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MOTILITY AND SPECIFIC FEATURES OF MOVEMENTS OF *Lepomis gibbosus* L. SPERMATOZOA

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A B S T R A C T. Motility of spermatozoa was studied in *Lepomis gibbosus* L. inhabiting streams heated by the effluents of the Dolna Odra and Pomorzany power stations. Studies revealed a high percentage (95%) of active spermatozoa, a very long (about 15 minutes, including the oscillatory movement) duration of their motility, and a characteristic trajectory of spermatozoa movement in water (148 s-long progression consisting of linear and circular movements). It is suggested that prolonged duration of spermatozoa motility and characteristic movements observed at that time are adaptations aimed at increasing efficiency of fertilisation of eggs upon the spawning grounds, which eventually enhance the potential for biological and territorial expansion of the species, new in Polish ichthyofauna.

Key words: *Lepomis gibbosus*, MOTILITY OF SPERMATOZOA

INTRODUCTION

Lepomis gibbosus Linnaeus, 1756, is a native species in the east part of North America, from New Brunswick in Canada to Georgia, USA. Few records of the species have been also reported from the west part of North America.

At the end of the 19th century (in 1877 according to Lorecco), *L. gibbosus* was brought to France from Canada. Over the last 100 years, the species was introduced into other European countries, including Poland (Welcomme 1981; Cihař 1992).

The species is classified in Poland as rare (Balon 1964). As of the early 1980's, reports of *L. gibbosus* being caught by anglers and professional fishermen have been more and more frequent (Hesse and Przybyszewski 1985). Some of the first records were reported from the vicinity of the Pomorzany power station in Szczecin. At present the species is most abundant in the Dolna Odra power station cooling water, and is gradually expanding over the entire area of the lower Odra (J. Filipiak, personal communication). The increasing population size is most probably related to an increase in water temperature, particularly in winter, the increase having been observed since the Dolna Odra power station became operative.

L. gibbosus is a predatory species. Major diet components include insect larvae, small crustaceans, and molluscs. It eagerly feeds also on eggs and fry of other fish (Trautman 1957; Schrenkeisen 1963). The species, once its population has reached an appropriate size, may become an important competitor for other predatory fish species occurring in the area; moreover, an excessive population growth may adversely affect reproduction of other commercially valuable fish species in the lower Odra, downstream of Gryfino.

Thus, the reproduction of *L. gibbosus*, including egg fertilisation, is one of the most important factors affecting the species population size in a given area. This prompted the author to study the reproduction of the "immigrant from the west" in question, and to focus on biological peculiarities of its reproductive cells. Detailed knowledge of this kind might be helpful in elucidating causes of the considerable, as shown by the literature, potential for biological and territorial expansion of the species. The present paper addresses the question of sperm behaviour during activation.

MATERIALS AND METHODS

The study embraced *L. gibbosus* spermatozoa collected from males entering the spawning grounds and caught in 1995 from the effluent canal of the Dolna Odra power station. The fish were obtained from two catches made a week apart with an electric power device; the males caught were kept in cages situated in the same canal. They were in the midst of spawning, at the "leaking" stage.

Spermatozoa motility was measured twice at the Szczecin Agricultural University Fisheries Experimental Station situated in the vicinity of the canal, so there was no need to transport the fish or their sexual products. The sperm obtained from a total of 13 males was examined.

The sperm was squeezed out onto an hour glass. Samples contaminated with blood, urea, and faeces were rejected.

Spermatozoa motility and behaviour were examined directly after collection, under a light microscope (Amplival, Carl Zeiss Jena) at 250x magnification, using a modification of a method described by Tomasik (1973). About 10 μl drop of water from the spawning ground in the canal (27^{o}C) was placed on a glass slide in the microscope stage. A droplet of sperm (estimated at about 0,05 μl by comparison with the pipetted volume of 1 μl) was placed using a pointed wooden stick on the bottom side of a cover

glass. For practical reasons, the sperm sample was merely a trace on the cover glass. The latter was placed on the slide so as to put the sperm in contact with the water. Once the contact was achieved (activation), the spermatozoa movement was timed. The percentage of moving spermatozoa was assessed up to 10%, and the movement types (progressive, with turbulent and quiet phases, and oscillatory) were timed to 0,5 s. The duration of movement types and phases was measured (Formicki *et al.* 1989, 1990) in the following way: 1) progressive movement, including a) turbulent phase: from activation until less than 50% of the spermatozoa moved this way; and b) quiet phase: from the end of the turbulent phase until less than 50% of the spermatozoa moved this way; 2) oscillatory movement; from the end of the progressive movements until the cessation of motility (when 5% of the spermatozoa in the sample performed this movement). The combined duration of progressive and oscillatory movements formed the total time of spermatozoa motility.

The sperm collected from each male was examined three times and an arithmetic mean of the three observations was calculated.

The data obtained were analysed statistically using the Statgraphics v. 6.0 Manugistics^{TM} (USA) software package.

RESULTS

The spermatozoa began to move as soon as they got in touch with water (activation), the percentage of activated gametes being very high (95 ± 4.3%) (Table 1). The mean duration of the progressive movement was 148 ± 41.2 s, i.e., 15.9% of the total time of motility; the total duration of motility (including oscillatory movements) was 929 ± 451 s (100%) (Table 1, Fig. 1). The oscillatory movement of spermatozoa obtained from one of the fish was observed for as long as 1800 s after activation (Fig. 1); that was the maximum deviation from the mean found when analysing different types and phases of sperm motility. In the remaining cases, maximum and minimum deviations from the mean remained at a similar level.

When timing the motility, the spermatozoa performing the progressive movements at the turbulent phase were observed to move along straight lines, while during the quiet phase (regardless of the much lower intensity of movements), the linear movements were accompanied by uncoordinated circular movements with a relatively long radius, and spiral movements. Moreover, of an interest was a very long

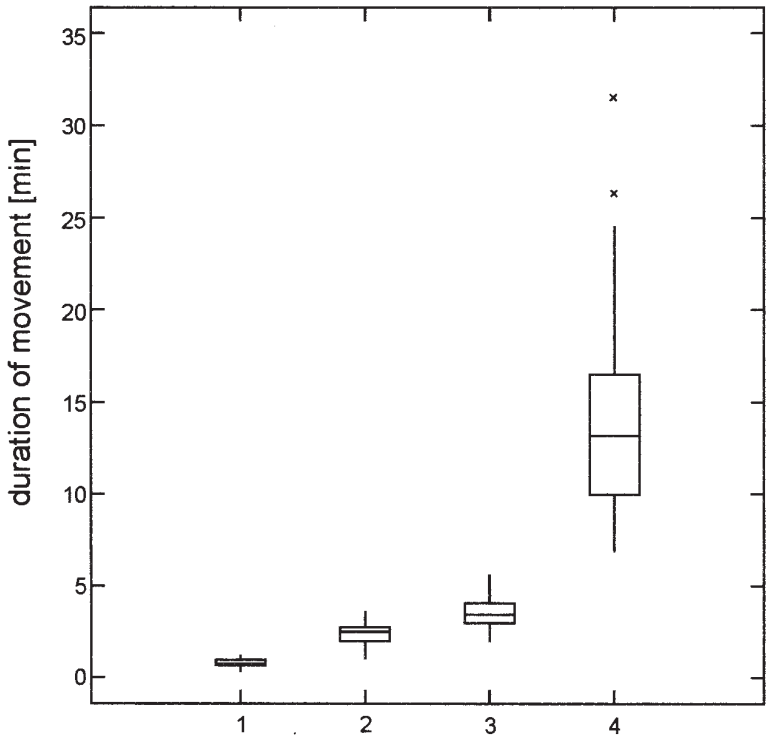

Fig. 1. Duration of *Lepomis gibbosus* spermatozoa motility, with particular reference to different types and phases of movement. Progressive movement, turbulent (1) and quiet (2) phase; intermediate phase (3); total duration of movement (4); duration of various phases and types of movement as measured from activation.

phase, intermediate between the progressive and typically oscillatory movements. During that time, the spermatozoa were performing a jerking oscillatory movements forward, that is a spermatozoon oscillated to and from, but at the same time moved very slowly forward. This phase was included into the oscillatory movement.

DISCUSSION

The duration of spermatozoa motility in fish with external fertilisation is known to vary within a very wide range (Stoss 1983). Sperm of some salmonids, after dilution with fresh water, perform progressive movements lasting from several to tens seconds: 10-15 s in *Oncorhynchus keta* (Smirnov 1975), 23 s in *Salmo gairdneri* (Billard 1978), 39 s in *Salmo trutta* (Tomasik 1973), and 48 s in *Hucho hucho* (Formicki et al.

TABLE 1

Movement of *Lepomis gibbosus* spermatozoa

			$\bar{x}$	%	Variance	SD
Percentage of active spermatozoa (%)			95.5		18.27	4.27
Duration of sperm movement (s) and (%)*	Duration of progressive movement	turbulent phase	47.4	5.1	256.09	16.00
		turbulent and quiet phase	148.5	15.9	1693.94	41.16
	Oscillatory movement	intermediate phase	218.9	23.5	3448.41	58.72
		total time of motility	929.9	100.0	204457	452.17

**duration of various phases and types of movement as measured from activation*

1989). It should be, however, remembered that total duration of spermatozoa motility (including oscillatory movement) lasts in rainbow trout and in trout 68 s and 55s respectively, and in huchen 132 s (Tomasik 1973, Formicki et al. 1989). The duration of spermatozoa motility (progressive and total – including oscillatory movement) of other freshwater fishs like *Coregonus albula* and *Thymallus thymallus* is 67.5 s up to 133.5 s, and 26 s up to 105 s respectively (Winnicki and Formicki 1993, Formicki *et al.* 1993).

The study was carried out under conditions which were intended to resemble as much as possible those in the natural habitat of *L. gibbosus*. As already mentioned in the Materials and Methods, observations were made in proximity of the site where males had been captured, immediately after sperm collection, and using water from the natural habitat. Under such conditions, when put in contact with water, spermatozoa of *L. gibbosus* moved for a relatively long time in comparison with the duration of motility of spermatozoa of other species of freshwater fish, as the intensive progressive movements took 148.5 s, and total duration of motility was 929 s. It can be assumed that the movement would still be prolonged, should a salt solution be used. Generally, initiation of spermatozoa motility in salt solutions instead of water reduces osmotic stress for the sperm cells and slightly prolongs duration of motility (Billard and Cosson 1992). On the other hand, spermatozoa moving in water can suffer extensive osmotic damage, the damage leading eventually to rupture of the cell membranes (Billard 1983). It cannot be ruled out that such a situation (slightly increased salinity) occurs under natural conditions, as numerous freshwater fish in the River Odra estuary feed and spawn in a zone where fresh and saline waters undergo partial mixing. That, however, does not unduly affect reproduction of *L. gibbosus*.

Our study revealed interesting spermatozoa movements along paracircular trajectories at the quiet phase of the progressive movement, and jerky oscillations at the

beginning of the oscillatory movement phase. This is not a completely new phenomenon in fish, as similar spermatozoan trajectories were observed in perch, whose spermatozoa changed the trajectory of their movements from linear to nonlinear (Lahnsteiner et al. 1995). On the other hand, rainbow trout spermatozoa change their movement pattern from circular to large trajectories, to resume the circular movements toward the end of the motility phase (Boitano and Omoto 1992). Salinity changes do not affect the spermatozoa movement pattern in rainbow trout (Billard and Cosson 1992, Boitano and Omoto 1992).

The data obtained and observations made during this study provide grounds for the suggestion that high activation rate and long duration of motility of *L. gibbosus* spermatozoa in water (at 27°C!), specific features of different movements, and the trajectories (circular and sinusoidal) of the spermatozoa increase their chances of finding the eggs and reaching the egg micropyle opening, which should warrant high fertilisation rate on the spawning ground and indirectly explain the potential for biological expansion of the species under study.

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STRESZCZENIE

RUCHLIWOŚĆ PLEMNIKÓW BASSA SŁONECZNEGO (*LEPOMIS GIBBOSUS* L.) I JEJ SPECYFIKA

Badano ruchliwość plemników bassa słonecznego, *Lepomis gibbosus* L. u osobników bytujących w podgrzanych przez elektrownie "Dolna Odra" i "Pomorzany" ciekach wodnych. Stwierdzono wysoki odsetek aktywnych plemników – 95%, bardzo długi okres ich ruchliwości – około 15 min (łącznie z ruchem wahadłowym) oraz charakterystyczny przebieg drogi (ruch postępowy – 148 sekund) wykonywanej przez plemniki w wodzie – prostoliniowy i okrężny. Wysuwa się przypuszczenie, że tak długi czas aktywności plemników oraz specyfika wykonywanych przez nie ruchów w tym okresie sprzyjają skuteczności zapłodnienia jaj na tarlisku, a w sumie, biologicznej i terytorialnej ekspansywności tego nowego dla polskich wód gatunku ryb.

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