Research Report for 8th STSM - AQUAGAMETE COST

STSM Title	Standardization of sperm quality assessment of different aquatic species
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Home Institution	Proiser R+D, Valencia, Spain
Host institutions	1 - Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Szent István University, Gödöllő, Hungary Supervisor: Dr. Ákos Horváth
	2 - Department of Aquaculture, University of Dubrovnik, Croatia Supervisor: Prof. Branko Glamuzina
Period	2016 April 18th until May 20th
WG(s)	WG1 – Techniques for evaluation of gametes quality

1. Research Data

Initially, this application proposed a collaboration with two AQUAGAMETE partners: Department for Aquaculture at University of Dubrovnik (Croatia) and Laboratory of Reproduction & Physiology (LRP-IMBBA) at the Hellenic Center for Marine Research (Greece). The planned activities were supposed to be carried out with sperm from Oyster species and four perciform fish (*Argyrosomus regius*, *Umbrina cirrosa*, *Polyprion americanus* and *Seriola dumerili*). However, due to logistical problems it was not possible to visit the LRP-IMBBA, and to keep the objectives of this study was necessary to choose another fish species with reproduction season during the STSM period. Thus, in this study, sperm samples were collected from Adriatic grayling (*Thymallus thymallus*).

2. Research Results

It is essential to define a standard method to assess the fish sperm quality and minimize the differences between the results obtained by different laboratories, and transfer from academia to industry. This study presents preliminary validation of CASA software for two aquatic species, Adriatic grayling (*Thymallus thymallus*) and European flat oyster (*Ostrea edulis*). To attain this goal, different technical and data processing methods were tested: 1) Spermtrack® reusable chambers (10 µl or 20 µl), and 2) frame rates (≤ 400 fps).

Grayling specimens (n = 7) were a F1 generation raised in captivity at the facilities of the Ribiška družina Tolmin in Tolmin, Slovenia. Mature oyster (n = 9) were collected in Bistrina, Mali Ston Bay in Dubrovnik (Croatia), and transported to the laboratory of the University of Dubrovnik. Sperm motility analysis of grayling and oysters males showed significant increase up to 200 fps ($P < 0.05$), which means that the best conditions for sperm analysis of both species is the highest frames per second. In the case of grayling males, it is also better use the counting chamber with greater depth (ST 20). However, for both species it was not possible to find the maximum velocity of spermatozoa, and thus obtain a accurate track of spermatozoa cells. So, it would be necessary to configure the ISAS system to analyse video recordings up to 400 fps.

The most important differences in the sperm motility parameters between grayling and oyster males were the instant speed or VCL. Oyster sperm showed lower VCL values than grayling sperm. These results could be linked directly to the reproductive strategies. Oyster adopted internal reproduction, and do not need sperm with a capacity to swim very fast to find the oocytes. On the other hand, grayling adopted external reproduction, and therefore the spermatozoa need to swim as fast as possible.

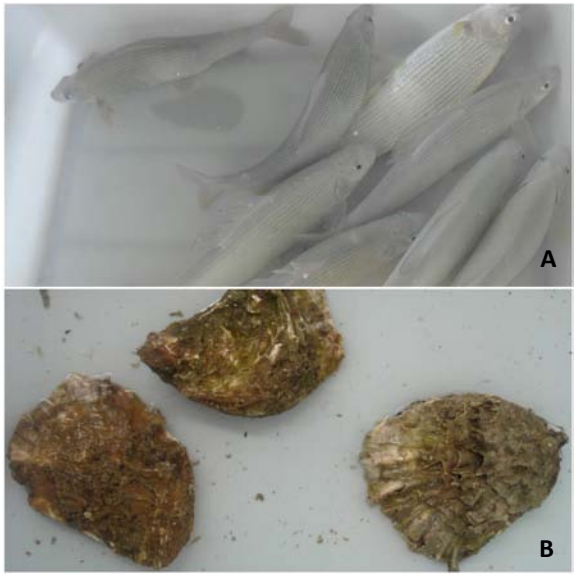


Fig. 1: Mature specimens of (A) Adriatic grayling (*Thymallus thymallus*) and (B) European flat oyster (*Ostrea edulis*).

Fig. 2: Workplace with a full ISAS system created by Proiser R+D. The CASA system was composed by UB203i phase contrast microscope, video camera of 500 fps, counting chambers (Spermtrack® 10 and 20 µL) and a computer with ISAS software.

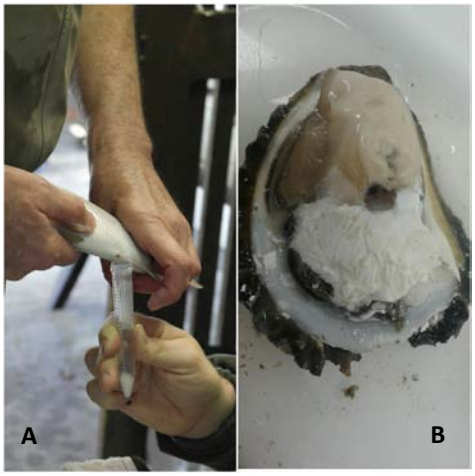


Fig. 3: Collection of sperm samples of sexually mature males of (A) grayling and (B) oysters specimens.

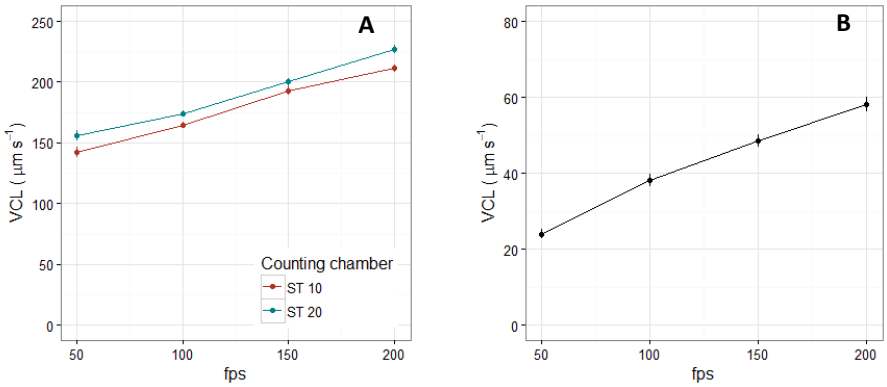
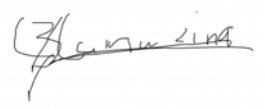


Fig. 4: Sperm motility analysis of grayling and oysters species. (A) The effect of frames per second (fps) and counting chambers (Spermtrack® 10 µL, ST 10, and 20 µL, ST 20) in curvilinear velocity (VCL) of grayling sperm. (B) The effect of fps on VCL parameter of oysters sperm, using a counting chamber Spermtrack® 10 µL.

How to STSM hosts, the undersigned, herewith confirm that the applicant and the host institutions have agreed on all research results.



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Dr. Akos Horváth