ACTA ICHTHYOLOGICA ET PISCATORIA

Vol. V, Fasc. 1 Szczecin, 1975

Krzysztof GORYCZKO, Lucjan TOMASIK

Physiology

AN INFLUENCE OF MALES ON THE VIABILITY AND FERTILIZATION DEGREE OF TROUT EGGS

WPŁYW SAMCÓW NA PRZEŻYWALNOŚĆ I STOPIEŃ ZAPŁODNIENIA IKRY TROCI (*SALMO TRUTTA TRUTTA* L.)

River Laboratory of Inland Fisheries Institute
Department of Anatomy and Embryology of Fishes
of Institute of Ichthyology

An attempt to estimate the quality of milt obtained from trout males stripped three times was made. The estimation was carried out through examining the spermatozoa motility and verified by analysing the viability and fertilization degree of the experimental roe.

INTRODUCTION

The present paper is aimed at finding an independent criterion of the sperm quality, the sperm being obtained from sea trout males (*Salmo trutta trutta* L). The evaluation was based on motility of sperm after contact with water. The salmonid spermatozoa, in general, show two stages of their movement, i.e., a pregressive movement and an oscillatory one, the latter observed when spermatozoa move "in place". According to many authors who agree on this point, only the progressive movement is significantly important in the fertilization. Moreover, the observations of many workers show the examination of spermatozoa movement including the duration (particularly its first stage) and intensity of movement as well as the motile/motionless spermatozoa ratio to be a practical quick test which, combined with the milt outer appearance, almost faultlessly allows to evaluate the milt suitability in fish culture.

An analysis of the viability of eggs fertilized with the investigated males sperm served to verify the estimation of the above kind.

MATERIAL AND METHODS

The experimental material consisted of the four years-old sea trout spawners (*Salmo trutta trutta* L.) reared in ponds belonging to River Laboratory of Inland Fisheries Institute in Gdańsk–Oliwa. 9 males were chosen at random and tagged in order to their better identification. All the data concerning the experimental males are given in Table 1.

Table 1

Sizes of experimental trout males (*S. trutta trutta*)

Tag No	Length (cm) (l. caud)	Weight (g)
1	36	520
2	43	790
3	42	820
4	37	510
5	42	800
6	34	440
7	36	520
8	36	510
9	43	910

The males were being stripped three times: on Nov. 8, 17 and 22. A special care was taken to prevent any contamination of the milt obtained. To assess the sperm motility, each time a triple milt sample was taken from each male using a blunt end of a wooden stick. The sample was then placed on a coverslip, this being immediately put onto a slide with a drop of water and placed under a pre-focused microscope (magnification 15x20) (Tomasik, 1973). A stop-watch was set on once the water drop got in contact with the sperm sample. The duration of both the total spermatozoa movement (disregarding singular spermatozoa of a delayed activation or of a longer-lasting movement) and its stages (the progressive and oscillatory movement) were noted. A relative number of activated spermatozoa versus immobile ones was approximately recorded. The mean values were then calculated from obtained in this way triplicate results of sperm motility measurements of each fish individual.

During each spawning, the sperm from 9 males studied served to fertilize separately eggs samples taken from 3 females. The latter were randomly chosen for each experimental spawning, those of eggs of poor quality being rejected. The experimental spawning proceeded in the following way. Milt was taken from each male to a labelled cristallizer. The eggs were stripped from every one of the three females into an individual labelled basin, the roe obtained being then divided into 9 parts (87–208 eggs in one part) placed in separate labelled vessels. The milt taken from 9 males served to fertilize the egg samples from 3 females, each egg sample being fertilized with three drops of sperm. The experiment described is presented schematically in Table 3.

Having fertilized and rinsed the roe, each sample was placed in a separate compartment of a specifically adjusted Californian apparatus (a typical one comprised 36 compart-

ments). A place for each sample was fixed at random using a table of random figures. Since the experiment had been planned in order to determine the quality of sperm expressed in percentage fertilization of eggs, the observations were made until the "eyed" stage of eggs was attained. The dead eggs were currently picked out and then fixed in Bouin's fluid. Once the eggs became eyed, they were rinsed; the number of non-whitened and non-fertilized eggs was recorded. A whitened roe was picked out during the incubation, preserved eggs being examined to determine the embryonic developmental stage. The latter was determined according to Devillers'scale (Domurat, 1964).

The experimental egg viability expressed in % was angularly transformed to allow its statistical treatment. A double-factor variance analysis with two elements per a sub-group as well as that with one element were applied (Ruszczyc, 1970).

RESULTS

Table 2 summarizes the results of examining the motility of sperm obtained in three subsequent collections from 9 experimental males. A tendency toward a decrease in the

Table 2

Motility of sperm (sec.) from subsequent strippings of each trout individual (progressive movement in numerator, oscillatory one in denominator)

Date / Male No	8.11.1972	17.11.1972	22.11.1972
1	38.7 $\frac{21.7}{17.0}$	43.0 $\frac{22.7}{20.3}$	44.7 $\frac{19.7}{25.0}$
2	32.3 $\frac{22.7}{9.6}$	38.7 $\frac{19.0}{19.7}$	80.0 $\frac{9.0}{71.0}$ a)
3	49.7 $\frac{30.0}{19.7}$	40.0 $\frac{19.7}{20.3}$	38.0 $\frac{18.3}{19.7}$
4	42.0 $\frac{23.0}{19.0}$	42.3 $\frac{21.3}{21.0}$	52.3 $\frac{21.7}{30.6}$
5	41.3 $\frac{23.3}{18.0}$	41.7 $\frac{20.0}{21.7}$	36.7 $\frac{19.0}{17.7}$
6	45.7 $\frac{24.7}{21.0}$	36.3 $\frac{18.7}{17.6}$ b)	57.0 $\frac{20.3}{36.7}$
7	40.0 $\frac{25.3}{14.7}$	39.3 $\frac{21.0}{18.3}$	42.3 $\frac{19.0}{23.3}$
8	38.3 $\frac{24.3}{14.0}$	35.0 $\frac{17.7}{17.3}$ c)	38.7 $\frac{19.3}{19.4}$
9	45.7 $\frac{25.3}{20.4}$	38.0 $\frac{20.0}{18.0}$	— d)

a) most spermatoza become activated only after several seconds from contact with water
b) ca 70 – 80% of spermatozoa move; weak intensity of movement
c) slightly weaker intensity of movement
d) milt of loose consistence

sperm progressive movement duration in the subsequent strippings is seen from the table, the oscillatory movement period tending to elongate.

The case of No 9 male needs an explanation. The sperm motility test after the third spawning yielded a negative result in spite of sixfold replication; the sperm, however, gave good results in fertilization (Table 3). Since the third stripping of milt is concerned, most probably we were dealing with an individual of a poor condition; its spermatozoa when brought in contact with water are motile only for a very short period (less then 4–5 sec.) just in time of handling the mount under the microscope, thus elapsing the observer's attention. When in contact with eggs, the sperm motility period undoubtedly becomes elongated on account of the ovarian fluid (actually the cavital one in salmonids) which, diluted with water, acts as a sperm motility stimulator, whereas in trout and in most the salmonids (Turdakov 1965; Ginsburg 1968; among others) it is an independent activator of the spermatozoa.

The results of the experimental roe incubation are presented in Table 3.

The statistical analysis of the egg viability from fertilization until the eyeing stage, conducted in a double-factor system (males – periods) of two elements per a sub-group (males in periods) showed no significant influence either of males or the spawning order on the viability. Also the interaction (males x periods) was insignificant (Table 4).

On the other hand, the double factor (males-females) variance analysis of one element per a sub-group (Table 5) proved a highly significant influence of females and a significant one of males on the eggs viability. In view of the principal aim of the experiment, which comprised an assessment of the experimental males' sperm quality based, among other things, on the egg viability, an additional statistic treatment was introduced in an analogous system, the egg fertilization percentage being assessed (Table 3 – figures in brackets) instead of the viability. It should be pointed out, however, that fertilized eggs cannot be distinguished from the non-fertilized ones in the early embryonic developmental stages of salmonids (Soin 1953). Therefore in the present report the percentage fertilization was determined for eggs passing the 10th stage of Devillers scale (Domurat 1964). Thus the calculated egg fertilization degree is erroneous since the roe grains deteriorated before reaching the 10th grade were not taken into consideration, undoubtedly leading to an overestimation of the fertilization degree as determined in the present paper.

The statistical treatment of the egg fertilization degree showed, when considered in a double-factor system of two elements per a subgroup (Table 6) a highly significant influences of males and the spawning order (periods) with a simultaneously high interaction (males x periods). In a system of one element per a sub-group (Table 7) a significant (close to highly significant) influence and a highly significant one of males and females, respectively, were revealed. A highly significant interaction (Table 6) results most probably from the females influence, which, disregarded in the double-factor system, in the single-factor one (Table 7) turned out to be highly significant (much more so than that of males).

When analysing the percentage fertilization of eggs collected each time, i.e., on Nov. 8, 17 and 22, with the multiple dispersion test (Ruszczyc 1970), a highly significant diffe-

Table 3

Percentages of the egg viability from fertilization to eyeing. (Figures in brackets reflect the percentages of fertilization)

♀ \ ♂	1	2	3	4	5	6	7	8	9	mean viability ♀
					First spawning 8 XI					
I	90.2(92.1)	95.9(100)	97.9(99.0)	98.0(100)	97.1(100)	98.2(100)	93.1(99.0)	100(100)	95.9(99.0)	96.2(98.9)
II	90.9(100)	98.3(100)	99.0(100)	94.3(100)	100(100)	100(100)	98.3(99.2)	98.1(100)	100(100)	97.7(99.9)
III	72.4(100)	73.3(100)	60.9(97.7)	61.0(100)	74.1(100)	72.3(100)	73.1(99.0)	65.2(100)	54.5(98.2)	67.0(99.3)
										mean for the period (99.3) 85.7
					Second spawning 17 XI					
IV	93.1(97.4)	91.7(96.3)	85.2(96.3)	83.8(99.0)	82.7(99.0)	65.9(92.8)	78.5(97.2)	85.8(99.0)	74.8(100)	81.9(97.7)
V	90.8(100)	91.0(97.3)	93.2(98.5)	92.3(99.2)	92.0(100)	71.7(99.2)	93.3(99.2)	94.3(98.6)	78.4(100)	88.2(99.1)
VI	99.4(100)	99.4(99.4)	97.6(99.4)	100(100)	97.5(98.8)	97.5(99.0)	95.1(98.4)	96.6(99.4)	91.7(100)	97.1(99.3)
										mean for the period (98.6) 88.9
					Third spawning 22 XI					
VII	86.9(86.9)	68.1(68.1)	100(100)	98.3(98.9)	99.4(99.4)	100(100)	98.9(98.9)	100(100)	95.5(95.5)	93.5(93.5)
VIII	46.2(47.2)	55.0(55.0)	99.0(100)	100(100)	80.5(81.3)	100(100)	99.0(99.0)	99.0(100)	77.3(77.3)	81.3(81.1)
IX	48.5(48.5)	82.3(83.4)	96.8(97.4)	95.7(95.8)	71.6(71.6)	99.4(99.5)	95.2.(95.2)	99.0(99.5)	82.6(83.2)	83.8(84.3)
	mean viability ♂									
	77.1(86.1)	82.4(87.1)	91.2(98.9)	90.6(99.1)	87.7(93.8)	88.1(98.8)	91.2(98.4)	92.4(99.5)	82.2(94.0)	mean for the period (86.3) 86.0

Table 4

Double–factor variance analysis with two elements per a sub–group of percentage viability of eggs obtained from 9 females stripped three times (3 individuals each time) and fertilized separately by 9 males

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
total	80	12742.30	–	–
males	8	1538.40	192.30	1.40
periods	2	49.90	24.95	0.18
interactions (males x periods)	16	3726.10	232.88	1.69
error	54	7427.90	137.55	

Table 5

Double–factor variance analysis with one element per a sub–group of percentage viability of eggs obtained from 9 females and fertilized separately by 9 males

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
total	80	12742.30	–	–
males	8	1538.40	192.30	2.33*
females	8	5924.20	740.52	8.98**
discrepancy	64	5279.70	82.49	–

Table 6

Double–factor variance analysis with two elements per a sub–group of percentage fertilization of eggs obtained from 9 females stripped three times (3 females each time) and separately fertilized by 9 males

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
total	80	9266.1	–	–
males	8	1575.2	196.9	5.89**
periods	2	2323.8	1161.9	34.79**
interaction (males x periods)	16	3561.3	222.6	6.66**
error	54	1805.8	33.4	–

Table 7

Double–factor variance analysis with one element per a sub–group of percentage fertilization of eggs obtained from 9 females and separately fertilized by 9 males

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
total	80	9266.1	–	–
males	8	1575.2	196.9	2.64*
females	8	2907.7	363.5	4.87**
discrepancy	64	4783.2	74.7	–

rence between the third (Nov. 22) spawning on one side and the first and second ones (Nov. 8 and 17) on the other was revealed (Table 8). The males were then arranged, according to mean percentages of eggs fertilized by them, in a lowering order (Table 3). A multiple dispersion test was applied also to determine the significance of differences between the males; the results are shown in Table 9.

Table 8

Analysis of differences between percentages of fertilization of eggs obtained in three periods: ++ higly significant difference (p. 0.01), – insignificant one

	$\bar{x}$	$\bar{x}$ –86.3	$\bar{x}$ –98.6
First spawning (Nov. 8)	99.3	(3) 13.0**	(2) 0.7 –
Second spawning (Nov. 17)	98.6	(2) 12.3**	
Third spawning (Nov.22)	86.3		

The significant differences were evident only between the best and the worst males.

CONCLUSIONS

The results of the experiment indicate to a fact that females are much more responsible for the roe viability and fertilization percentage than the males used in the spawning. However, significant differences were found in the experimental males' milt capability to fertilize the eggs. Also a decreasing fertilization percentage was found for the eggs from subsequent strippings (Nov. 8, 17 and 22), the differences between the two first and the last one being highly significant. The decrease resulted not only from a lowering quality of sperm obtained from the males used three times, confirmed by a tendency to shorten the spermatozoa progressive movement duration (Table 2) but also from a lower quality of eggs obtained from females stripped later (Nov. 17 and 22). A highly significant interaction (males x periods) (Table 6) would confirm this interpretation of the results.

Table 9

Experimental males arranged in a lowering order according to a mean fertilization percentages obtained from them.
Significance of differences between males:
++ highly significant (p. 0.01); + significant (p. 0.05); – insignificant;
figures in brackets show dispersion between means $\bar{x}$

Male No	$\bar{x}$	$\bar{x}$ – 86.1	$\bar{x}$ – 87.1	$\bar{x}$ – 93.8	$\bar{x}$ – 94.0	$\bar{x}$ – 98.4	$\bar{x}$ – 98.8	$\bar{x}$ – 98.9	$\bar{x}$ – 99.1
8	99.5	(9) 13.4^{++}	(8) 12.4^{++}	(7) 5.7^{-}	(6) 5.5^{-}	(5) 1.1^{-}	(4) 0.7^{-}	(3) 0.6^{-}	(2) 0.4^{-}
4	99.1	(8) 13.0^{++}	(7) 12.0^{++}	(6) 5.3^{-}	(5) 5.1^{-}	(4) 0.7^{-}	(3) 0.3^{-}	(2) 0.2^{-}	
3	98.9	(7) 12.8^{++}	(6) 11.8^{++}	(5) 5.1^{-}	(4) 4.9^{-}	(3) 0.5^{-}	(2) 0.1^{-}		
6	98.8	(6) 12.7^{++}	(5) 11.7^{++}	(4) 5.0^{-}	(3) 4.8^{-}	(2) 0.4^{-}			
7	98.4	(5) 12.3^{++}	(4) 11.3^{++}	(3) 4.6^{-}	(2) 4.4^{-}				
9	94.0	(4) 7.9^{++}	(3) 6.9^{+}	(2) 0.2^{-}					
5	93.8	(3) 7.7^{++}	(2) 6.7^{+}						
2	87.1	(2) 1.0^{-}							
1	86.1								

In the experimental system assumed by the authors, the suitability of the method involving motility of spermatozoa as an indicator of the sperm quality failed to be proved sufficiently. The fertilized eggs' viability analysis as the method of a sperm quality assessment is not very accurate, either, on account of the fertilized eggs' mortality. On the other hand, in spite of some methodological inaccuracies resulting from the fertilization degree impossible to be estimated in the early embryonic developmental stages, the analysis of the percentage egg fertilization obtained allowed to detect differences in the experimental males' sperm quality.

ACKNOWLEDGEMENT

The authors wish to thank Mrs. Cecylia Cykowska M.Sc. for her assistance in determining the embryonic developmental stages of eggs, and Dr. Ludosław Drelichowski for his valuable help in the statistical treatment of the results.

REFERENCES

Domurat J., 1964: Embryonic development of the Rainbow Trout (Salmo irideus Gibb.) and the Brown Trout (S. trutta L.) in a non-aqueous medium. – Zoologica Poloniae, **14**, 3–4: 125–151.

Ginsburg A.S., 1968: Oplodotvorenije u ryb i problema polispermii. Izd. „Nauka", Moskva.

Ruszczyc Z., 1970: Metodyka doświadczeń zootechnicznych. [Methods in zootechnical experiments]. PWRiL, Warszawa.

Soin S.G., 1953: O razvitii neoplodotvorennoj ikry lososevych ryb. – Rybnoje Choziajstvo, **29**, 5: 55–58.

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

Turdakov A.F., 1965: Dejstvije veščestv ikry i ovarjalnoj židkosti na spermatozoidy ryb. – Vopr. Ichtiol., **5**, 2(35): 302–314.

Translated: mgr Teresa Radziejewska.

WPŁYW SAMCÓW NA PRZEŻYWALNOŚĆ I STOPIEŃ ZAPŁODNIENIA IKRY TROCI (*Salmo trutta trutta* L.)

Streszczenie

Celem pracy było znalezienie obiektywnego kryterium jakości mlecza samców doświadczalnych troci (*Salmo trutta trutta* L.). Oceny mleczu dokonywano drogą badania ruchliwości plemników poszczególnych osobników w 3-krotnych wytarciach. Weryfikację metody stanowiły: analiza przeżywalności ikry zapłodnionej przez spermę badanych samców oraz zapłodnienie ikry wyrażone w %. Obserwację zapłodnionej ikry prowadzono do stadium zaoczkowania. Ikrę zbielałą w trakcie inkubacji wybierano i utrwalano, a następnie badano w celu określenia stopnia rozwoju zarodkowego. Przeżywalność zapłodnionej ikry, jak również zapłodnienie ikry wyrażone w % poddano opracowaniu statystycznemu.

Rezultaty doświadczenia wykazały, że tak przeżycia ikry, jak i procent jej zapłodnienia w znacznie wyższym stopniu są zależne od samic niż od samców. Stwierdzono również jednak istotne różnice

w zdolności zapłodnienia ikry przez mlecz. Wykazano także fakt zmniejszania się procentu zapłodnienia ikry pochodzącej z kolejnego tarła, co najprawdopodobniej wynika nie tylko z obniżenia jakości mlecza w miarę kolejnych wycierań, ale także i z obniżenia jakości ikry, pochodzącej od samic wycieranych w późniejszych okresach.

Uzyskane rezultaty wskazują, zdaniem autorów, iż metoda oceny jakości mleczu poprzez analizę przeżywalności zapłodnionej nim ikry jest zbyt mało dokładna, ze względu na występowanie śmiertelności zapłodnionej ikry. Analiza uzyskanego procentu zapłodnienia ikry, mimo braków metodycznych wynikających z niemożności określenia stopnia zapłodnienia w bardzo wczesnych fazach rozwoju embrionalnego, pozwoliła na uchwycenie różnic jakości spermy samców doświadczalnych.

ВЛИЯНИЕ САМЦОВ НА ВЫЖИВАЕМОСТЬ И СТЕПЕНЬ ОПЛОДОТВОРЕНИЯ ИКРЫ КУМЖИ (*Salmo trutta trutta* L.)

Р е з ю м е

Основной целью работы является оценка качества молок подопытных самцов с трёхкратным выцеживанием. Оценку проводили путём исследования подвижности сперматозоидов. Для проверки правильности такой оценки провели анализ выживаемости и степени оплодотворения экспериментальной икры. Наблюдения над оплодотворённой икрой проводили до наступления стадии глазка. Икру, побелевшую в процессе инкубации, выбирали и фиксировали, а затем исследовали с целью определения степени эмбрионального развития. Выживаемость экспериментальной икры и оплодотворение её выражены в процентах и подвергнуты статистическому анализу.

Результаты опыта показали, что как выживаемость икры, так и процент её оплодотворения в значительно большей степени зависят от самок, чем от самцов. Одновременно установлены, однако, существенные различия в возможности оплодотворения икры молоками. Кроме того, установлен факт снижения процента оплодотворения икры последующего нереста, что вероятнее всего, вытекает не только из снижения качества молок по мере совершения последующих нерестов, но и из снижения качества икры самок, нерестящихся позднее. По мнению авторов, полученные результаты показывают, что метод оценки качества молок путём анализа выживаемости оплодотворённой этими молоками икры является недостаточно точным, т.к. отмечалась смертность уже оплодотворённой икры. Анализ полученного процента оплодотворения икры, несмотря на его методические недостатки, вытекающие из невозможности определения степени оплодотворения в более раниие стадии эмбрионального развития, позволил, однако, констатировать разницы в качестве спермы подопытных самцов.

Address:

Dr Krzysztof Goryczko, dr Lucjan Tomasik
Instytut Rybactwa Śródlądowego
Instytut Ichtiologii AR
71-550 Szczecin
ul. Kazimierza Królewicza 4
Polska – Poland

Received: 14 III 1975 r.