ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XIX, Fasc. 2 Szczecin 1989

Krzysztof FORMICKI[1], *Mieczysław KOWALEWSKI*[2], *Andrzej SOBOCIŃSKI*[1], *Lucjan TOMASIK*[1], *Aleksander WINNICKI*[1]

Embryology

MOTILITY OF *HUCHO HUCHO* L. SPERMATOZOA AFTER ACTIVATION

RUCHLIWOŚĆ PLEMNIKÓW GŁOWACICY (*HUCHO HUCHO* L.) PO AKTYWACJI

[1] Institute of Ichthyology, Academy of Agriculture, Szczecin
[2] Polish Anglers Association Station at Łopuszna

Observations of the Danube salmon spermatozoa motility and spermatocrit were conducted. Sperm was tested immediately after spawning and during its prolonged storage.

INTRODUCTION

The specialized literature, so far, has no data on complex studies of *Hucho hucho* L. spermatozoa and conducted surveys were fragmentary only. Haempel (1913), for example, had observed motility of *Hucho huchò* L. spermatozoa since their activation in water and noted it to last up to 45 sec. According to Scheuring (1928), time when *Hucho hucho* L. spermatozoa stayed motile reached up to 76 sec (progressive motion up to 30 sec.) depending on the spermatozoa quality; geting shorter with prolonged storage of sperm. Pavlik (1957) confirmed Haempel's results, while Harsanyi, cited by Holčik et al. (1984) found the *Hucho hucho* L. spermatozoa to be motile up to 120 sec after their activation in water of 10°C. Analysis of Danube salmon spermatozoa with electron microscope proved their structure to be typical for salmonids, with lack of acrosome and eccentric situation of the flagella in relation to the head (Radziun and Tomasik, 1985).

Difference in conclussions, in just few papers dealing with the *Hucho hucho* L. spermatozoa motility problem, induced us to under-take complex, to some extent,

studies. Besides we wanted to find out if and to what extent observations done by others on fish spermatozoa motility (Nomura, 1964; Yoshida and Nomura, 1972; Holtz et al., 1977), dividing whole motility period into separate phases of progressive motion and pendular motion, can be applied to *Hucho hucho* L. spermatozoa.

MATERIAL AND METHODS

Surveys were conducted in 1985 in the Polish Anglers Association Station at Łopuszna and than repeated in 1988, Sperm from 11 and 6, respectively, mature *Hucho hucho* L. males was analysed. Males, whose ejaculates were tested, were measured and their age determined.

Surveys included determination of total motility time of spermatozoa after getting in touch with water, distinguishing the length of each phase of the motion; namely – progressive motion random (turbulent) and circular (quiet) – according to motion's intensity and pendular motion (Fig. 1). For all samples a relative number of active spermatozoa (percent of motility) and a relative volume of spermatozoa within a sperm sample = "spermatocrit" according to the method by Winnicki and Tomasik (1976), were determined.

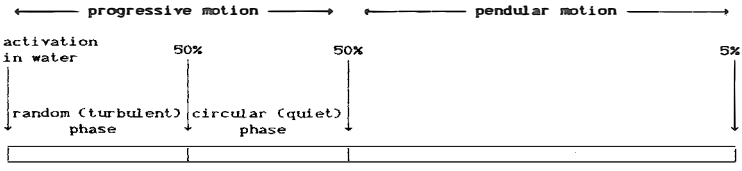

Fig. 1. Motion of *Hucho hucho* L. spermatozoa after activation (explanation in text)

A total time of spermatozoa motility was number of seconds from an activation moment up to time when there was not more than 5% spermatozoa with pendular motion within the sample. A progressive motion time started with activation process and lasted to the moment when number of spermatozoa with this type of motion dropped below 50%. Limit of a random (turbulent) phase was determined analogically. Difference between total motility time and progressive motion time was pendular motion time (Fig. 1). Number of active spermatozoa was determined on spermatozoa transition from random (turbulent) phase into a circular (quiet) one.

The spermatozoa motility tests, according to the method by Tomasik (1973) were conducted with optic microscope (enlargement 200 x). Time of each phase was measured with stoper with 0.5 sec accuracy. Observations were carried out in a cold room, at the temperature around 5°C, the same as the one in water tanks, were *Hucho hucho* L. males were being kept (to avoid heating up a sample while on microscope stage).

Spermatocrit was estimated by hematocrit centriruge (5000 rpm; for 5 min.). Each result of all the tested parameters for each individual is an average of three succesive measures.

Some of the ejaculates were transported, in cooled thermos container (temp. while transported 5°C±1°), to the laboratory at the Institute of Ichthyology, where surveys on spermatozoa motility in the tap water, at +8°C were being continued. Obtained results were worked out statistically.

RESULTS

Spermatozoa tested were distinguished by relatively high activity (about 70% of active spermatozoa) with spermatocrit average being 16.7% (Tab. 1). Progressive motion of spermatozoa, extended in time, lasted about 48 sec., where 26 sec was an average time for preliminary phase of that motion – a random (turbulent) one (that is 55% of a total translatory motion). Progresive motion was then replaced by a relatively long lasting pendular motion (84%, that is 64% of total motility time of spermatozoa).

Table 1

Spermatoza motility and spermatocrit of Danube salmon, *Hucho hucho* L.

Specification	Longitudo caudalis (cm)	Age (years)	Spermatozoa activation (%)	Motion time (sec.)*			Spermatocrit (%)
				progressive		pendular	
				random (turbulent) phase	circular (quiet) phase		
n	17	17	17	10	17	17	17
$\bar{x}$	81.7	7.9	69.0	26.1	47.9	131.7	16.7
SE	3.6	0.6	5.4	2.2	3.4	8.0	1.5

* spermatoza motion time measured since activation moment

Interesting data were provided by surveys on the spermatozoa motion phases after 24 and 48 hours cold storage (+5°C) of sperm samples, transported then to the laboratory in Szczecin (Tab. 2). Since 24 hours of cold storage a gradual reduction of progressive motion and pendular motion periods was observed, down to about half of its prime value after 96 hours of storage. After 216 hours spermatozoa of only one male showed sings of activity. There was not any sings of a random (turbulent) phase of spermatozoa motion observed already 24 hours after the ejaculates caollection. Quite possibly this phase, then, is too short to observe it (within few seconds necessary to get ready for microscopic observations).

Table 2

Motility of Danube salmon, *Hucho hucho* L. spermatozoa, when stored

Time since spermatozoa colleciton (h)	Specification	Spermatozoa activation	Motion time (sec.)***		
			progressive		pendular
			random (turbulent) phase	circular (quiet) phase	
24*	n	15	10	15	15
	$\bar{x}$	28.2	25.1	42.9	118.2
	SE	5.96	2.74	4.3	10.7
48*	n	12		12	12
	$\bar{x}$	21.5	–	32.3	102.7
	SE	5.18		2.54	17.71
96**	n	7		6	7
	$\bar{x}$	34.3	–	24.5	52.3
	SE	5.05		1.58	6.9
144**	n	6		6	6
	$\bar{x}$	24.8	–	24.8	51.0
	SE	7.5		1.74	8.1
192**	n	2		2	2
	$\bar{x}$	20.0		20.5	47.0
216**	n	1	–	–	not numerous

* surveys at the spawning ground

** surveys after transportation to the laboratory

**** spermatozoa motion time measured since activation moment

DISCUSSION

Observations done, so far, on time of activated *Hucho hucho* L. spermatozoa motion were not numerous and not too precise. Authors were interested only in the spermatozoa motion itself, not mentioning its character and phases. Scheuring (1928) was the only one, who had taken into consideration a progressive motion and pendular motion of *Hucho hucho* L. spermatozoa.

Our observations, repeated twice, in long intervals, on individuals from the same environment, under the same conditions, indicated the motility time of *Hucho hucho* L. spermatozoa to last up to 230 sec. with the average motility time being 132 sec. That is longer than it was observed by Scheuring (1928) and Harsanyi cited by Holčík et al.

(1984) according to whom motility was observed up to 120 sec. Therefore, *Hucho hucho* L. spermatozoa activity lasts longer than the one observed for other salmonids. A progressive motion is the most essential from fertilization point of view. According to Smirnov (1954, 1963, 1975) progressive motion of pink Salmon (*Oncorhynchus gorbuscha*) spermatozoa oscilated between 30 and 55 sec., while Caga and Chorevina (1984) found it to last up to 80 sec., at temp. 6 to 9°C. Spematozoa of *Oncorhynchus keta* were in progressive motion for 10 to 15 sec. after their activation in water of temp. 10°C (Smirnov, 1975). The same type of motion for spermatozoa of *Salmo gairdneri* lasted 23 sec. (Billard, 1978) while for *Salmo trutta* average progressive motion time was 42 sec. (Szymelfenig, 1979).

Our experiments let us to distinguish two phases within a progressive motion; a random (turbulent) phase with rapid straight forward movements of spermatozoa and a circular (quiet) one where the motion vibly slows down and enables to distinguish individual spermatozoa in rectilinear motion and then rotatory one. That rotatory motion can result from eccentric situation of flagella to the head of spermatozoon (Turdakov, 1972; Radiun and Tomasik, 1985).

Lately conducted surveys on *Thymallus thymallus* let to distinguish also, two phases within a progressive motion (work in print). However, a random (turbulent) phase is very short (few seconds), while the same phase for *Hucho hucho* salmon took longer (20 to 30 sec.) and was visibly pronounced. We are to admit, that differences in time of spermatozoa motion measures by Haempel (1913) and Pavlik (1957) on one side and Harsanyi (Holcik et al., 1984) and us on the other were, probably, due to the progressive motion measures taken only by the first. According to Scheuring (1928) a progressive motion time was 30 sec. with total motion time (76 sec.) somewhat shorter than the one we noted. However he transported his samples in container cladded with ice, while our experiments were conducted immediately after spawning, in the same water fish males were taken from, that is almost in natural conditions. Only the second part of the experiment (Tab. 2) was conduted on sperm transported to Szczecin.

Our surveys proved the total progressive motion time for *Hucho hucho* spermatozoa is somewhat longer than for other salmonids, being similar to the one for pink salmon. The only exception found was *Salmo gairdneri* for whom that type of motion was relatively very short. A particularly long pendular motion time was characteristic for *Hucho hucho* spermatozoa.

Relative volume of *Hucho hucho* spermatozoa in sperm (spermatocrit) is rather low when compared with other salmonids. Winnicki and Tomasik (1976) stated, for example, the spermatocrit of mature *Salmo trutta* males to range from 20 to 50%. According to various authors spematocrit valud of *Salmo gairdneri* ranged from 10 to 40% (Bouck and Jacobson, 1976; Tomasik et al., 1981; Piironen and Hyvärinen, 1983).

Those essential differences and sometimes surprisingly high spermatocrit values (up to 40 and more %) can be presumbsly refered to differences in methods used to collect sperm for surveys. It is known, that "leaking" males have "more diluted" sperm than males artificially stripped when mechanical pressure is to be applied.

Hence, for the mature Danube salmon (*Hucho hucho*, L.) males percent of spermatozoa activation is rather high (70%), while spermatocrit relatively low (17%). Immediately after spawning a random (turbulent) phase of progressive motion is cearly distinguished and the total motion time of spermatozoa equals to 130 sec. During cold storage of sperm, spermatozoa stay viable for a long time.

REFERENCES

Billard R., 1978: Changes in structure and fertilizing ability of marine and freshwater fish spermatozoa diluted in media of various salinities. – Aquacult., 14: 187–198.

Bouck G.R., J. Jacobson, 1976: Estimation of Salmonid Sperm Concentration by Microhematocrit Technique. – Trans. Amer. Fish. Soc., 105: 534–535.

Caga I.L., N.B. Chorevina, 1984: Ekologicekije osobennosti vosproizvodstwa kety, nerki i gorbusi. – Biologia moria, no 6: 27–31 (in Russian).

Haempel O., 1913: Fische I. Allgemeine anatomisch-physiologische übersicht. 11. Die Fortpflanzunsorgane. – in: Hildheimer M.: Handbuch der Biologie der Wirbeltiere, Stuttagrt, Verl. Ferd. Enke: 65–74.

Holcik J., K. Hensel, J. Niesladnik, 1984: Hlavatka. Bratislava, Veda (in Czech).

Holtz W., J. Stoss, S. Bütykükhatipoglu, 1977: Beobachtungen zur Akivierbarkeit von Forellenspermatozoen mit Fruchtwasser, Bachwasser und destillierten Wasser.– Zuchthygiene, 12: 82–88.

Nomura M., 1964: Studies on reproduction of rainbow trout Salmo gairdneri, with special reference to egg taking. VI – The activities of spermatozoa in different diluents, and preservation of semen. – Bull. Jap. Soc. Sci. Fish., 30: 723–733.

Pavlik I., 1957: Umely vyter hlavatek. – Cs. rybarstvi, 12: 71 (in Czech).

Piironen J., A. Hyvärinen, 1983: Composition of milt of some teleost fishes. – J. Fish. Biol., 22: 351–361.

Radziun K., L. Tomasik, 1985: Ultrastructure of Hucho hucho (L.) spermatozoa. – Acta Ichthyol. Pisc., 15(2): 130–140.

Scheuring L., 1928: Weitere biologische und physiologische Untersuchungen an Salmonidensperma. – Zool. Jb., Abt. zool. physiol., 45: 651–705.

Smirnov A.I.: 1954: Nabludenija nad producirovanijem molok tichookeanskimi lososiami. – Izv TINRO, 42: 159–164 (in Russian).

Smirnov A.I.: 1963: Producirovanije spermy tichookeanskimi lososiami roda Oncorhynchus. – Vopr. ichtiol., 3: 84–98 (in Russian).

Smirnov A.I., 1975: Biologija, rozmnoženije i razvite tichookeanskich lososej, Moskwa, Izd. Moskovsk, Universiteta (in Russian).

Szymelfenig M., 1979: Wpływ wody morskiej o różnych zasoleniach na plemniki i jaja pstrąga tęczowego (Salmo gairdneri Rich. 1836) i troci wędrownej (Salmo trutta trutta L. 1758. [Effect of sea water of different salinity on spermatozoa and eggs of Salmo gairdneri Rich. 1836 and Salmo trutta trutta L. 1758.] – Zesz. nauk. Wydz. Biol. i Nauk o ziemi Uniw. Gdańskiego (Oceanografia), 6: 129–146.

Tomasik L., 1973: Specific and individual differences in motility between salmonid spermatozoa. – Acta Ichthyol. Pisc., 3, 1: 11–17.

Tomasik L., A. Sobociński, A. Winnicki, 1981: Spermatokryt jako wskaźnik w badaniach nasienia ryb. [Spermatocrit as an indicator in surveys of fish semen]. In main surveys conducted at the Faculty of Marine Fisheries and Food Technology in 1970–1980 Szczecin, Summary: 110–114.

Turdakov A.F., 1972: Vosproizvoditelnaja sistema samcov ryb. – Izd. Ilim. Frunze. (in Russian).
Winnicki A., L. Tomasik, 1976: "Spermatocrit" as a method for biological evaluation of fish sperm. – Acta Ichthyol. Pisc., 6, 2: 3–8.
Yoshida T., M. Nomura, 1972: A substance enhancing sperm motility in the ovarian fluid of rainbow trout. – Bull. Japan. Soc. Scient. Fish., 38: 1073.

Translated: Dr E. Daczkowska-Kozon

Formicki Krzysztof, Kowalewski Mieczysław, Sobociński Andrzej.
Tomasik Lucjan, Winnicki Aleksander

RUCHLIWOŚĆ PLEMNIKÓW GŁOWACICY (*HUCHO HUCHO* L.) PO AKTYWACJI

STRESZCZENIE

W latach 1985 i 1988 przeprowadzono obserwacje ruchliwości i spermatokrytu plemników 17 samców głowacicy (*Hucho hucho* L.). Stwierdzono, że względna ilość aktywnych plemników w spermie jest znaczna (średnio 70%), a spermatokryt (względna objętość plemników w spermie) niski (średnio 16.7%) w porównaniu z innymi rybami łososiowatymi. Bezpośrednio po tarle, wśród zaaktywowanych plemników wyraźnie zaznaczona jest faza burzliwa ruchu postępowego. Całkowity czas ruchu plemników wynosi średnio 130 sek, z czego na ruch postępowy przypada ok. 37% (śr. 48 sek.).

Plemniki w spermie przetrzymywanej w temp. 8°C zachowują żywotność do 216 godz.

Author's address:
Prof. dr hab. Aleksander Winnicki
Dr Krzysztof Formicki, Dr Lucjan Tomasik,
Mgr inż. Andrzej Sobociński
Akademia Rolnicza
Instytut Ichtiologii
ul. K. Królewicza 4
71-550 Szczecin (Poland)

Mgr inż. Mieczysław Kowalewski
Polish Angling Union
Fishery Station at Łopuszna
34-432 Łopuszna 192, Poland

Received: 1988.09.23