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Black Sea Turbot Sperm Motility Depending on the Dilution of Seawater

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Black Sea turbot (*Scophthalmus maximus* L.) is valuable commercial fish, a promising subject for mariculture, and one of the important components of the Black Sea ecosystem. Semen quality assessment is important for understanding the status of the spawning population of this species.

Black Sea turbot is a typical broadcast spawner with external fertilization. In marine fish with external fertilization the osmolality of semen is significantly lower than the osmolality of seawater. The initiation of sperm motility is possible with a slight dilution of semen with seawater [Cosson, 2019].

The success of fertilization is the most reliable characteristic of the spermatozoa quality (provided there are high-quality female gametes). It is influenced by many abiotic and biotic factors. The sperm fertilizing ability depends on the volume, sperm concentration, and the composition of the seminal fluid (the content of ions, lipids, and proteins), as well as the characteristics of spermatozoa motility: the speed and duration of movement [Cosson, 2008 ; Gallego, 2018].

The most appropriate methods for determining the viability of sperm cells are functional tests that determine the characteristics of sperm movement: the percentage of motile sperm, speed and duration of activity. The ratio and methods of sperm dilution, the different solutions in which activation takes place, and even the method of application on a glass slide can influence the spermatozoa speeds and the motility duration [Fauvel, 2010 ; Cosson, 2020].

This work aimed to determine the effect of the sperm dilution on the main characteristics of motility, namely on the speed, the proportion of motile sperm, and the retention of activity during the time after activation.

Individual sperm characteristics were studied from samples obtained from three turbot males from a natural population. To study the characteristics of sperm motility, 0.1 mL of sperm was diluted in a ratio of 1:10 and 1:100 with seawater. In the experiment, video recording of sperm movement was carried out in fresh sperm smears in triplicate (dilution was carried out in separate containers for each replicate) taken from the plate both immediately after dilution and after 5, 10, 15, 20 min.

The average speed VCL of sperm immediately after activation with seawater when diluted in a ratio of 1:10 was $(85 \pm 20) \mu\text{m}\cdot\text{s}^{-1}$, the maximum $123 \mu\text{m}\cdot\text{s}^{-1}$, and the average percentage of motile sperm in all samples was 80 %; for sperm diluted in a ratio of 1:100, $(46 \pm 16) \mu\text{m}\cdot\text{s}^{-1}$, maximum $97 \mu\text{m}\cdot\text{s}^{-1}$, and the average percentage of motile spermatozoa in all samples was 28 %. After 20 minutes for 1:10 VCL was $(61 \pm 20) \mu\text{m}\cdot\text{s}^{-1}$, maximum speed $90 \mu\text{m}\cdot\text{s}^{-1}$, and percentage 66 %; for 1:100 VCL was $(36 \pm 10) \mu\text{m}\cdot\text{s}^{-1}$, maximum $38 \mu\text{m}\cdot\text{s}^{-1}$, and 27 %.

We found that in turbot the sperm motility characteristics are the best when diluting semen with seawater in a ratio of 1:10; also, sperm move longer at high speeds, and the proportion of motile sperm is higher. It can be assumed that the best fertilization rate will occur at a 1:10 dilution.

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