USE OF LAPAROSCOPY FOR THE EVALUATION OF THE REPRODUCTIVE APPARATUS OF EEL (ANGUILLA ANGUILLA)

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Abstract
In this paper we evaluate the application of laparoscopy for the assessment of the reproductive status of the European eel, *Anguilla anguilla*, with the final goal of recognizing the mature stage of the gonads. Laparoscopy was carried out on 20 anesthetized eels, using a 1.9 mm rigid cystoscope. Gonads were visualized and histological samples were collected with a forceps and showed presence of gametes at different maturity stages. The application of laparoscopy can be powerful to evaluate sex and to establish the reproductive status of the gonads of eel.
Keywords: Fishes, Reproduction

Introduction
Laparoscopy is a useful diagnostic and therapeutic tool in human and, recently, in veterinary medicine. There are only few reports on laparoscopy application in teleosts [1]. This study is aimed at evaluating in eel the use of laparoscopy to analyze the reproductive tract and to detect the maturation stage of the gonads.

Material and Methods
Laparoscopy was performed on 20 eels (Anguilla anguilla) 60 cm medium length. The study was performed with a rigid cystoscope, a pump for air insufflation and a video device; a forceps was used to collect the bioptic samples. The fish were anesthetized in a tank containing MS 222 0.3 mg/L, and then placed on a grid out of the water; the anesthesia has been maintained conveying water containing MS 222 0.4 mg/L through a small aspiration pump in the oral cavity of the fish, using an i.v. flow regulator; the water has never been recycled. Laparoscopy was carried out placing fish on dorsal decubitus. A pneumocoelom was performed in order to separate the abdominal wall from the viscera. A small paramedian surgical incision, close to the anal pore, allowed the introduction of the Veress needle, linked to the insufflator through an i.v. flow regulator. The laparoscopic surgery door was made with the same procedure about 1 cm cranially from the first incision. After the examination, the coelom was emptied of air and treated with Enrofloxacin 14 mg/Kg; the laparoscopic entry sites were sutured with Vicryl (from 5-0 to 2-0). The fish were then placed again in the tank and the recovery was reached few minutes later. The bioptic samples were processed for paraffin-embedding. Slices were stained with Ematoxylin-Eosin. The follow-up showed a quick recovery and excluded the presence of post-surgery lesions as well as functional alterations within 3 months.

Results
During the laparoscopy the gonads were visualized as paired organs attached to the swimbladder. The histological examination showed presence of gametes at different maturity stages in both sexes. Ovaries were characterized by oocytes at different stage of maturation. Previtellogenic ones were characterized by a reduced size, an evident nucleus and a dense and deeply stained cytoplasm. Vitellogenic oocytes included ova with nucleus still evident and one or more layer of vacuoles filled with lipid yolk, and large ova with vacuoles and stained cytoplasmic material (protein yolk) covering the nucleus. The testes were characterized by Sertoli cells and primordial germ cells.

Discussion
One of the major challenges in fish reproduction is the precise assessment of the gonadal maturity stage before hormone conditioning, necessary for stimulating gamete release in several farmed species [2]. Generally fish not showing sexual dimorphism are sexed through direct observation of gonads, sacrificing the fish, therefore this technique is not reliable for endangered species or expensive fish such as broodstock; radio-immuno assays for determine sex hormone concentration, even if not lethal, is difficult and expensive, and not very sensitive in case of low levels of sex hormones e.g. young fish or onset of reproductive activity [3, 4]. The laparoscopy showed a big potential allowing direct visualization of the gonads and a quick examination. The main problems of this procedure are the cost of the devices, the high expertise and training of the staff and the small size of many teleost fish with consequent potential injuries to coelom organs/cavity.

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