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INFLUENCE OF NaCl SOLUTIONS AT VARIOUS CONCENTRATIONS ON HARDENING OF EGG MEMBRANES OF TROUT – *SALMO TRUTTA* L.

WPŁYW ROZTWORÓW NaCl O RÓŻNYM STĘŻENIU NA TWARDNIENIE OSŁONEK JAJOWYCH U TROCI – *SALMO TRUTTA* L.

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The influence of NaCl solutions on the hardening process of egg membranes of the trout, *Salmo trutta* L. was investigated.

Experiments have proved that the processes connected with absorption of water and with hardening of the membranes may take place even in a habitat of a considerable salinity, but their initiation must be preceded by at least a short period of immersion of the egg in fresh water.

It is the authors' intention to try to explain the phenomenon of a different effect of solutions of various concentrations on the egg membranes as well as the increase of the membrane hardening velocity in NaCl solutions in comparison with the standard.

Great interest is being given to the attempts made at accustoming fresh water fishes to life in brackish waters (**Karpevič** and **Dorošev**, 1964; **Bartel**, 1969; **Żukowski**, 1973). On the one hand, it is the development of hydrotechnical facilities and the increasing pollution of water connected with the growth of industry, that restrict the natural range of the appearance of many fish species, mainly of two-habitat salmonid and acipenserid fishes, which become deprived of their natural spawning grounds. On the other hand, however, there is a possibility now of improwing the qualitative composition of the ichthyofauna in highly productive brackish water basins which may become fishing grounds or even cultivation centres for valuable fish species (**Rass**, 1965; **Landrein**, 1973).

Although the fry and adult fishes become readily accustomed to the altered living conditions (**Willer** and **Trahms**, 1942; **Landrein**, 1973), attempts aimed at fertilizing and incubation of fish eggs in brackish or diluted sea water usually ended with a failure or were successful only in water of a 3–5‰ salinity. This refers among others, to herbivorous cyprinid fishes, *Ctenophyryngodon idella* and *Hypophtalmichthys molitrix* – (**Dorošev**, 1964; **Rykova**, 1964, 1966) as well as to the American centrarchid fishes, *Micropterus salmoides* and *Lepomis macruchirus* – (**Tebo** and **McCoy**, 1964). In Poland, attempts of incubation of *Salmo gairdneri* eggs in the water from the Baltic were taken up. Although on the whole it proceeded without any disturbances, the fertilization process alone was not successful (**Wiktor**, 1973; **Żukowski**, 1973).

There is a justified suggestion that the causes connected with the incubation failure of salmonid fishes eggs in their early development stages (just after fertilization) are to be found in mechanisms for regulating the water and ionic exchanges which take place in the egg at that time. As is well known, the egg membranes of the salmonid fishes, in their initial development stage, can be permeated by water and salt (**Bogucki**, 1930), but in a short time they increase their strength rapidly and become almost completely impermeable (**Zotin**, 1961). In spite of a lot of research work in this field (**Bogucki**, 1930; **Zotin**, 1955, 1958, 1961; **Kalman**, 1959; **Fischer**, 1963; **Winnicki**, 1968; **Potts** and **Rudy** 1969; **Winnicki** and **Cykowska**, 1973; and many others) not all of the problems involved have been explained so far. Among others things, explanation of the role of various ions in these processes is required.

The present work is aimed at determining the influence of various NaCl contents in water upon the hardening process of the trout, *Salmo trutta* L. egg membranes.

MATERIAL AND METHODS

The experiments were carried out in November and December in 1972 and 1973. Eggs from trout, *Salmo trutta* L. were used as experimental material. The fishes from which the eggs and milt were taken had been caught in the Rega river (near their spawning ground) and in the Vistula river (near the mouth) and kept in basins with fresh water for a period of three months.

The eggs and milt were taken to the laboratory in vacuum flasks without any water in them at a temperature of not more than 8°C. Fertilization was carried out not earlier than 10 to 12 hours from the moment of obtaining the eggs, which, as it has been proved earlier (**Cykowska** et. al., 1973). does not influence on the development of the eggs. Fertilized eggs were at first placed in water and after a short time (0.5; 1; 3 and 10 minutes) taken to 5‰, 10‰ and 15‰ solutions of NaCl (0.09; 0.18; 0.27 M NaCl).

After 12 and 60 hours from the moment of fertilization the strength of the egg shells by a modified **Schäperclaus'** method (1940) was measured. For a check-up test eggs which developed the whole time in water (check-up I) as well as eggs that were taken to NaCl solutions (check-up II) immediately after fertilization were used. The water temperature during the experiments amounted to 10°±1°C.

RESULTS

Data regarding the strength of trout egg shells after 12 and 60 hours after fertilization have been compiled in Table 1. It follows from the table that Rega river trout eggs kept in a 5‰ solution of NaCl had an increase of strength by about 75% of the maximum value already in the sample that had been taken over after a 0.5 min. stay in water. In the case of eggs kept in 10‰ and 15‰ solutions, such a high increase of the shell strength appeared only in samples that had been previously kept in water for at least 3 minutes.

A higher increase of the egg shell hardening velocity in NaCl solutions can be observed in comparison with the check-up I i.e. with those egg shells that had been kept in water from the very beginning. This was independent of the concentrations of those solutions. Eggs that had been taken over to NaCl solutions from water 3 minutes after fertilization attained a nearly maximum shell strength as early as after 12 hours, whilst the eggs of check-up I attained only 65% of that value in the same period of time. After 60 hours the strength of egg shell was nearly the same in the samples and in the check-up I.

As far as the Vistula trout eggs are concerned, an increase of their shell strength was found to take place only in those samples that had been previously kept in water for at least 3 minutes. The maximum strength of those eggs in the samples as well as in the check-up I, was nearly twice lower than of the Rega trout eggs. In this case such considerable difference in the hardening velocity between the shells in the samples and in the check-up I did not take place.

The shells of eggs that were kept in the NaCl solution of the check-up II during the whole duration of the experiment did not increase their strength at all. Those eggs did not absorb any water either.

DISCUSSIONS AND CONCLUSIONS

The results obtained in this work permit to state that whilst the water absorption itself by the egg after fertilization is possible in an environment with considerable salinity, the initiation of this process can take place only in water with a salinity lower than 3 to 4‰ and in a period of less than 1 minute. This statement corrects **Zotin's** (1958, 1961) viewpoint regarding the length of the period for the stay of the egg in non-salted water indispensable for the initiation of the water absorption process. It is also in conformity with the results obtained by other authors later (**Winnicki** and **Cykowska**, 1973).

As regards to the raised, in relation to the standard (eggs developing in non-salted water) shell strength increasing velocity of eggs subjected to incubation in NaCl solutions at various concentrations, it may be concluded that the initiated water absorption process becomes slower as soon as the eggs are taken over to NaCl solutions. (**Potts** and **Rudy** share the same opinion, 1969). The shell hardening enzyme which is present at that time in the periviteral fluid may, as it is believed by **Winnicki** and **Bartel** (1967), act with a higher intensity owing to its increased concentration and shorten the strength of egg shell raising period from 2 days to 12 hours. That phenomenon takes place regardless of

Table 1

Strength (g) of the egg membranes of *Salmo trutta* L.
placed for a certain period in water and than incubated in NaCl solutions

Strength measurement time (hours)		12			60		
NaCl concentration (‰)		5	10	15	5	10	15
Time of the eggs stay in water (min)	**Eggs of the trout from the Rega river**						
	0.5	3100	230	240	3500	300	260
	1.0	3650	460	240	3600	500	270
	3.0	4050	4100	3000	4000	4100	4100
	10.0	4100	4100	4000	4000	4100	4050
	check-up I	2600	2600	2600	4000	4000	4000
	check-up II	250	230	240	250	240	230
	Eggs of the trout from the Vistula river						
	0.5	200	200	180	180	180	180
	1.0	230	190	180	290	280	200
	3.0	1250	1200	1200	1750	1700	1600
	10.0	1850	1200	1200	2000	1850	1650
	check-up I	1200	1200	1200	1700	1700	1700
	check-up II	170	180	180	170	180	180

the NaCl concentration in all the eggs that had been taken over to the solutions 3 minutes after their fertilization.

Such explanation, however, is not fully satisfactory as regards to the differences of strength between the eggs that were placed in a 5‰ NaCl solution 0.5 to 3 minute after they had been fertilized and those taken to solutions of a higher concentration after the same period of time. Maybe there is some other factor which inhibits the action of the membrane hardening enzyme. This, however, can be explained only after thorough investigation in future. It would appear necessary to determine the time period precisely, during which the hardening enzyme acts upon the shells. This however, is not a simple matter because the effect of such action becomes visible only after several minutes.

With consideration given to the above statements, incubation of an egg fully filled with water seems to be possible in brackish water or even in sea water. The same is the case with the absorption process, provided that the initiation takes place in fresh water. In spite of those possibilities considerable practical effects cannot be expected on their basis. This is first of all due to economical considerations and because of the fact that not all the factors which may have an influence on the developing eggs are known yet.

As the experiments on the Vistula trout were carried out with eggs from one female only, the results obtained can only have an ancillary significance. The differences in the strength of egg shell could be caused, for instance, by the physiological condition of the female beyond its standard. The fact of keeping the fish in basins with fresh water for a few month could also be the cause of those differences.

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WPŁYW ROZTWORÓW NaCl O RÓŻNYM STĘŻENIU NA TWARDNIENIE OSŁONEK JAJOWYCH U TROCI – *SALMO TRUTTA* L.

Streszczenie

Badano wpływ roztworów NaCl o różnym stężeniu na proces twardnienia osłonek jajowych u troci, *Salmo trutta* L.

Znalazły potwierdzenie dane o tym, że procesy wchłaniania wody i twardnienia osłonek mogą zachodzić nawet w środowisku o znacznym zasoleniu, lecz dla ich zainicjowania jajo musi przynajmniej na krótki okres czasu znaleźć się w wodzie słodkiej.

Autorzy podejmują próbę wyjaśnienia zjawiska odmiennego wpływu roztworów o różnych stężeniach na osłonki jajowe oraz wzrostu szybkości twardnienia osłonek w roztworach NaCl w porównaniu z normą.

ВЛИЯНИЕ РАСТВОРОВ NaCl РАЗЛИЧНОЙ КОНЦЕНТРАЦИИ НА ОТВЕРДЕВАНИЕ ОБОЛОЧКИ ИКРИНОК У КУМЖИ (Salmo trutta L.)

Р е з ю м е

Исследовали влияние растворов NaCl различной концентрации на процесс отвердевания оболочки икринок у кумжи (Salmo trutta L.). При этом подтвердились данные, свидетельствующие о том, что процессы поглощения воды и отвердевания оболочек могут происходить и в среде со значительной солёностью, однако для их возникновения икринка должна по крайней мере на непродолжительное время оказаться в пресной воде.

Авторы делают попытку выяснить явления разного влияния растворов различной концентрации на оболочки икринок, а также увеличения скорости отвердевания оболочек в растворах NaCl по сравнению с нормой.

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