

## DAILY PH FLUCTUATIONS IN THE GASTROINTESTINAL TRACT OF SENEGALESE SOLE AND GILTHEAD SEABREAM JUVENILES IN RELATION TO DIFFERENT FEEDING PROTOCOLS

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### Introduction

Gastrointestinal tract (GIT) pH is an important factor involved in gut enzymatic capacity and the consequent bioavailability of ingested nutrients, solubilization of proteins and minerals, and modulation of the gut microbiota. Moreover, GIT pH may be affected by several factors including fish age, feeding schedule, and food quality, among others. More accurate pH measurements are needed to have a general picture of the complex processes occurring during the feed transit and, therefore, to define efficient feeding protocols. Nevertheless, the information on the luminal pH through the digestive tract in fish is scarce. Therefore, the aim of the present work was to know the ionic luminal pattern under different feeding conditions in two important aquaculture species, with very different digestive anatomies, Senegalese sole (*Solea senegalensis*) and gilthead seabream (*Sparus aurata*).

### Materials and Methods

Juveniles from *S. senegalensis* and *S. aurata* ( $10 \pm 0.3\text{g}$  and  $17.66 \pm 0.38\text{g}$ , respectively) were randomly distributed into 4 groups with 2 replicates, in 200-L tanks, with flow-through water system at  $19.5 \pm 1.0^\circ\text{C}$  and a photoperiod of 11L/13D. Juveniles were fed on experimental diets (2% of the fish wet weight) and with different daily feeding protocol. For Senegalese sole: protocol 1) one daily meal at 8:30 hours (local time);, 2) six meals during daylight at 08:30, 10:00, 12:00, 14:00, 16:00 and 18:00 h; 3) six meals during night at 20:00, 22:00, 24:00, 02:00, 04:00 and 06:00; and 4) 12 meals during 24 h (at the times mentioned in protocols 2 and 3). For gilthead seabream: protocol 1) one daily meal at 08:30 h; 2) three meals during daylight at 08:30, 13:30 and 18:30 h; 3) five meals during daylight at 08:30, 10:30, 13:30, 16:30 and 18:30; and 4) continuous feeding during daylight. In Senegalese sole and gilthead seabream, 5 and 7 points of sampling were chosen during a 24 h cycle, respectively. pH was measured in different segments of the GIT, using a Micro pH Electrode as described in Yúfera and Darías (2007).

### Results

In both species, a clear pH change was observed during the 24 h cycle in all the segments of the GIT and in the four feeding protocols. In *S. senegalensis*, little acidification capacity (min. pH = 6.80) was observed. In all protocols, anterior intestine demonstrated neutral pH, while medium and posterior intestine showed slightly alkaline pH (Fig. 1). In *S. aurata*, stomach showed considerable acidification in all the protocols (min pH = 3.49). However, low pH levels were not maintained during 24 h, but increased in some moments of the day depending on the feeding protocol (Fig. 2).

### Discussion and Conclusion

Our results highlight the inter-specific variability in acidification strategies. The negligible acidification of Senegalese sole stomach was confirmed, as previously addressed in Yúfera and Darías (2007) by checking only fed and fasted fish, demonstrating that optimal gastric conditions for pepsin activation are hardly attained and, otherwise under real physiological pH, that pepsin activity is highly overestimated in this species. On the other hand, in gilthead sea bream, it seems that the pH pattern is affected by the feeding protocol as was already observed in this species (Yúfera et al., 2014) attracting many studies on nutrition and chronobiology, although nothing is known about the effect of feeding frequency on the daily rhythms of the gastric digestion process. In this article, we investigated daily rhythms in stomach fullness, gastric and intestine pH, as well as pepsin activity and expression of pepsinogen and proton pump in juvenile fish under three different feeding protocols: (A.

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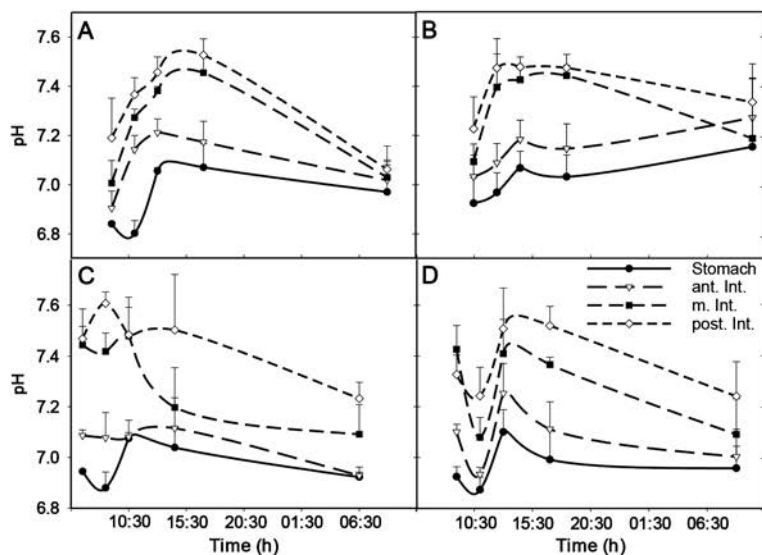


Fig. 1. pH (mean  $\pm$  SEM) in different segments of Senegalese sole GIT with feeding protocols 1 (A), 2 (B), 3 (C), and 4 (D). The grey areas indicate the dark period.

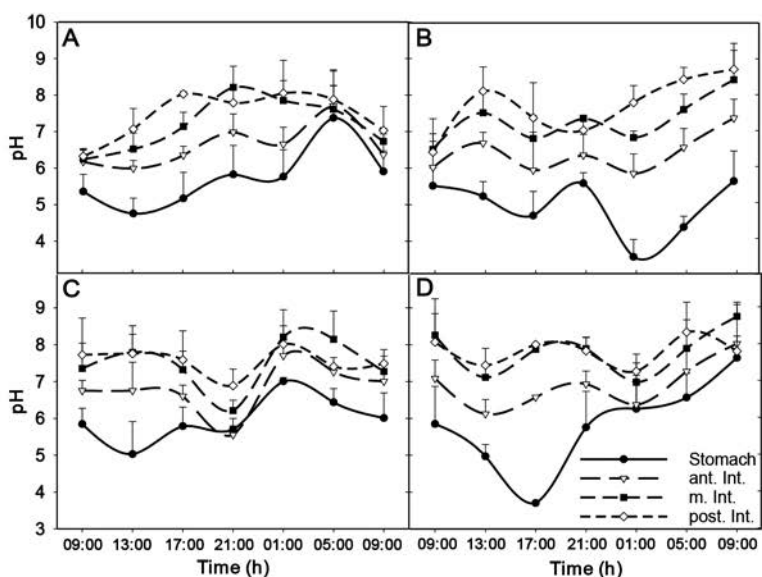


Fig. 1. pH (mean  $\pm$  SEM) in different segments of gilthead seabream GIT with feeding protocols 1 (A), 2 (B), 3 (C), and 4 (D). The grey areas indicate the dark period.

## References

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