

SUMMARY

The conservation status of the species studied in this thesis (European eel and pufferfish) is currently frail, thus the main goal of this research was to develop, improve and apply several techniques and protocols with the aim of increasing the knowledge about their sperm biology, improving their reproductive performance and even helping future breeding in captivity.

The reproductive performance of the males is often assessed through the sperm motion parameters analysed by the CASA system, so first we focused on how to standardize this technique in terms of procedural and biological settings. In this respect, we laid the foundations for applying a standard method to assess sperm quality in fish, making it possible for sperm studies to be compared both intra- and inter-laboratories using the proper CASA settings.

Secondly, with the aim of improving the reproductive performance of European eel males, 3 thermal regimes (two of them variable: T10 and T15; and one of them constant: T20) and 3 hormonal treatments (hCG, hCG_{rec} and PSMG) were assessed based on different sperm quality parameters. In the case of the thermal regimes, our results demonstrated that the onset and progression of spermiation are strongly influenced by water temperature, with treatment T20 showing the best results in all the sperm quality parameters. In the case of hormonal treatments, hCG_{rec} produced the best results in all the sperm quality parameters, becoming an economically profitable/viable treatment and an effective alternative to the standard hCG treatment often used to induce spermiation in eel species.

A preliminary physiological study regarding the changes to the main ions involved in the fish sperm activation process was carried out. Our results showed that intracellular concentrations of Ca²⁺ and K⁺ increased upon eel sperm activation, while pH gradually decreased

over time, thus it is likely that all of them play an important role in the initiation of sperm motility in the European eel, as with other marine and freshwater teleosts.

In the second part of this thesis, which focuses on the pufferfish, an in-depth study into the sperm of this species was carried out for future application in aquacultural matters. A short-term storage method for pufferfish sperm was developed, enabling us to preserve the sperm quality parameters for a relatively long time period (7 days) compared to fresh sperm samples. Moreover, the effects of both the osmolality and the ion composition of the activation media on the sperm motion parameters were evaluated, concluding that both factors play an essential role in the initiation of sperm motility of pufferfish sperm and probably, in marine fish sperm.

Finally, *in vitro* fertilization trials were developed to ascertain how the quantity and quality of male gametes affects the fertilization and hatching rates. We demonstrated that sperm/egg ratio and sperm quality are strongly related factors, suggesting that both should be taken into account as unique interrelated elements. In addition, coefficients of correlation among all the spermatozoa motion parameters provided by a CASA system and fertilization and hatching rates were estimated for the first time in a marine species. Spermatozoa velocities showed the highest coefficients (≥ 0.80), suggesting that the kinetics of the spermatozoa are a key factor in the fertilization process.