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Embryology

EFFECT OF SALINITY ON MOTILITY AND VIABILITY OF SALMONID SPERMATOZOA

WPŁYW ZASOLENIA NA RUCHLIWOŚĆ I ŻYWOTNOŚĆ PLEMNIKÓW RYB ŁOSOSIOWATYCH

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Spermatozoa of trout (*Salmo trutta* L.), lake trout (*S. trutta m. lacustris* L.), rainbow trout (*S. gairdneri* Rich.), and brook trout *(Salvelinus fontinalis (Mitch.))* kept at 8–10°C without water in various diluting fluids behaved in all media more or less similarly up to 8 hrs after stripping. Spermatozoa viability as tested after 20 and 32 hrs was found to decrease with time, the rate of this decrease (a reduction in number of active spermatozoa and – to a lower degree – a shorter period of motility) depending on a fish species and medium used.

INTRODUCTION

Effects of salinity on motility of fish spermatozoa has been the focus of attention of many workers; their results, however, are hardly comparable. Various solutions were used as experimental media: NaCl, physiological fluid, Ringer's solution, sea water, etc. Furthermore, sea water from various localities differed in its chemical composition. The results of tests were heavily dependent on thermal conditions as well. Finally, almost all

the results were biased owing to the methods used, based mostly on a visual observation of spermatozoa moving in the microscope field of vision. All those factors contributed to considerable discrepancies in results and differences of opinions as to the activation time, mechanisms underlying activation, favourable or deleterious influence on various compounds.

Spermatozoa of freshwater and anadromous fishes may be activated in a fairly wide range of salinities, which does not imply that equally favourable conditions are provided by every salinity value in the range. Numerous authors are of opinion that the most suitable medium for the activation of spermatozoa of those fishes is found in fresh or brackish (up to 2°/₀₀ salinity) water (i.a., Borodin, 1898; Scheuring, 1925; Stroganov, 1938; Ivlev, 1940; Beljajev, 1957; Drabkina, 1961; Turdakov, 1970a); it is assumed that the most suitable medium, given all or nearly all spermatozoa being activated, is the one in which the spermatozoa have the longest motility time, the progressive movements being mainly considered here.

Other workers maintain the salinity of 2–3°/₀₀ (Tanasijčuk and Vonokov, 1955, 1956; Makejeva and Belova, 1975) or slightly higher, from 3–4°/₀₀ up to 8°/₀₀ (Huxley, 1930; Ellis and Jones, 1939; Havelka et al., 1956; Gostejeva, 1957; Habeković and Fijan, 1962; Dorošev and Gorelov, 1964; Gorelov, 1966; Kosorić and Vuković, 1966; Popova, 1968; Kunin, 1970; Rykova, 1970; Turdakov, 1970b; Stoss et al., 1977) to be most suitable for fish spermatozoa. Rucker (1949) observed the *Oncorhynchus nerka* spermatozoa to have a slightly longer motility time in physiological fluid and Ringer's solution compared to fresh water.

Few authors only studied effects of NaCl solutions (free of any additional ions such as contained, for instance, in diluted sea water or Ringer's solution) on salmonid spermatozoa (Reighard, 1893 after Ginsburg, 1968; Scheuring, 1923, 1925; Gaschott, 1925; Werner, 1934 after Ginsburg 1968; Winge and Ditlevsen, 1937; Rucker, 1949; Terner and Korsch, 1963; Stoss et al., 1977). Studies on salinity effects on spermatozoa were spurred, among other things, by the frequently observed fact of an activity period elongation in media other than natural; hence attempts to explain the mechanisms of this phenomenon and to apply it to the fish culturist's practice.

Owing to the above-mentioned discrepancies and differences of opinions, the present studies involved as uniform conditions as possible, thus enabling the results to be compared for different salmonid species.

Additionally, attempts were made to follow the effects of NaCl and sea water on activation of spermatozoa kept for some time (24 – 30 hrs) at temperatures below 10°C. A number of papers (i.a., Tomasik, 1974) indicated to a feasibility of such short-term storage of sperm at 0–10°C. In fact, it is often necessary to transport or store fish genital products (roe and milt) for a short time. This aspect of the problem has never been discussed.

MATERIAL AND METHODS

The studies presented were carried out in 1970–1973 on spermatozoa of trout (*Salmo trutta* L.) from the Rega River in Trzebiatów, lake trout (*S. trutta m. lacustris* L.) from the Wdzydze Lake, and rainbow (*S. gairdneri* Rich.) and brook (*Salvelinus fontinalis* (Mitch.)) trout from ponds of the Inland Fisheries Institute's River Field Laboratory, Gdańsk–Oliwa.

Sperm was obtained from a total of 18 live, mature, 3–4 yr old males. Only pure milt of a uniform, medium-dense texture was used. Sperm samples were transported to the Szczecin Laboratory in sealed glass vials in an insulated vessel, the temperature on transport not exceeding 8–9°C. Owing to a rather long duration of the transport the experiments were commenced 8 hrs after stripping. In the laboratory, equal amounts of sperm were stored in identical vials (sperm: vial volume ratio of 1:4) at 8–10°C.

Immediately after delivery of samples, and then after 12 and 24 hrs (i.e., 20 and 32 hrs after stripping), spermatozoa motility was assessed using the method described by Tomasik (1973), the duration of progressive and oscillatory movements being registered with a stop-watch. Spermatozoa motility was observed in tap water, sea water (from the Pomeranian Bay, 7.5–8°/₀₀ salinity), and NaCl solutions of 2, 4, 8, 12, and 20 g/l (i.e., from 2 to 20°/₀₀). The experimental media temperature was about 16°C. An approximate number of motile spermatozoa was determined and the milt samples were assigned to one of the following three groups: a) all or nearly all spermatozoa (75–100%) motile; b) about half of the spermatozoa (40–60%) motile; c) only a few spermatozoa (to 15%) motile, in which case no differentiation was made between progressive and oscillatory movements. Every measurement was made in triplicate and an arithmetic mean calculated.

RESULTS

The results are presented in four graphs (Figs. 1–4), each illustrating the two fundamental objectives of the experiment: effect of various diluting fluids on spermatozoa activation and viability (ability to become activated) of the spermatozoa kept at least for 32 hrs at 8–10°C. The spermatozoa activation patterns observed in tap water after delivery to the laboratory (= 8 hrs after stripping) was taken as a reference. When all or nearly all spermatozoa were activated, trout and lake trout spermatozoa were motile for 55–65 sec., 1/3 of which time being oscillatory movements, while 50–80 sec. of motility, 1/2 of which being oscillatory, were observed for rainbow and brook trout.

Lake trout (*S. trutta m. lacustris*) – Fig. 1.

The spermatozoa motility time in fresh water, 2 and 4°/₀₀ NaCl, and in sea water 8 hrs after stripping is basically equal in all the individuals, the time being shorter in 8°/₀₀ NaCl, where the number of activated spermatozoa declined by half. In 12°/₀₀ NaCl, only some of the spermatozoa moved, none moving in 20°/₀₀ NaCl. The pattern was virtually repeated after 20 hrs. After 32 hrs in 8°/₀₀ NaCl the spermatozoa activity decreased; in

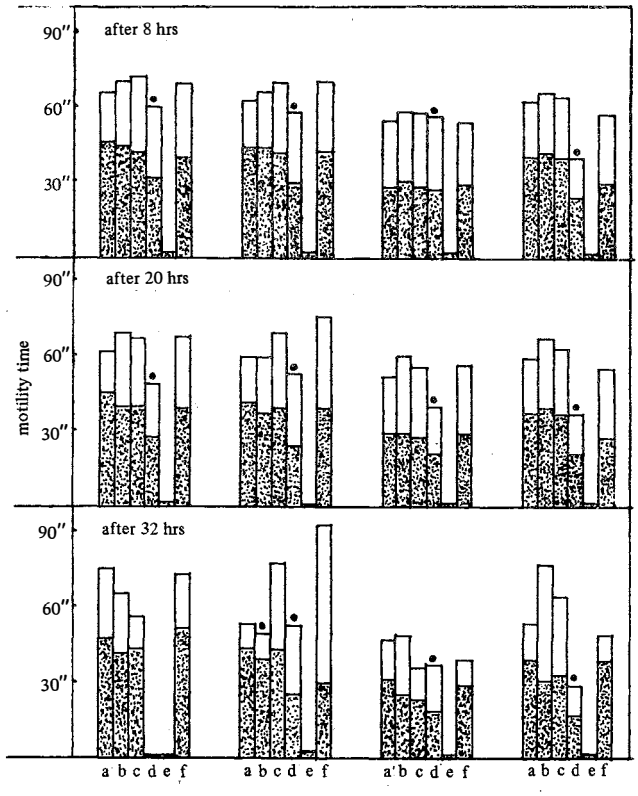

Fig. 1. Lake trout (*Salmo trutta m. lacustris*) spermatozoa motility 8, 20, and 32 hrs after stripping (progressive movements; oscillatory movements; about 50% spermatozoa motile; a) fresh water; b) 2‰ NaCl; c) 4‰ NaCl; d) 8‰ NaCl; e) 12‰ NaCl; f) Baltic sea water; I–IV: males stripped)

the remaining solutions the progressive movements period was slightly shortened, oscillatory movements being shortened or prolonged. Certain small differences in motility of spermatozoa from various males began to be apparent.

Trout (*S. trutta*) – Fig. 2

Spermatozoa motility parameters in all the individuals and in all the extenders studied were similar to those of lake trout. It was after 32 hrs that the spermatozoa activity clearly diminished not only in 8‰ NaCl, but also in 2 and 4‰ solutions (the progressive movements stage decreased slightly, but the oscillatory one was greatly disturbed and the number of active spermatozoa was reduced). Two individuals (I and III) showed their spermatozoa activity time in sea water to be longer than in the remaining media and to increase with time; after 32 hrs it was twice as long as in fresh water.

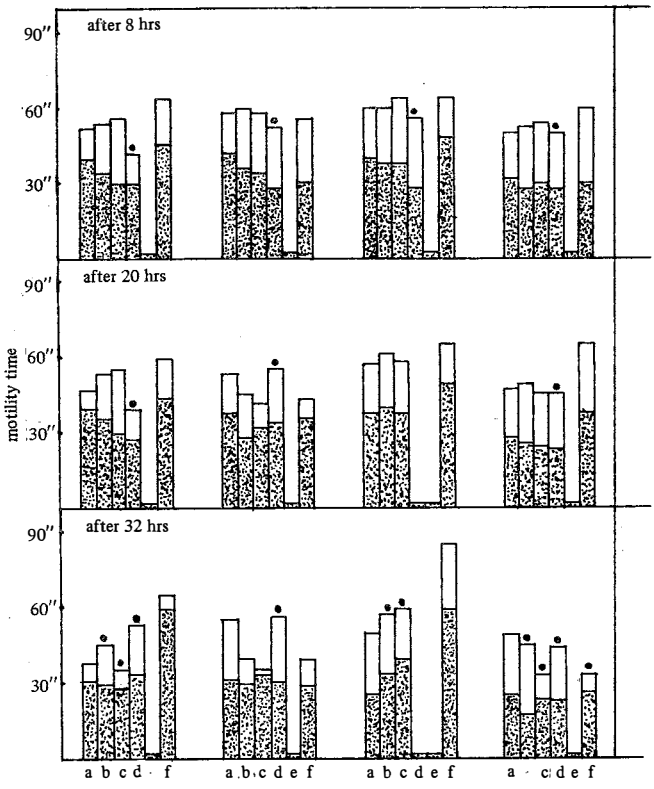

Fig. 2. Trout (*Salmo trutta*) spermatozoa motility 8, 20, and 32 hrs after stripping (for explanations see caption to Fig. 1)

Rainbow trout (*S. gairdneri*) – Fig. 3

Differences in motility of spermatozoa from various males became obvious as early as after 8 hrs. In 12°/oo NaCl, a half of the spermatozoa from male I were motile as opposed to few motile spermatozoa from the remaining males. In 8°/oo NaCl, all the male I spermatozoa, a half of the males II, V, and VI ones, and some of the males III and IV ones moved. All the spermatozoa proceded to move in 2 and 4°/oo NaCl, sea and fresh water, the motility time in each case being similar to one another and to the reference. After 20 hrs the males II and III spermatozoa activity decreased markedly in all media except fresh water. Activity of other spermatozoa was slightly affected; however, the male I spermatozoa revealed a decrease in their viability in all media except fresh water (only a half of all spermatozoa moving). After 32 hrs, a normal activity, in terms of the duration of progressive movements and number of active spermatozoa was retained only

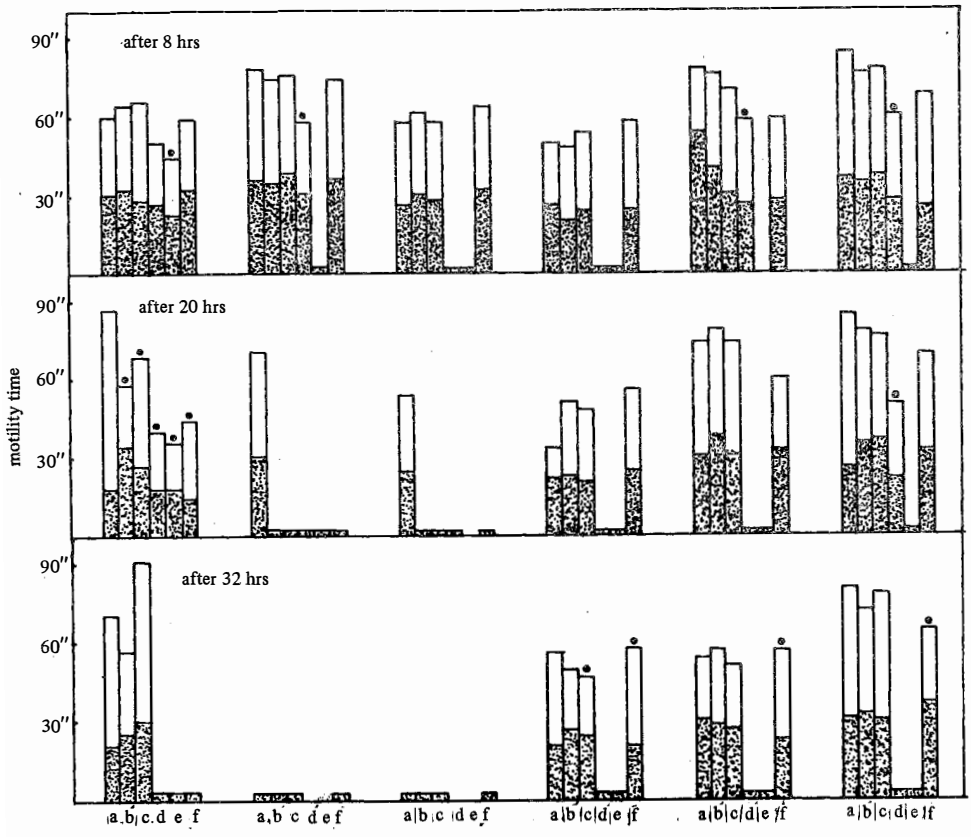

Fig. 3. Rainbow trout (*Salmo gairdneri*) spermatozoa motility 8, 20, and 32 hrs after stripping (for explanations see caption to Fig. 1)

by those spermatozoa from males I, IV, V, and VI kept in fresh water and in 2 and 4‰ NaCl. A marked drop in sperm activity was revealed in the remaining samples.

Brook trout (*Salvelinus fontinalis*) – Fig. 4

The spermatozoa activity 8 hrs after stripping is similar in all samples. In 8‰ NaCl a half of all spermatozoa moved, only some of them performing movements in 12‰. The activity in sea water was the same as in fresh water. After 20 hrs, a slight decrease in activity was observed in all media, a number of active spermatozoa being clearly reduced in 8‰ NaCl. During subsequent 12 hrs this decline in activity became still more pronounced, spermatozoa from various males – kept in different media – showing differing viability.

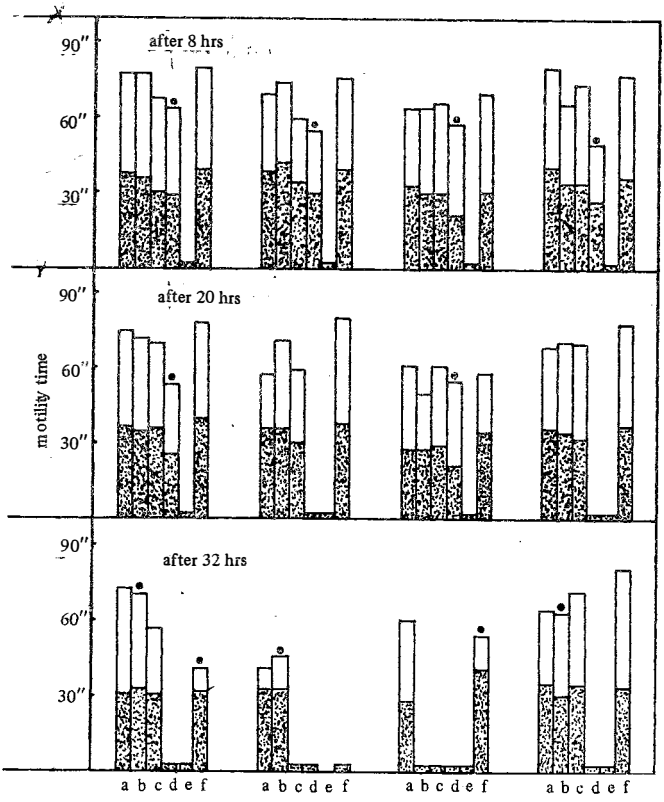

Fig. 4. Brook trout (*Salvelinus fontinalis*) spermatozoa motility 8, 20, and 32 hrs after stripping (for explanations see caption to Fig. 1)

DISCUSSION

When testing motility of spermatozoa of all four salmonid species 8 hrs after stripping, the duration of motility stage in spermatozoa from various males (provided the spermatozoa were motile at all) was found to differ only slightly in the media used, at least in terms of progressive movements. Oscillatory movements and a relative number of active spermatozoa changed more markedly, particularly in more concentrated solutions. It is therefore difficult to choose, among fresh water, 2 and 4°/₀₀ NaCl, and Baltic water, a medium most suitable for the spermatozoa activation. A markedly lower number of spermatozoa becomes activated in 8°/₀₀ NaCl than in sea water of 8°/₀₀ salinity, the motility time being shorter in the first case as well.

An interesting phenomenon of a greater motility and longer activity of spermatozoa of the salmonid species tested in a complex medium such as Baltic sea water when compared

to a NaCl solution of a similar concentration is not treated in the literature in a sufficient depth. Petit et al. (1973), while studying effects of osmotic pressure (using sea water and various NaCl concentrations) focused their attention directly on fertilization; they found the ionic composition of sea water to bear no significant influence. Gorelov (1966) emphasized the importance of osmotic pressure for spermatozoa activation; Dorošev and Gorelov (1964) pointed out to a fact that not only the ambient osmotic pressure, but also a ionic composition of various salts and their particular chemical properties affect spermatozoa activity and viability. This problem was also touched upon by Scheuring (1925), Gaschott (1925), Schlenk and Kahman (1938), Stroganov (1938), Elster and Mann (1950), Dorošev (1964, 1967), Turdakov (1962, 1970b).

With respect to a salinity limit critical for the activation of spermatozoa of freshwater and anadromous fishes, most authors consider the range of 9–15°/₀₀ to include such a limiting value (Borodin, 1898; Scheuring, 1925; Ivlev, 1940; Tanasijčuk and Vonokov, 1955, 1956; Drabkina, 1961; Dorošev and Gorelov, 1964; Gorelov, 1966; Kosorić and Vuković, 1966, 1968; Ginsburg, 1968; Makejeva and Belova, 1975; Stoss et al., 1977). Karpevič, (1966) gives the salinity value of 7.6°/₀₀ to be the upper limit for the activation of *Ctenopharyngodon idella* spermatozoa, various concentrations of the Black Sea water being tested in his studies. The results reported by Turdakov, (1970a, b) are different in that his lethal limit for cyprinid spermatozoa motility in NaCl solutions is 12–24°/₀₀; as high as 28°/₀₀ is his upper limit for the trout species tested in various concentrations of Ringer's. The NaCl concentration range of 8–12°/₀₀ is, in the light of the present results, the upper limit for the activation of spermatozoa of the four salmonid species tested. In 8°/₀₀ NaCl a half of all the spermatozoa were still motile, while only a few moved in 12°/₀₀. The above statement does not rule out a possibility of some spermatozoa being motile in higher (16–20°/₀₀) NaCl concentrations, which may result from not only a higher viability of some spermatozoa, but also from, e.g., inhomogenous mixing of milt with an extender and a slow increase in the concentration of the latter within a spermatozoa aggregation.

No unequivocal proof was arrived at for certain findings referred to in the Introduction (Ellis and Jones, 1939; Rucker, 1949) concerning an increase in the spermatozoa activity (elongation of motiliy time) associated with a slight increase in salinity. However, for spermatozoa of two trout males (I and III), a longer motility time in sea water was found, compared to fresh water and NaCl solutions.

Having been held at 8–10°C for 20 and 32 hrs after stripping, the spermatozoa examined showed their activity to be altered differently in various species. Trout and lake trout spermatozoa retained their activity after 20 hrs almost intact; changes, observed after 32 hrs, involved a reduction in number of active spermatozoa in 8,4, and 2°/₀₀ NaCl and – to a lower extent – a shorter duration of progressive and disturbed oscillatory movements. Spermatozoa kept in fresh and sea water altered their activity to a low degree only. In case of rainbow and brook trout, the changes were visible after 20 hrs, becoming more pronounced after 32 hrs. The rainbow trout males yielded spermatozoa activity

varying greatly. The spermatozoa of the two species lost a good deal of their viability in sea water. A slight decrease only was recorded in the activity in fresh water and 2‰ NaCl.

As already mentioned in the Introduction, there is a lack of data on behaviour of deactivated spermatozoa stored for a long time in various media. Only Scheuring (1925) found trout spermatozoa stored for 24 hrs in Tyrod's fluid to have a longer motility time than in tap water, i.e., contrary to their behaviour immediately after stripping.

The present work shows the viability of spermatozoa of the four species tested to decrease with time. The rate of this decrease depends upon a species and a medium used: trout and lake trout spermatozoa for a longer time maintain their activity in more saline media than rainbow and brook trout spermatozoa. There are no indications of any extender used (NaCl solutions and sea water) being more suitable than fresh water for a short-term maintenance of spermatozoa activity, although it can be seen that the spermatozoa of all four species investigated, trout and lake trout in particular, become activated equally well after 32 hrs in Baltic sea water and in fresh water.

The observations described allow to draw a further conclusion: there are compounds other than NaCl, present in natural waters, that play an extremely important role in the processes discussed (the test solutions of NaCl were made with distilled water).

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WPŁYW ZASOLENIA NA RUCHLIWOŚĆ I ŻYWOTNOŚĆ PLEMNIKÓW RYB ŁOSOSIOWATYCH

Streszczenie

Plemniki troci (*Salmo trutta* L.), troci jeziorowej (*Salmo trutta m. lacustris* L.), pstrąga tęczowego (*Salmo gairdneri* Rich.) oraz pstrąga źródlanego (*Salvelinus fontinalis* (Mitch.)) przetrzymywano bez wody w temperaturze 8–10°C. Po upływie 8, 20 i 32 godz. od momentu wytarcia mierzono czas ruchu oraz określano ilość aktywnych plemników w wodzie słodkiej, roztworach NaCl o stężeniu 2,4, 8 i 12‰ i w wodzie morskiej z Bałtyku (zasolenie 7,5–8‰). Do 8 godz. po wytarciu nie zaobserwowano istotnych różnic w zachowaniu plemników poszczególnych gatunków we wszystkich mediach. W miarę upływu czasu żywotność plemników zmniejszała się, a tempo tych zmian (spadek liczby aktywnych plemników i w mniejszym stopniu skrócenie okresu ruchliwości) było zależne od gatunku ryby i rodzaju środowiska. Plemniki troci i troci jeziorowej zachowują dłużej większą żywotność w środowiskach o wyższym zasoleniu niż plemniki pozostałych gatunków. Plemniki badanych ryb, a zwłaszcza troci i troci jeziorowej aktywują się w wodzie morskiej z Bałtyku prawie równie dobrze jak w wodzie słodkiej, co wskazuje na to, że ważną rolę odgrywają tu inne poza NaCl substancje występujące w wodach naturalnych.

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ВЛИЯНИЕ СОЛЁНОСТИ НА ПОДВИЖНОСТЬ И ЖИЗНЕДЕЯТЕЛЬНОСТЬ СПЕРМИЕВ ЛОСОСЁВЫХ РЫБ

Р е з ю м е

Спермии кумжи (Salmo trutta L.), озёрной форели (Salmo trutta m. lacustris L.), радужной форели (Salmo gairdneri Rich.) и американской палии (Salvelinus foutinalis (Mitch.)) содержали без воды при температуре 8–10°C. Через 8, 20 и 32 часа после вымета измеряли время движения и определяли количество активных спермиев в пресной воде, растворах NaCl при концен-

трации 2, 4, 8 и 12‰ и в морской воде из Балтийского моря (солёность 7,5-8‰). До 8-и часов после вымета не обнаружено существенных разниц в поведении спермиев отдельных видов во всех средах. По мере истечения времени жизнедеятельность спермиев уменьшалась, а темп этих изменений (уменьшение количества активных спермиев и, в меньшей степени, сокращение времени подвижности) зависел от вида рыбы и типа среды. Спермии кумжи и озёрной форели дольше сохраняли повышенную активность в среде с более высокой солёностью, чем спермии остальных видов. Спермии исследуемых рыб, а прежде всего кумжи и озёрной форели, активизируются в морской воде из Балтийского моря почти так же хорошо, как в пресной воде. Это указывает на то, что важную роль играют здесь кроме NaCl другие субстанции, содержащиеся в естественных водах.

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