

Małgorzata *BONISŁAWSKA*, Krzysztof *FORMICKI*, Aleksander *WINNICKI*

Fish embryology

SIZE OF EGGS AND DURATION OF EMBRYOGENESIS IN FISHES

WIELKOŚĆ JAJ A CZAS TRWANIA EMBRIOGENEZY RYB

Department of Fish Anatomy and Embryology, Agricultural University of Szczecin, Poland

Studies were undertaken on the relationship between egg size and duration of embryogenesis in four freshwater fish species (*Salmo trutta* L., *Esox lucius* L., *Gasterosteus aculeatus* L., *Leucaspis delineatus* Heck.). Analyses were carried out in a comparative sense (different taxa) as well as with respect to eggs of the same species, or even laid by the same female.

In addition to understandable differences of egg size between particular species, considerable differences were observed as regards egg size in fish of the same species, and even originating from the same female. In extreme cases the differences in egg size of the same female reached up to 11 percentage points (linear size) and over 40 (volume)!

A strict relationship was found between duration of embryonic development and egg size: the smaller the egg, the shorter its embryogenesis. Biological meaning of this phenomenon has been discussed.

INTRODUCTION

Scientific literature on the relationships between length of embryonic development and environmental factors is fairly abundant. Fish culturists are those most interested in these problems.

It seems that size of the egg cell, and thus—of the whole egg, represents one of the elements that might improve methods of incubating fish eggs in artificial conditions, especially in case of economically most valuable species, which are certainly worth paying more attention to. This was probably one of the reasons why so many scientists looked for the relationships between egg size and female age or size in a variety of fish species. Unfortunately, majority of these analyses ended up with stating simple facts, with no deeper insight to their biological determinants, nor possible consequences for the fish breeders (Suworow 1954; Terlecki and Kempieńska 1956; Kaj and Włoszczyński 1957; Schäperclaus

1961; Steffens 1963; Backiel 1964; Belanina 1964; Penczak and Przasnyska 1969; Bartel 1971a; Papała et al. 1998; Dlaboga et al. 1998).

Of special interest are the studies concentrated on variations of egg size within the same species. It was found that egg size may be affected by:

- fecundity (Zotin 1961; Smolej 1966);
- type and amount of food (Bartel 1971b);
- breeder strain—studies on salmonids (Pope et al. 1961) and rainbow trout (Bartel 1971b);
- selection procedures (Svärdson 1949; Scott 1962);
- environmental conditions (Moskalenko 1956) and others.

Rass (1953) found that egg size was a permanent tag for the order, suborder, family, subfamily, and species (in spite of the fact that egg size in a given species diminished as spawning season progressed, the amplitude of these variations did not change).

Also Bagenal (1971) dealt with the observed changes of fish egg size depending on the season, place of spawning, and reproductive cycle of marine fishes (46 species) with pelagic or demersal eggs, and of freshwater fishes (27 species), and confirmed a well-known seasonal trend of decreasing size of marine fish eggs. Tables presented by this author, based on the data obtained by a variety of other authors, clearly show that the egg volume in particular species may vary within a wide range.

Kaj and Lewicka (1962) stated that eggs in particular brown trout ovaries (*Salmo trutta* L.) differed in size. They also observed a clear effect of egg size (in controlled physico-chemical conditions) on the rate of embryogenesis and size of hatched larvae.

The same conclusions were presented, based on the differences in the weight of eggs collected from the same salmon females, by Privolnev (1953), and then Steffens (1963) and Galkina (1969) for rainbow trout.

Wallace and Aasjord (1984) also dealt with considerable differences of egg size in *Salvelinus alpinus* L. (egg diameter 3.8–5.8 mm) and concluded that larvae hatched from bigger eggs were bigger, showed higher growth rate, were less susceptible to diseases, showed lower mortalities, and had proportionally bigger yolk sac than larvae hatched from smaller eggs. What is surprising, is that these authors found no relationship between duration of embryogenesis and egg size.

Flipping through the most important papers on great differences observed as to fish egg size, both on a comparative basis (different taxa) and within eggs laid by the same female (not speaking of females of the same species), one reaches the conclusion that so far there has been no satisfactory explanation of the biological meaning of this phenomenon.

Usually the authors state facts, with no deeper explanation of the possible effects of differences in eggs size on embryogenesis in particular species. Notwithstanding this,

a regularity becomes fairly evident of the dependence between duration of embryonic development and egg size, both with respect to different species as well as eggs laid by one female.

A few questions arise: what is the mechanism of these relationships, what are their consequences for fish breeders, and—the most interesting question—what is the biological meaning of this phenomenon?

The aim of the study was to carry out a detailed analysis of egg size: between particular fish species, within one species, and from single females, and to attempt finding out possible mechanisms which determine relationships between egg size and duration of embryogenesis and possibly elucidating biological meaning of this phenomenon.

MATERIAL AND METHODS

The material consisted of the eggs of the following fish species:

- Brown trout (*Salmo trutta* L.) from 1998/99. Sexual products were obtained from spawners caught in the Wieprza River.
- Three-spined stickleback (*Gasterosteus aculeatus* L.) and white aspe (*Leucaspius delineatus* Heck.) from spring 1998. Fertilized eggs of stickleback were collected together with nests from the bottom of Lake Krzemień, and spawners of white aspe were caught in the same lake.
- Pike (*Esox lucius* L.) from spring 1999—fish ready to spawn originated from the Vistula River.

Eggs of brown trout, white aspe, and pike were fertilised with the “dry” method.

Incubation of brown trout and pike eggs was carried out in flow-through apparatuses in the aquarium room of the Department of Fish Anatomy and Embryology, in water with constant oxygen content and the mean temperature of 10.3°C for brown trout, and of 12.9°C for pike.

Eggs of three-spined stickleback (carefully removed from the nests) were placed in glass vessels containing lake water and additionally oxygenated. Eggs of white aspe were incubated in the same conditions.

Water temperature in a field hatchery in Izdebnó, in which studies were carried out, was maintained at the level of $20 \pm 2^\circ\text{C}$ in 24 h cycle, being close throughout the experiment to water temperature in the lake littoral.

Measurements of brown trout eggs were made using a measuring microscope because the eggs had a thick, opaque membranes. Then eggs from particular females were divided into three size fractions and incubated separately in the same flow-through apparatuses, taking note of the moment of larvae hatching in particular egg fractions.

Measurements of pike, three-spined stickleback, and white aspe eggs took advantage of the fact that eggs had transparent membranes and were small. They were carried out using a set consisting of a microscope with two $\times 2$ Nikon objectives, connected with a high resolution digital camera CCD, a monitor, and a VCR. The microscope picture (magnification $\times 100$) was controlled by a monitor and registered on video tapes. Multiscan[®] v. 6.07 computer programme was used to perform precise measurements of the two egg and egg cell diameters (longer and shorter) on the video picture. Arithmetical means were calculated from the two values. This method of egg measuring was proposed by Bartel (1971a). It can be well taken as sufficiently precise to calculate the size of the egg itself and of the inside egg cell.

Duration of embryonic development was determined using degree-days D° or degree-hours H° (the product of the number of days, or hours, of embryogenesis duration and the mean daily temperature (Embrey 1934)), with special attention given to maintaining the temperature at levels optimal for the species.

The results were treated statistically using the programmes: Excel 97, Statgraphics[®] v. 6.0 and Harvard Graphics.

RESULTS

It was found that duration of embryonic development of each species, measured in degree-days (D°) and—to obtain higher accuracy—in degree-hours (H°), was strictly related to egg size. And thus:

1. In fish of autumn spawning—brown trout (*Salmo trutta* L.) (eggs usually very big: 4.25–6.56 mm)—duration of embryonic development in specific environmental conditions was very long, 365–455 D° (Fig. 1). Fish of spring spawning—pike (*Esox lucius* L.), and laying eggs in summer—three-spined stickleback (*Gasterosteus aculeatus* L.) and white aspe (*Leucaspis delineatus* Heck.), had much smaller eggs: pike from 2.38 to 2.88 mm in diameter, three-spined stickleback: 1.47–1.66 mm, white aspe: 1.14–1.31 mm. Duration of embryogenesis was much shorter: 96.2–103.6 D° (2307–2450 H°) in pike (Fig. 2), 99.2–101 D° (2382–2427 H°) in white aspe, in stickleback (90 D°).
2. Egg size differed within the same species (in females of different age and size):
 - a) brown trout—smaller female (weight 1.5 kg, total length 56 cm) produced small eggs: 4.25–5.4 mm, while bigger female (weight 3.9 kg, total length 79 cm) had much bigger eggs, with diameter 20 percentage points bigger than of eggs from smaller female, viz. 5.75–6.56 mm (Fig. 3).
 - b) Similar differences were observed in pike; a female aged 3.5 years and weighing 1.4 kg, considered to be of medium size, produced the biggest eggs (2.58–2.88 mm), while eggs of a very small female, probably spawning for the first time (weight

0.3 kg), and of a very big one (4.4 kg) and the oldest (5 years) were almost the same size (2.38–2.70 mm) (Fig. 4).

3. Egg size differed even within eggs obtained from the same female. In extreme cases these differences were much greater than the differences between particular individuals. Figs. 3, 4, 5, and 6 clearly show that size distribution of eggs collected from single females of the examined fish species was a normal one, with the highest frequencies of medium-sized eggs.

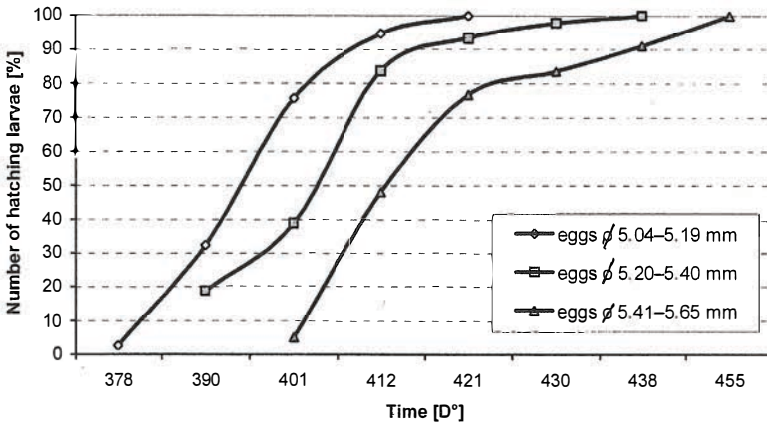


Fig. 1. Dependence between duration of embryonic development and size of eggs collected from the same brown trout (*Salmo trutta* L.) female

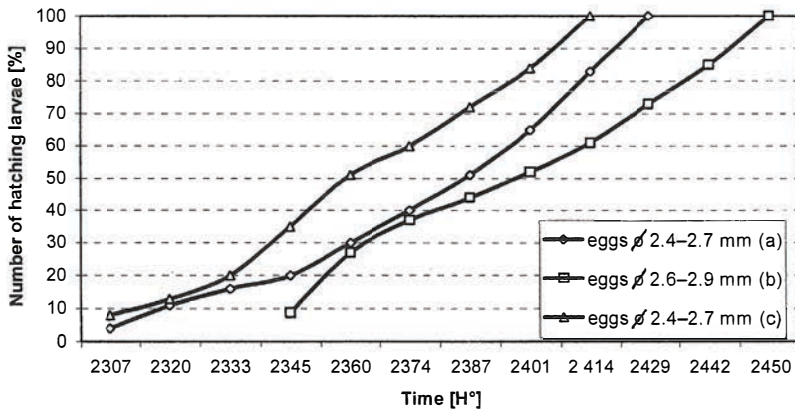


Fig. 2. Dependence between duration of embryonic development and size of eggs collected from three pike (*Esox lucius* L.) females: small one—weight 0.3 kg (a); medium one—weight 1.4 kg (b); big one—weight 4.4 kg (c)

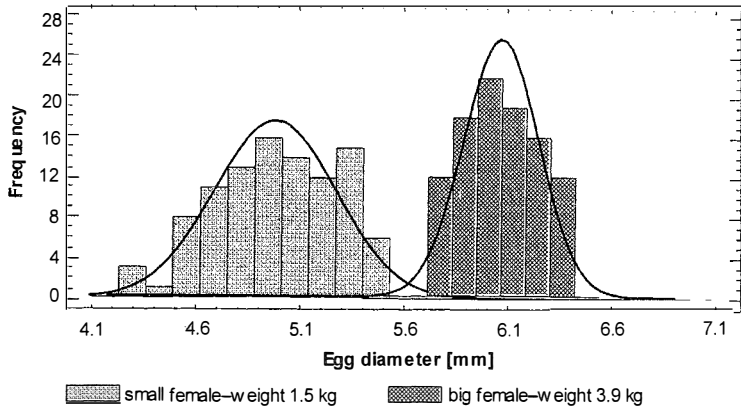


Fig. 3. Size frequency distribution of eggs collected from two brown trout (*Salmo trutta* L.) females

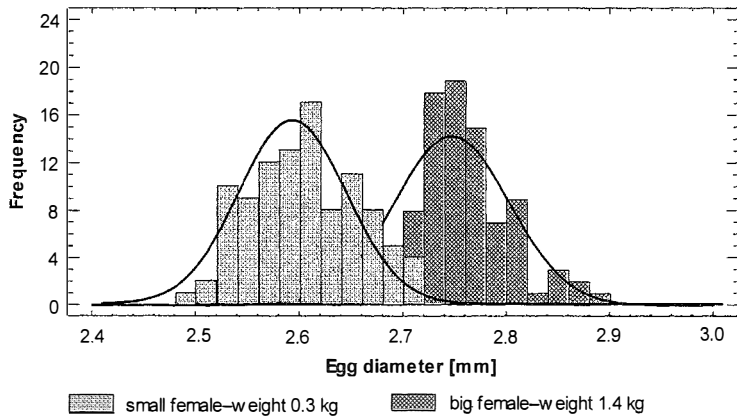


Fig. 4. Size frequency distribution of eggs collected from two pike (*Esox lucius* L.) females

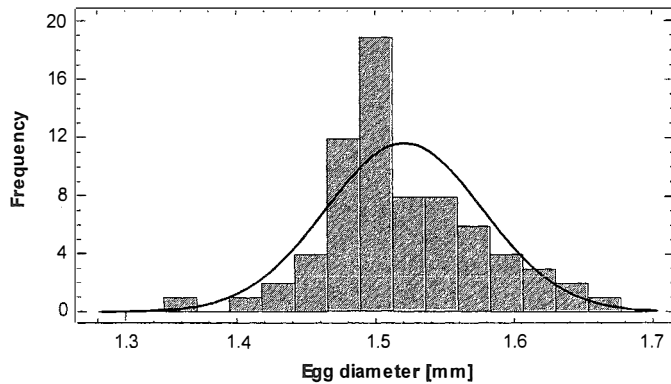


Fig. 5. Size frequency distribution of eggs collected from one three-spine-stickleback (*Gasterosteus aculeatus* L.) female

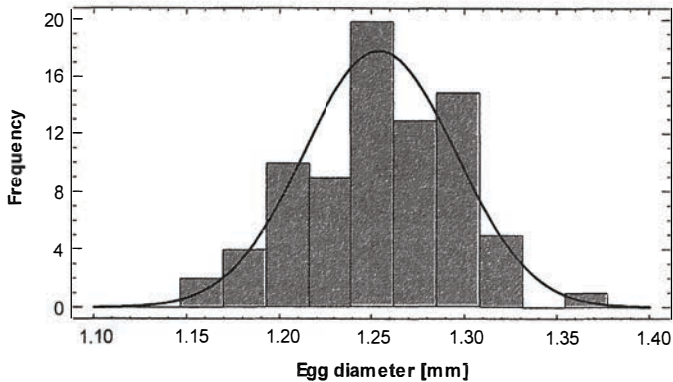


Fig. 6. Size frequency distribution of eggs collected from one white aspe (*Leucaspis delineatus* L.) female

DISCUSSION

Results obtained in our study confirm opinions of the majority of authors that size of fish eggs is generally highly diversified, and that there are considerable differences between and within species, and even within the same egg batch produced by the same female.

Notwithstanding this, when one reads ample literature on the subject it is difficult not to get an impression that the majority of the papers only states these obvious facts, whereas little attention is paid to the effects of egg size on the duration of embryonic development, although such an effect is readily seen in practically every single case. When analysing these relationships it seems worth mentioning a rather well known regularity that eggs of fish spawning in autumn-winter period tend to be bigger than of those spawning in spring and summer months. Even when autumn-spawning fish produce eggs of not such an extreme size (e.g. vendace), their structure is a little different from that of eggs produced by spring-spawning species. This difference refers to the size of the relative volume of yolk droplet in the perivitelline space. It is quite understandable that bigger eggs, with relatively bigger yolk volume, will develop at a slower pace than small eggs, having a relatively larger perivitelline space, and that their development measured in degree-days (D°) will be relatively longer. Perhaps it is also worth mentioning here that during embryogenesis of first type eggs, yolk is used to a lesser extent than in case of the latter egg type, in which the embryo uses almost the entire yolk volume. This makes the difference between the two egg types even more pronounced. Let's recall that newly hatched larvae of, say, cyprinids are almost devoid of yolk, whereas in case of salmonids only 10–15% of the yolk volume has been used by the embryo. Kamler and Kato (1983) observed that rainbow trout larvae hatched from smaller eggs used their yolk energy resources in a more economic way than those hatched from bigger eggs.

For the moment there is no satisfactory explanation of the mechanism underlying these phenomena; only obvious facts are noticed and registered. The essence of these phenomena, their material and physiological background, and biological meaning should be revealed by further studies.

Such studies are justified by a real need of gaining deeper knowledge on the mechanisms and subtleties making up reproduction strategy and species development. In case of economically valuable fishes which had also become almost domesticated animals, these problems need to be known also in order to improve incubation technologies. Because if there is a simple, frequently observed dependence between duration of embryonic development and eggs size, wouldn't it be justified to either initially segregate eggs of particular females and within the same batch with respect to their size, or to look for the methods to shorten hatching time (time lapse between the first and the last hatched larvae)? It is a common knowledge that egg hatching in case of autumn-spawning fishes and incubation in low temperatures may extend to several days, and this is certainly a drawback for the fish breeder.

It should also be mentioned here that the opinion formulated by Buznikov (1956) that technological mistakes are the only reason for extended period of larvae hatching in a given egg batch (incubation apparatus) does not seem to be justified. Explanation of many interrelations between egg size and rate of embryonic development regarded from a comparative level (different species) calls for many labour-consuming and precise studies. Only then it might be possible to formulate general conclusions on biological meaning of these differences.

It can be assumed that studies on the meaning of s/v index (ratio between egg surface and its volume), which have been recently initiated in our laboratory, will add to the knowledge of these problems.

CONCLUSIONS

Taking into account general ichthyological knowledge as well as the results obtained in this study, it can be already stated with high probability that considerable differences observed as to egg size within egg batches produced by the same female are a favourable feature for fish reproduction in nature, where periodical, short-term fluctuations of abiotic conditions, or an influx of pollutants might endanger development and hatching of the whole larvae population. The fact that there is a simple dependence between egg size and duration of their incubation, which extends the period of larvae hatching, ensures that at least some of them will be able to survive the dangers.

This seems to be the biological meaning of the described diversity of egg size, and it is also our general conclusion drawn from the results presented in this paper.

One could still discuss whether the phenomenon of considerable diversification of egg cell size (egg size), resulting in extended time during which the larvae leave egg envelopes and hatch, is a chance phenomenon or an evolutionary regularity. Whatever the answer (the latter seems more probable), the effect remains unchanged; it consists of increasing the survival chances for particular populations (group, race, species).

REFERENCES

- Backiel T.**, 1964: Pstrągi [The trouts]. PWRiL, Warszawa. (In Polish).
- Bagenal T.B.**, 1971: The interrelation of the size of fish eggs, the date of spawning and the production cycle. *J. Fish Biol.*, **3**: 207–219.
- Bartel R.**, 1971a: Pomiary średnicy jaj pstrąga tęczowego (*Salmo gairdneri* Rich.) [Measurements of diameter of the eggs of rainbow trout (*Salmo gairdneri* Rich.)]. *Rocz. Nauk. Roln.*, **93-H-3**: 7–17. (In Polish).
- Bartel R.**, 1971b: Czynniki decydujące o wielkości jaj pstrąga tęczowego (*Salmo gairdneri* Richardson) [Key factors affecting egg size in rainbow trout (*Salmo gairdneri* Richardson)]. *Rocz. Nauk. Roln.*, **93-H-4**: 7–33. (In Polish).
- Belanina T.N.**, 1964: O sviazi žirnosti samok, plodovitisti i kačestva ikry u bielomorskoj koriuški, *Osmerus eperlanus dentex natio dvinensis* Smitt [Relation between female body size, fertility of eggs quality in *Osmerus eperlanus dentex natio dvinensis*]. *Vopr. Ichtiol.*, **4**, 3 (32): 477–482. (In Russian).
- Buznikov T.A.**, 1956: Materialy po fizjologii vyluplenija embrionov kostistych ryb. [Materials on hatching physiology of embryos of teleost fishes]. *Trudy Soviešč. po Fizjologii Ryb, Tr. Sov. Ichtiol. Kom. AN SSSR*, **8**: 421–426. (In Russian).
- Dlaboga D., R. Bartel, K. Bieniarz, P. Epler**, 1998: Relation between egg size and body size and age of females in brook trout (*Salvelinus fontinalis* Mitchill). *Arch. Ryb. Pol.*, **6**, 1: 27–35.
- Emboly G.C.**, 1934: Relation of temperature to the incubation periods of eggs of four species of trout. *Trans. Am. Fish. Soc.*, **64**: 281–292.
- Galkina Z.I.**, 1969: Nieodnorodnost' zrieloj ikry radużnoj foreli (*Salmo irideus* Gibb.) [Heterogeneity of ripe eggs of *Salmo irideus* Gibb.]. *Izv. VNIORCH*, **68**: 187–196. (In Russian).
- Kaj J., K. Lewicka**, 1962: Wpływ wielkości ikry w jajnikach troci (*Salmo trutta* L.) na rozwój embrionalny i postembrionalny [The influence of the size of spawn in ovaries of *Salmo trutta* L. on the embryonic and postembryonic development]. *Rocz. WSR w Poznaniu*, **12**: 55–68. (In Polish).
- Kaj J., B. Włoszczyński**, 1957: Dojrzewanie płciowe i płodność ikrzyc szczupaka z dorzecza Warty jako podstawy do normowania wymiarów ochronnych [Sexual maturation and female spawners fertility of pike from Warta as a background for standardise the protective sizes]. *Pozn. Tow. Przyj. Nauk*, **3**, 5: 1–32. (In Polish).

- Kamler E., T. Kato**, 1983: Efficiency of yolk utilization by *Salmo gairdneri* in relation to incubation temperature and egg size. *Pol. Arch. Hydrobiol.*, **30**, 3, 271–306.
- Moskalenko B.K.**, 1956: Vlijanie mnogoletnich kolebanij reki Obi na rost, plodovitost' i rozmnożenie niekotorych ryb [The influence of long term water level changes of the Ob River on fertility and reproduction of some fishes]. *Zool. Žurn.*, **35**, 5: 746–752. (In Russian).
- Papała D., R. Bartel, K. Bieniarz, P. Epler**, 1998: Relation between Vistula sea trout (*Salmo trutta* L.) egg size and size of females. *Arch. Ryb. Pol.*, **6**, 1, 37–50.
- Penczak T, M. Przasnyska**, 1969: Przyczynek do znajomości biologii i morfologii *Alburnoides bipunctatus* (Bloch) [On biology and morphology of *Alburnoides bipunctatus* (Bloch)]. *Przegl. Zool.*, **13**, 1: 39–56. (In Polish).
- Privolnev T.I.**, 1953: Izmenienie viesa ikry lososia pri embrionalnom razvitii [Changes in salmon eggs weight during embryonic development]. *Izv. VNIORCH*, **33**: 4–9. (In Russian).
- Rass J. C.**, 1953: Značenje strojenija ikrinok i ličinek dla sistematiki ryb [The meaning of structure of eggs and larvae for fish systematic]. *Očerki po obščim voprosam ichtiologii*, 183–196. (In Russian).
- Schäperclaus W.**, 1961: *Lehrbuch der Teichwirtschaft*. Paul Parey, Berlin–Hamburg.
- Scott D.P.**, 1962: Effect of food quantity of rainbow trout, *Salmo gairdneri*. *J. Fish. Res. Bd Can.*, **19**, 4: 715–731.
- Smolej A.J.**, 1966: Plodovitost' sevanskich forelej [Fertility of trout]. *Vopr. Ichtiol.* **6**, 1 (38): 77–83. (In Russian).
- Steffens W.**, 1963: Eingrößen und Eizahlen der Regenbogenforelle (*Salmo gairdneri*). *Deutsch. Fisch. Ztg.*, **10**: 253–262.
- Suworow E.**, 1954: *Podstawy ichtiologii*. [Fundamentals of ichthyology]. PWN, Warszawa. (In Polish).
- Svärdson G.**, 1949: Natural selection and eggs number o in fish. *Ann. Rep. year 1948. Inst. Freshw. Res. Bd Drottningholm*, **29**: 115–122.
- Terlecki W., H. Kempnińska**, 1956: Sieja i sielawa [Whitefish and cisco]. PWRiL, Warszawa. (In Polish).
- Wallace J.C., D. Aasjord**, 1984: An investigation of the consequences of egg size for the culture of Arctic char, *Salvelinus alpinus* (L.). *J. Fish Biol.*, **24**: 427–435.
- Zotin A. J.**, 1961: Otnositelnaja plodovitost' i razmiery ryb [Fertility and sizes of fish]. *Vopr. Ichtiol.* **1**, 2 (19): 307–313. (In Russian).

Małgorzata BONISŁAWSKA, Krzysztof FORMICKI, Aleksander WINNICKI

WIELKOŚĆ JAJ RYB A CZAS TRWANIA EMBRIOGENEZY

STRESZCZENIE

Mierzono, a następnie inkubowano jaja czterech gatunków ryb: troci (*Salmo trutta* L.), szczupaka (*Esox lucius* L.), ciernika (*Gasterosteus aculeatus* L.) i słonecznicy (*Leucaspis delineatus* Heck.). Pomiary wykonywano w przypadku troci, (ze względu na małą przezroczystość osłonki jajowej), za pomocą mikroskopu pomiarowego, zaś wymiary jaj pozostałych gatunków uzyskiwano stosując zestaw składający się z mikroskopu (powiększenie $\times 100$) sprzężonego z kamerą CCD, monitorem, magnetowidem i komputerem.

Badania wykazały, że znaczne różnice wielkości występujące nie tylko u poszczególnych taksonów, ale także w obrębie tego samego gatunku (różne ikrzyce), a nawet w zbiorowiskach jaj pozyskanych od tej samej samicy.

Ustalono, że istnieje istotna zależność długości trwania rozwoju zarodkowego od wielkości komórki jajowej i w konsekwencji nie pozostaje bez wpływu na przebieg i czas wykluwania się larw w zbiorowisku (od pierwszej wyklutej larwy do ostatniej).

Rozważane są sens biologiczny tych zróżnicowań oraz konsekwencje hodowlane.

Received: 15 October 1999

Author's address:

Małgorzata Bonisławska MSc
Department of Fish Anatomy and Embryology
Agricultural University of Szczecin
Kazimierza Królewicza 4, 71-550 Szczecin, Poland