TRYPSIN-CHYMOTRYPSIN ALTERNATION AND OTHER DIGESTIVE ENZYMES DURING THE DAILY DIGESTION IN EARLY SENEGAL SOLE JUVENILES

N. Gilannejad 1, C. Navarro-Guillén 1, G. Martínez-Rodríguez 1, F.J. Moyano 2, M. Yúfera 1

1 Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), 11519 Puerto Real, Cádiz, Spain.
2 Dept. Biology and Geology, University of Almería, 04120-Almería, Spain

*N* E-mail: neda.gilannejad@icman.csic.es

Introduction
The improvement of diets and the utilization of their nutrients by growing fish is a major challenge in fish farming. In vitro experiments with customized bioreactors simulating the digestion conditions are considered as an easy-working and quick methodology for improving feed formulation. Nevertheless, it is first necessary to define realistic digestion conditions according to the target species. In a first step to advance in the integral knowledge of the digestive function in Senegal sole (*Solea senegalensis*), we have analysed the activity pattern of some key digestive enzymes during a 24 hours period in early juveniles with different daily feeding frequencies.

Materials and methods
Juvenile Senegal sole with an average weight of 10±0.3 g were reared in four 400-L tanks with flow-through water system with a temperature of 19.5 ± 1.0 °C and a natural light/dark photoperiod. Fish were fed daily on an experimental diet with a ratio of 2% fish wet weight with four different feeding protocol: a) one daily meal at 9:00 h (local time), b) six daily meals during daylight at 08:30, 10:00, 12:00, 14:00, 16:00, and 18:00 h, c) six daily meals during night at 20:00, 22:00, 24:00, 02:00, 04:00 and 06:00 and, d) 12 daily meals during 24 hour (at times mentioned in protocols b and c). The fish were acclimatized to these experimental conditions during two weeks and then were sampled every 4 hours during a 24 hours cycle for determination of the digestive enzymes activities. Sampled fish (five fish per sampled point and feeding condition) were anaesthetized, and then killed by anaesthesia overdose and dissected to remove the complete digestive tract. Fish and gut were weighed and trypsin, chymotrypsin, amylase, alkaline phosphatase, and lipase activity was determined according the methodology described in (Navarro-Guillén et al. 2015). All enzyme activities are expressed as Relative Fluorescence Units per gut protein content (RFU/gut protein content (mg) and data are shown as mean ± standard error of the mean.

Results and Discussion
Patterns of activity of the different digestive enzymes showed evident changes during the 24-h cycle (Fig. 1). The most remarkable change is that the activity of trypsin and chymotrypsin increased successively during day time and night time respectively. Other interesting finding is that the trypsin, chymotrypsin and alkaline phosphatase activities were lower and the amylase activity higher when the juveniles were fed with only one daily meal than with the other feeding protocols.

These results confirm that feeding frequency has a clear effect on the daily digestion in fish. Although the daily amount of food offered was the same in the four protocols, the proteolitic activity in Senegal sole juveniles is higher as more frequent is the food supply which probably result in a better nutrient utilization.

References

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Fig. 1. Activity pattern of different digestive enzymes during a 24 h cycle in early juveniles of Senegal sole with different daily feeding frequencies.