Ninth Call for STSM-Applications

COST Action FA1205: AQUAGAMETE - Assessing and improving the quality of aquatic animal gametes to enhance aquatic resources. The need to harmonize and standardize evolving methodologies, and improve transfer from academia to industry

Name: João Santana
STSM Reference: COST-STSM-FA1205-34548
Title: Evaluation of different cryoprotectants for spotted wolffish, Anarhichas minor (Olafsen, 1772) sperm cryopreservation.
Period: 2016-10-01 to 2016-10-15
Location: Mørkvedbukta Research Station, Nord University, Bodø, Norway
Host: Dr. José Beirão, Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway (jose.beirao-dos-santos@nord.no)

Description of the work
During the period of this COST Action I participated in the experiments performed by Dr José Beirão within the project WOLFSTORE. I learned how to manipulate spotted wolffish for sperm collection as well as the techniques to assess sperm quality. The spotted wolffish broodstock used in this experience were kept at Mørkvedbukta Research Station, Nord University. For gamete collection, fish were anaesthetised, and then, repeatedly massaged in the abdomen to release urine and faeces, after that, sperm was obtained by applying pressure in the upper lateral part of the abdomen. Sperm was collected using Pasteur pipettes with a little suction in fish's genital papillae. In spotted wolfish sperm is already active on stripping and is able to keep its motility for up to 48h. Only sperm samples with more than 50% motile sperm within one hour after collection ,as measured with Computer Assisted Sperm Analysis (CASA) software, were used in the different pools. At least 3 individual samples were used in each pool.
Different extender solutions were tested with two different sperm pools. The extender that presented less decrease in motility within 24h incubation time was the one recommended by Kime and Tveiten (2002) and was selected as cryopreservation extender.
To assess cryoprotectants toxicity, different cryoprotectants (DMSO; 1,2-Propanediol; and Methanol) at different concentrations (5, 10 and 20%) were tested. After the incubation time (2 min), motility and VCL (curvilinear velocity) were measured. Methanol presented higher motility and VCL values even at higher concentrations (20%), with no significant differences from the control group. Whereas, for DMSO and 1,2-Propanediol 10% concentration caused a decrease in the percentage of motile sperm and 20% caused a decrease of both the percentage of motile sperm and sperm velocity. Thus, the following cryoprotectans and concentrations were selected for the development of cryopreservation protocols (DMSO 5%; 1,2-Propanediol 5%; and Methanol 20%)The knowledge and data acquired in Bodø allows me to gather data to my master's thesis,

but mostly in further development of a cryopreservation protocol for spotted wolffish sperm, this way, solving one of the bottlenecks in reproductive management of this species.

Fig. 1 Broodstock observation in the tanks. Fig. 2 Wolffish's urogenital papilae. Fig. 3 Sperm collection Fig. 4 Sperm analysis in CASA system.

Confirmation

Dr. José Beirão (Faculty of Biosciences and Aquaculture, Nord University) certifies that João Santana (University of Algarve) has completed a Short-Term Scientific Mission awarded by the Cost Action F1205 in the period of October 1st to 15th, 2016.

José Beirão
(Host)

João Santana
(STSM applicant)