to 11–13°C and increase to the two remaining higher ranges proceeded at a constant rate of $\pm 0.5°C \cdot h^{-1}$.

Incubation in every water temperature was carried out in three aquaria each, every aquarium housing 300 eggs. At 6–h intervals, 15 eggs from each incubation temperature range were collected and fixed for histological assays.

Throughout the experiment, observations were made on live eggs to follow the development of ide embryos and to determine consecutive developmental stages.

The eggs, fixed and stored in 80% ethyl alcohol, were cut into 5 µm thick sections and stained with haematoxylin and eosin; triple staining of Azan–Heidenhain [2] was applied as well. A total of 790 microscopic slides were prepared.

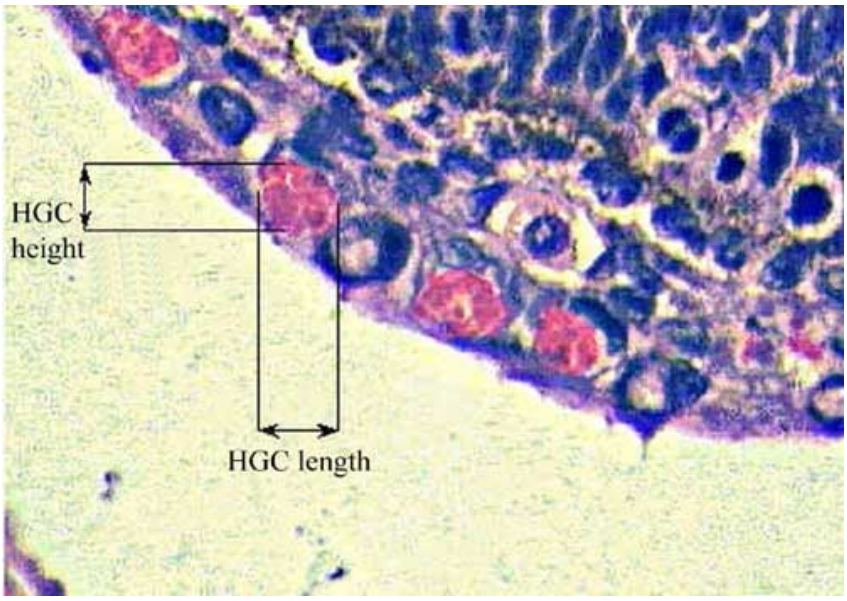
The number and distribution of HGCs were determined by examining the slides and counting the cells under a light microscope coupled with a computer. HGCs were measured (Photo 1) under a Nikon Alphaphot–2 YS2 microscope coupled with a Mintron camera and MicroScan for Windows v. 1.5 image analysis software. The HGCs volume was calculated from an elongated ellipsoid formula given by Blaxter and Hemple [3]:

$$V = 0.5236 \times l \times h^2$$

where l is the HGC length and h is the HGCs height.

The HGCs size data were tested for significance of incubation temperature effects with analysis of variance performed with the SAS statistical software package.

Photo 1. Measurements taken on hatching gland cells (HGCs) with MicroScan computer image analysis software (×640)



RESULTS

Timing of HGC appearance

HGCs on slides made from ide embryos were identified using methods applied to follow the timing of HGCs appearance in other fish species: salmonids (Salmonidae) studied by Yokoya and Ebin [25] and Yamagami [23], pike (*Esox lucius*) [10], as well as carp (*Cyprinus carpio*) and zander (*Stizostedion lucioperca*) [15, 16]. The triple staining technique allowed to identify HGCs which, when stained, turn orange. That colour was the basic criterion of telling HGCs apart from blue–staining mucus cells (Photos 2, 3).

Photo 2. Epidermis showing blue-stained mucus gland cells; Azan-Heidenhain staining technique (×600)

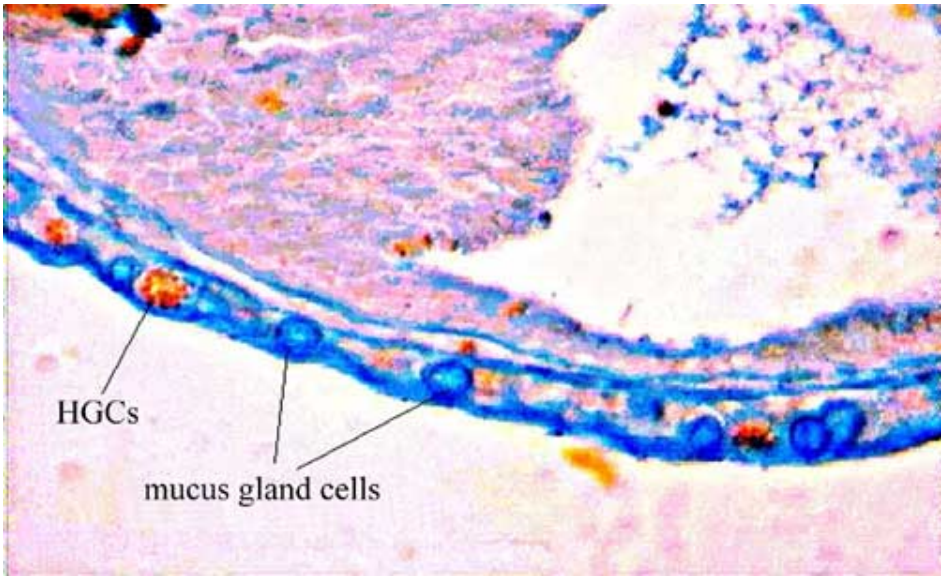
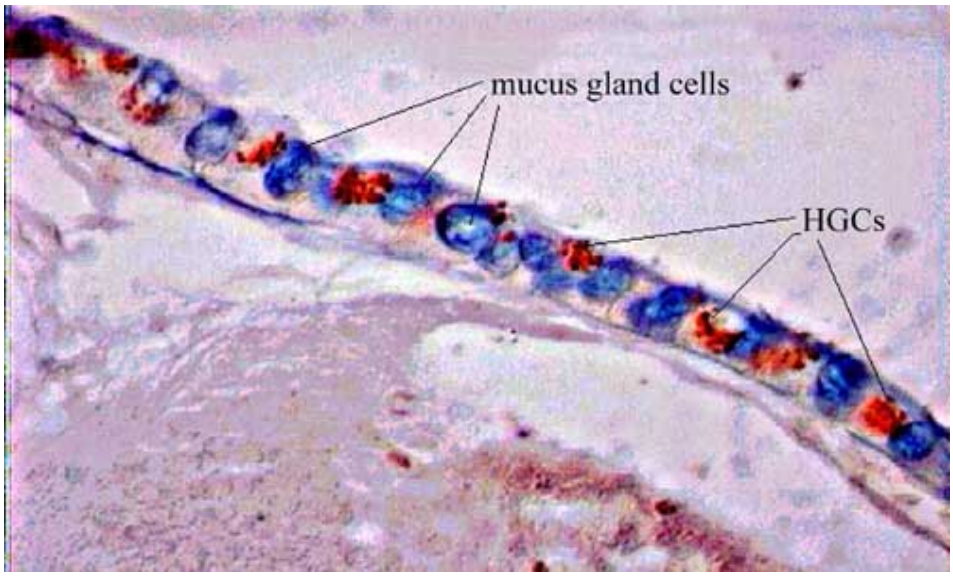


Photo 3. Epidermis showing orange-stained HGCs; Azan-Heidenhain staining technique (×600)



The timing of HGC appearance was found to depend on incubation temperature. The lowest temperature (11–13°C) resulted in a clear extension of the time before HGCs appeared. In the embryos incubated at that temperature, the cells appeared as late as after about 145 hours from fertilisation (before eyeing). The eggs became eyed as late as about 10 hours after HGCs appeared, *i.e.*, after 155 hours of incubation. At 15–16°C, close to the incubation optimum, HGCs appeared after about 110–115 hours of incubation (Photo 4). The timing overlapped with egg eyeing which happened after 105–110 hours of incubation. At the highest temperature (18–20°C), HGCs appeared 5–10 h after eyeing – after about 75 h of incubation (Photos 5, 6).

Photo 4. HGCs in epidermis of ide embryo incubated at 15-16°C, after 120 h of incubation (×600)

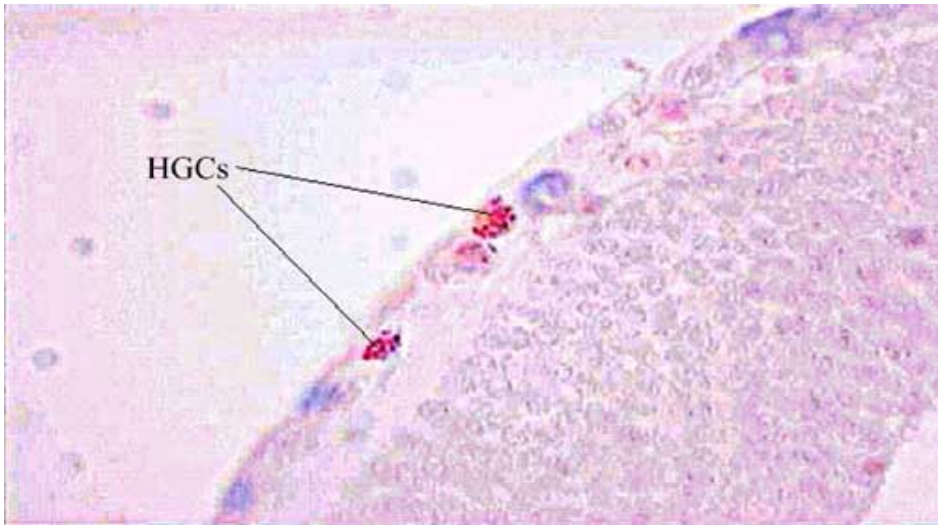


Photo 5. HGCs in epidermis of ide embryo incubated at 18-20°C, after 80 h of incubation (×600)

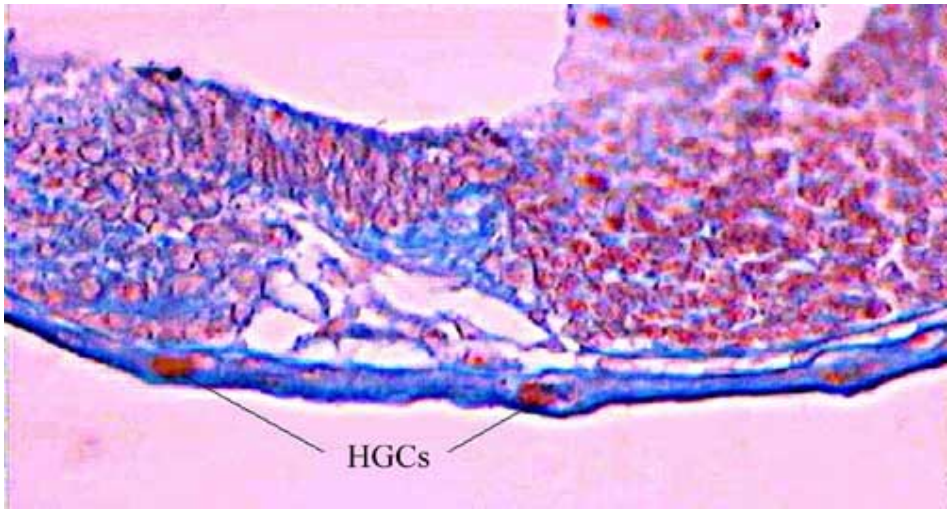
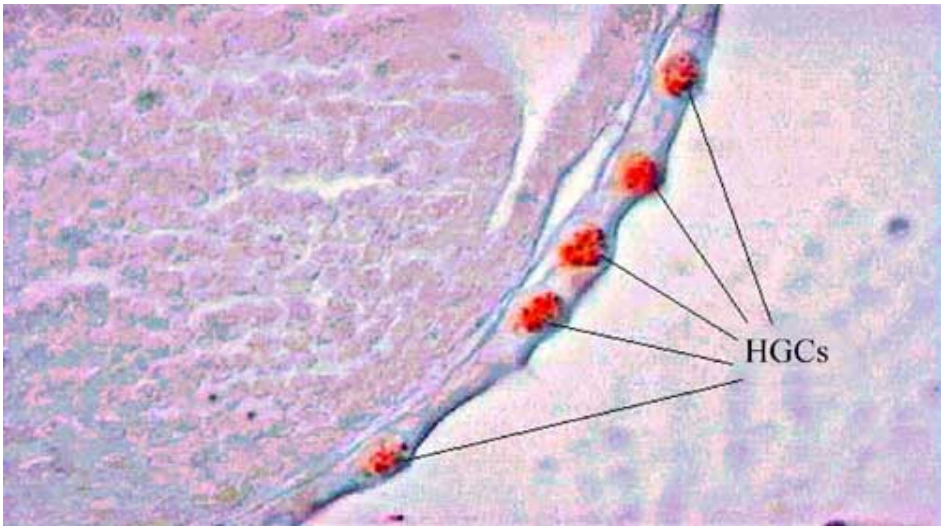


Photo 6. Densely arranged HGCs in epidermis of ide embryo incubated at 18-20°C, after 90 h of incubation (×600)



Number and distribution of HGCs

Examination of ide embryo histological slides and HGCs counts on individual cross–sections allowed to determine number of HGCs occurring in an embryo. Initially, the HGCs were relatively sparse and scattered in the embryo's epidermis. As the embryo grew, the number of HGCs increased as well, to reach 200–390 per embryo regardless of water temperature (Table 1). The small variation in the number of HGCs per embryo resulted most probably from individual variability among the embryos.

Table 1. Numbers of HGCs per ide Leuciscus idus (L.) embryos incubated in three water temperature ranges

Temperature [°C]	Number of HGCs	
	minimum – maximum	mean
11–13	200–390	292
15–16	215–385	293
18–20	215–380	299

Observations of the slides allowed to conclude that HGCs were present only in the anterior part of each embryo, the caudal part being devoid of them. The HGCs on the head occurred most abundantly in the frontal part and near the eyes (Photos 7 and 8 A, B, C, D). The HGCs on the yolk sac membrane were much less dense (Photo 9) and occurred only on that part of the sac closes to the head. In addition, HGCs were present also in the embryonic epidermis of the pectoral fins (Photo 10).

Photo 7. Localisation of HGCs in epidermis of ide embryo's head (×270)

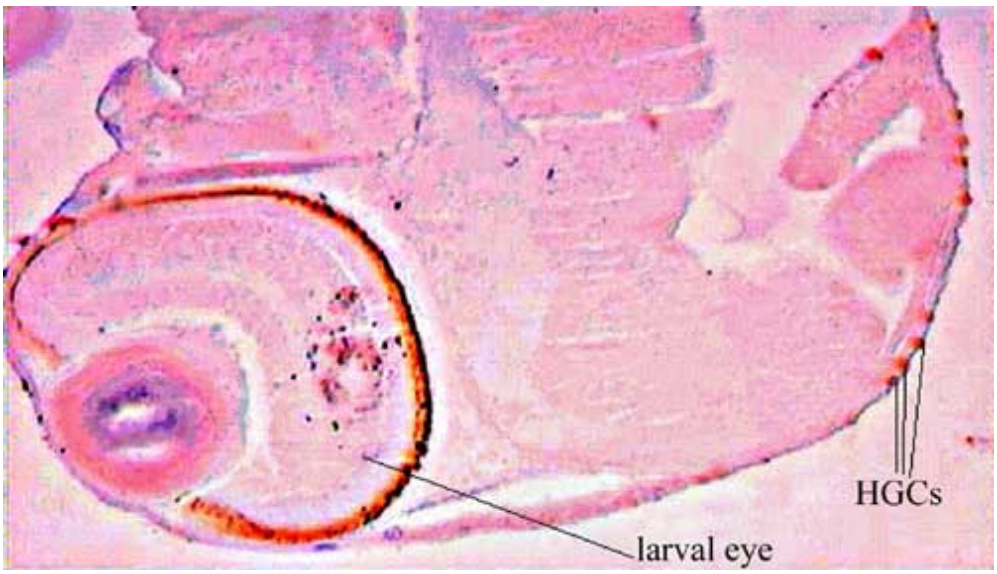
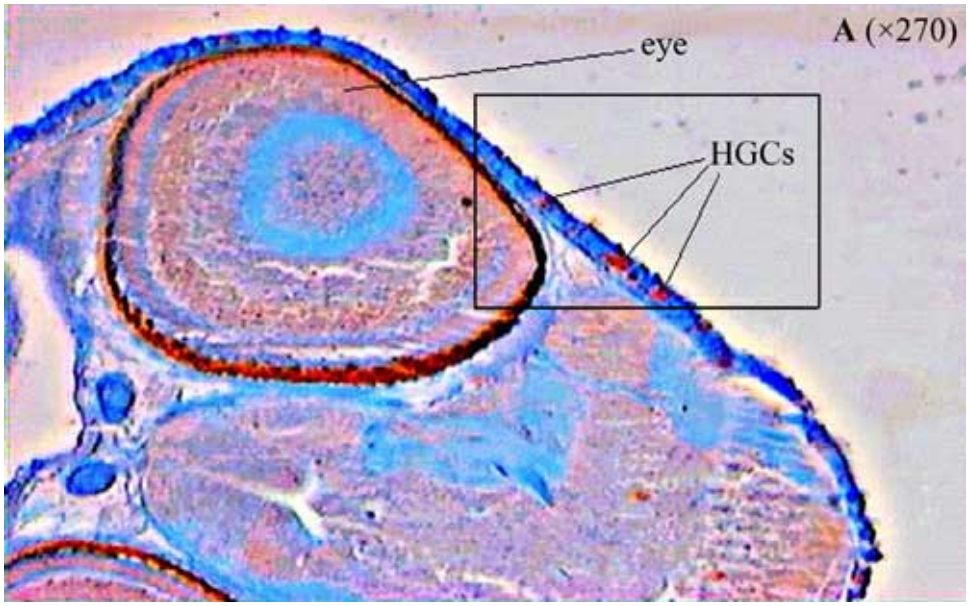
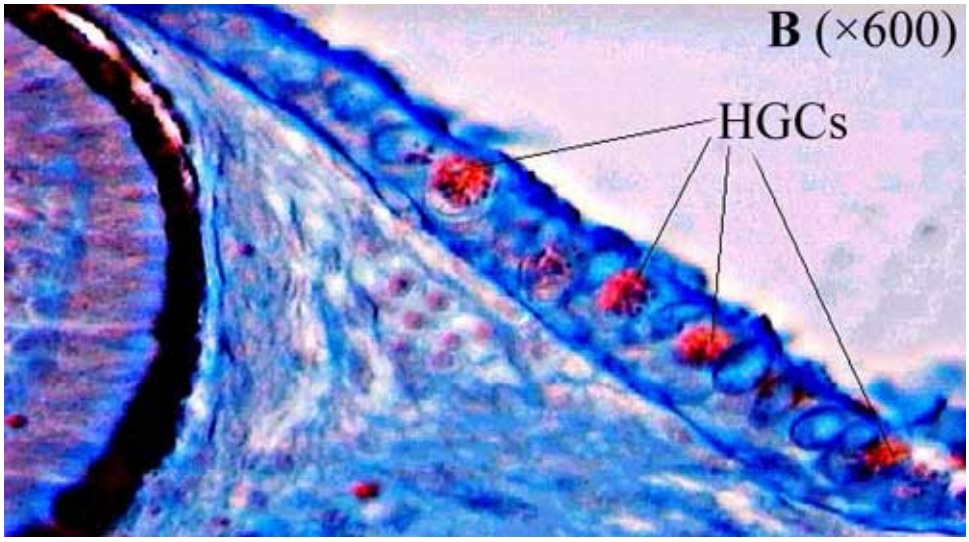
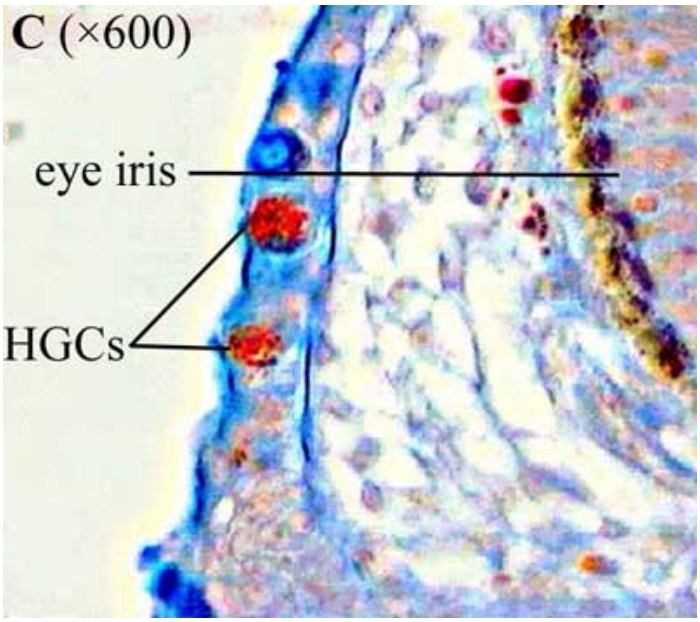


Photo 8 (A, B, C, D). Localisation of HGCs in epidermis of ide embryo near eyes







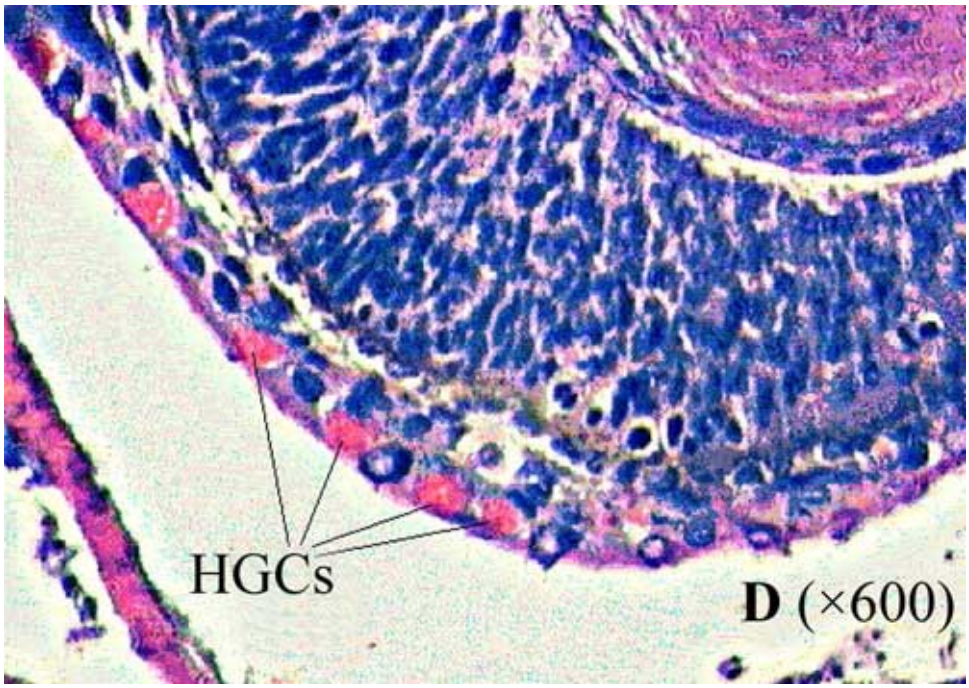


Photo 9. Localisation of HGCs in ide embryo's yolk sac membrane (×600)

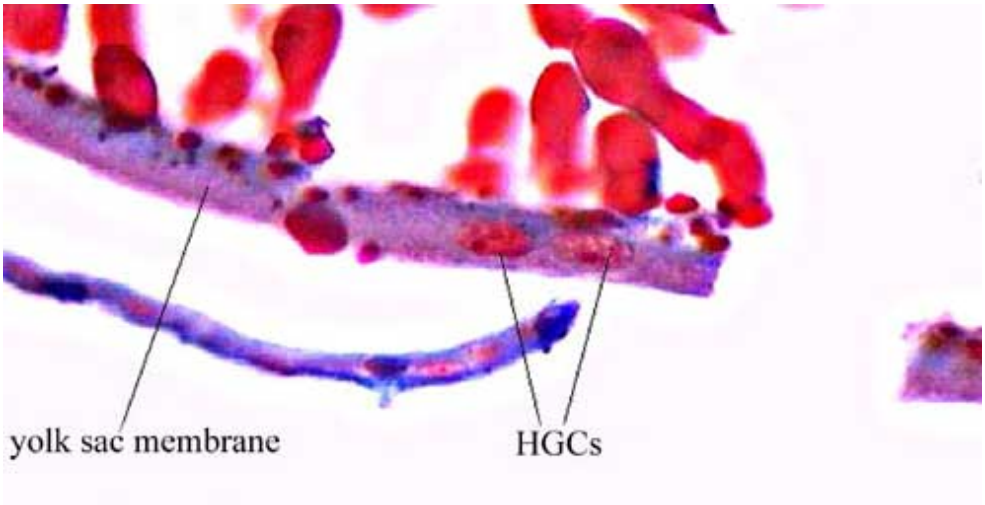
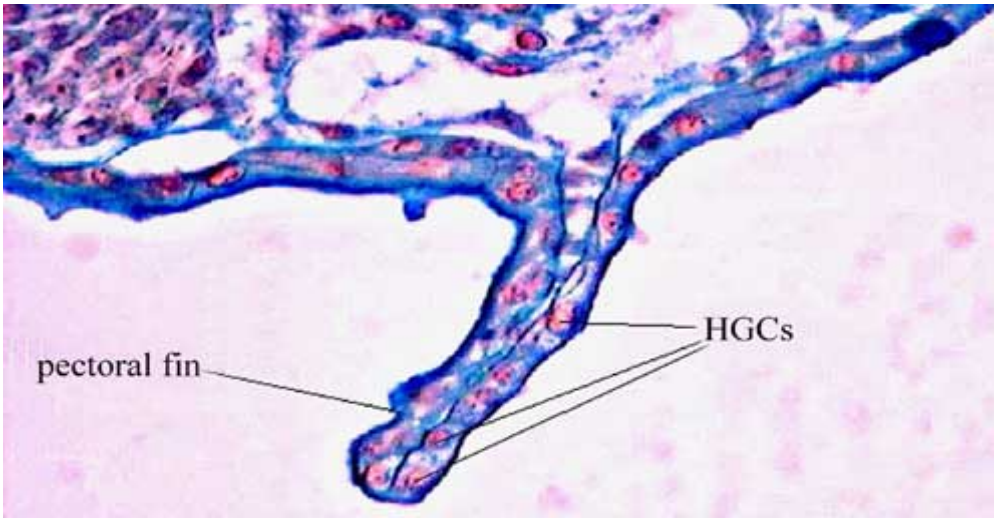


Photo 10. Localisation of HGCs in ide embryo's pectoral fin epidermis (×600)



HGCs size

Observations of microscopic slides and application of the image analysis system allowed to measure the length and height of the HGCs examined. The elongated ellipsoid volume formula made it possible to calculate the cell volumes. The HGCs dimensions were measured on the embryos after 114–130 hours of incubation at various incubation temperatures.

The larvae developing at 11–13°C had HGCs of 26.34 µm mean length, the value being lower than that found in the embryos incubated at 15–16°C (29.30 µm). The difference was highly significant (Table 2). The lowest HGCs mean length (23.92 µm) was characteristic of those ide embryos incubated at the highest water temperature (18–20°C) (Table 2).

Table 2. HGC dimensions in relation to water temperature

	11–13°C	15–16°C	18–20°C	Mean
N	390	360	180	
Length [µm]				
Mean	26.34 **B***	29.30 **A**	23.92 **C**	27.01
Standard error of the mean	0.28	0.26	0.48	0.19
Minimum	10.37	18.03	11.31	10.37
Maximum	40.10	43.29	48.27	48.27
Standard deviation	5.62	5.00	6.47	5.92
Height [µm]				
Mean	23.01 **B**	24.54 **A**	20.69 **C**	23.16
Standard error of the mean	0.27	0.21	0.34	0.16
Minimum	6.60	12.37	11.30	6.60
Maximum	41.52	36.12	39.61	41.52
Standard deviation	5.33	4.01	4.50	4.89
Volume [μm^3]				
Mean	8038.06 **B**	9694.58 **A**	5926.83 **C**	8270.67
Standard error of the mean	225.10	211.07	293.17	144.16
Minimum	257.96	1901.24	1008.23	257.96
Maximum	26438.31	25820.70	29016.41	29016.41
Standard deviation	4445.38	4004.82	3933.23	4396.16

***Means denoted with identical letters are not highly significantly different at $p \leq 0.01$.**

The embryos developing at 11–13°C showed HGCs having mean height of 23.01 µm, higher values being recorded for the HGCs of those embryos developing at the optimal temperature of 15–16°C (24.54 µm). The latter mean height was highly significantly different from those recorded in the remaining experimental groups (Table 2). The lowest mean height (20.69 µm)

was attained by HGCs present in the embryos incubated at 18–20°C and was highly significantly different from the mean heights recorded both in the embryos developing at 11–13 and 15–16°C (Table 2).

The ide HGC volume was clearly incubation temperature–dependent. At 11–13°C, the mean HGC volume was 8038.06 μm^3. The cells were smaller than those in the embryos developing at the optimal temperature (15–16°C) (the highest mean volume of 9694.58 μm^3) (Table 2). The lowest mean HGCs volume (5926.83 μm^3) was found at 18–20°C, the value being highly significantly different from those recorded in the other experimental groups (Table 2).

DISCUSSION

As reported in the available literature on fish reproduction physiology, the timing of HGCs appearance is closely related to the moment in time at which the eye pigment appears and the heartbeat and blood circulation begin [12, 13, 20].

The present study showed that the water temperature at which the ide eggs were incubated was clearly controlling the timing of HGCs appearance. The cells emerged more or less at the time when the eggs became eyed. The higher was the incubation water temperature, the earlier the pigment in the embryo's eye appeared and the heartbeat began. At 18–20°C, this occurred as early as 75 h after incubation and a few hours before eyeing. At the lowest temperature, 11–13°C, HGCs showed up as late as after 145 h, while eyeing was complete after 155 h. It was only at the optimum temperature of 15–16°C that the eye pigmentation preceded the HGCs appearance by a few hours, after about 110–115 hours of incubation. The first HGCs in zander kept at 18–20°C appeared after 70–74 h from fertilisation, while in carp kept at 20°C they emerged as early as after 40 h of development [15, 16]. As reported by DiMichele *et al.* [5], HGCs in *Fundulus heteroclitus* appeared, as they did in the ide in this study, at the moment of eye pigmentation emergence and heartbeat and blood circulation commencement. Similar results were obtained by Hagenmaier [6] in the rainbow trout (*Salmo gairdneri*) and by Ostaszewska [15, 16] in zander and carp.

Observations on the ide embryos under the microscope showed HGCs to be located, regardless of the incubation temperature, in the anterior part of the body and in the frontal wall of the yolk sac. These results are in agreement with results of studies carried out by Križanovski [11] and Ostaszewska [15, 16] on embryonic development of carp and zander. HGCs occurred on the yolk sac surface in pike (*Esox lucius*), rainbow trout, and *Brachydanio rerio* [8, 21, 22]. Occasionally, the gland cells appeared in the ide pectoral fin epidermis. A similar arrangement of HGCs in carp was observed also by Ostaszewska [16]. The caudal part of the embryo lacked HGCs altogether.

The number of HGCs found in the ide embryos varied from 200 to 390 per embryo and was independent of incubation temperature. A similar number of HGCs in *Barbus schuberti* was reported by Willemse and Denuce [22]. Embryos of zander [15] and *Brachydanio rerio*, *Danio malabaricus*, and *Moenkhausia oligolepis* [22] carried about 200 HGCs per embryo. On the other hand, the number of HGCs per embryo in carp was found to range from 400 to 600 [16], while much higher numbers (more than 1000 HGCs per embryo) were typical of embryonic pike and *Plecoglossus altivelis* [9, 21]. Rosenthal and Iwai [17] found embryos of *Clupea harengus* to support from 1500 to 2000 HGCs per embryo.

The results obtained in this study showed the ide HGCs to be 24–29 µm long (mean length of about 27 µm) and 20–24.5 µm high (mean height of 23 µm). The wide inter–specific size variability of HGCs is genetically controlled. The *Clupea harengus* HGCs were observed to be 16–30 µm long and 10–14 µm high [17], while they attained about 20 µm in rainbow trout [8] and 10–15 µm in pike [18, 21]. On the other hand, larvae of *Plecoglossus altivelis* carried small HGCs which attained as little as 7–8 µm in size [9], somewhat larger cells (14 µm) being observed in larval *Oryzias latipes* [24]. In Poland, Luczynski et al. [14] determined the HGCs size in ablen (*Coregonus albula*) and whitefish (*C.lavaretus*) to vary from 11 to 20 µm. On the other hand, Ostaszewska [15, 16] found the hatching–enzyme producing cells to be 16–20 µm long and 5–7 µm high in zander and 15 µm long and 8.5 µm high in carp.

The literature lacks reports on incubation temperature effects on HGCs size. The results of this study showed that the ide HGCs size did depend on water temperature prevailing during the embryonic development. The largest HGCs were observed in the embryos developing at 15–16°C; their mean length, height, and volume were 29.3 µm, 24.54 µm, and 9694.58 μm^3, respectively. This could be taken as evidence that the temperature in question was optimal for the embryos' development and allowed the cells to attain the maximum size. The smallest gland cells were supported by the ide embryos developing at 18–29°C; their mean length, height, and volume were 23.92 µm, 20.69 µm, and 5926.83 μm^3, respectively.

SUMMING UP

The timing of HGCs appearance in ide was incubation temperature–dependent. The earliest to appear (75 h after fertilisation) were HGCs in the embryos incubated at the highest water temperature (18–20°C). HGCs in those embryos incubated at the lowest water temperature (11–13°C) took the longest to appear (145 h of incubation).

The ide embryo HGCs were located in the anterior part of the body: on the head, near eyes, on pectoral fins, and on the frontal part of the yolk sac. No HGCs were detected on the trunk and caudal part of the larvae.

Individual ide embryos supported from 200 to 390 HGCs each, the number of HGCs per individual being similar in all the embryos, regardless of the incubation temperature.

Incubation temperature was found to affect the HGCs size. The highest values of HGCs size parameters were recorded in the embryos incubated at 15–16°C, a temperature range regarded as optimal for controlled reproduction of the species under study.

REFERENCES

1. Backiel T., Horoszewicz L., 1970, Temperature and fish, Wydaw. Inst. Ryb. Srod. Olszt. 41: 1–25, [in Polish].
2. Baginski S., 1965, Microscope Techniques. A Practical Manual for a Microscope User, PWN, Warszawa, [in Polish].
3. Blaxter J.H.S., Hemple G., 1963, The influence of egg size on herring larvae (*Clupea harengus*), J. Cons. Int. Explor. Mer. 28: 211 – 240.
4. DiMichele L., Taylor M. H., 1981, The mechanism of hatching in *Fundulus heteroclitus*: development and physiology, J. Exp. Zool. 217: 73–79.
5. DiMichele L., Taylor M.H., Singleton R., 1981a, The hatching enzyme of *Fundulus heteroclitus*, J. Exp. Zool. 216: 133–140.
6. Hagenmaier H.E., 1974, Proceedings: Enzymatic hydrolysis as a mechanism of hatching in the teleost, *Salmo gairdneri* Rich. Hoppe–Seylers, Zeitschrift. für Physiologische Chemie 355: 1202–1202.

7. Hagenmaier H.E., 1974a, The hatching process in fish embryos. V. Characterization of the hatching protease (chorionase) from the perivitalline fluid of the rainbow trout, *Salmo gairdneri* Rich. as metalloenzyme, Wilhelm Roux' Arch. 175: 157–162.
8. Hagenmaier H. E., 1974b, Zum Schlupfprozess bei Fischen. VI. Entwicklung, Struktur und Funktion der Schlupfdrüsenzellen bei der regenbogenforelle, *Salmo gairdneri* Rich. Z. Morph. Tiere 79: 233–244.
9. Iwai T., 1962, Studies on the *Plecoglossus altivelis* problems: Embriology and histophysiology of digestive and osmoregulatory organs, Bull. Misaki Mar. Biol. Inst. Kyoto Univ. 2: 1–101.
10. Kotljarevskaja H.V., 1969, Process vyluplenija u ščuki *Esox lucius* L., Vopr. Ichtiol. 9: 116–127.
11. Krizanovski S.G., 1949, Ekolo–morfologičeskije zakonomernosti razvitia Kaprovych, viunovych i somovych ryb (Cyprinoidei i Siluroidei), Tr. Inst. Morf. Ziv. I: 5–392.
12. Luczynski, M. 1985, Survival of *Coregonus albula* (L.) (Teleostei) embryos incubated at different thermal conditions, Hydrobiologia 121: 51–58.
13. Luczynski M., Dlugosz M., Szutkiewicz B., Kirklewska A., 1984, The influence of the incubation temperature on body length and the yolk sac volume of *Coregonus albula* embryos, Acta hydrochim. hydrobiol. 12 (6): 627–640.
14. Luczynski M., Hosaja M., Dabrowski K., 1986, Hatching gland cells in *Coregoninae* embryos, Z. Angew. Zool. 73: 63–73.
15. Ostaszewska T., 1989, Hatching gland cells in embryos of pikeperch (*Stizostedion lucioperca*), Ann. Wars. Agric. Univ., Anim. Sci. 24: 3–5.
16. Ostaszewska T., 1998, Development of unicellular hatching glands in carp (*Cyprinus carpio* L.), Ann. Wars. Agric. Univ., Anim. Sci. 34: 19–28.
17. Rosenthal H., Iwai T., 1979, Hatching glands in herring embryos, Mar. Ecol. Prog. 1: 123–127.
18. Schoots A. F. M., Denuce J. M., 1981, Purification and characterization of hatching enzyme of the pike (*Esox lucius*), Int. J. Biochem. 13: 591–602.
19. Schoots A. F. M., 1982, Enzymatic hatching of fish embryos. PhD Thesis, Catholic Univ. Nijmegen; The Netherlands.
20. Schoots A.F.M., De Bont R.G., Van Wys G.J.J.M., Denuce J.M., 1982a, Evidence for a stimulating effect of prolactin on teleostean hatching enzyme secretion, J. Exp. Zool. 219: 129–132.
21. Schoots A.F.M., Opstellen R.J.G., Denuce J.M., 1982b, Hatching in the pike *Esox lucius* L.: Evidence for a single hatching enzyme and its immunocytochemical localization in specialized hatching gland cells, Devel. Biol. 89: 48–55.
22. Willemse M.Th.M., Denuce J.M., 1973, Hatching glands in the teleosts, *Branchydanio rerio*, *Danio malabaricus*, *Moenkhausis oligolepis* and *Barbus schuberti*. Develop. Growth and Differ. 15: 169–177.
23. Yamagami K., 1981, Mechanisms of hatching in fish: Secretion of hatching enzyme and enzymatic choriolysis, Am. Zool. 21: 459–471.
24. Yamamoto M., 1975, Hatching gland and hatching enzyme. In Medeka (killifich). Biology and Strains, Keigaku Publ. Co. Tokyo.
25. Yokoya S., Ebina Y., 1976, Hatching glands in salmonid fishes, *Salmo gairdneri*, *Salmo trutta*, *Salvelinus fontinalis* and *Salvelinus pluvius*, Cell Tissue. Res. 172: 529–540.

Submited:

Jacek Rechulicz
Department of Biological Bases of Animal Production
Agricultural University of Lublin
Akademicka 13, 20–935 Lublin, Poland
Phone: +81 445 66 28
e-mail: jarech@ursus.ar.lublin.pl