# Effect of two different pulses of *Shewanella putrefaciens* Pdp11 on *Solea senegalensis* larvae performance


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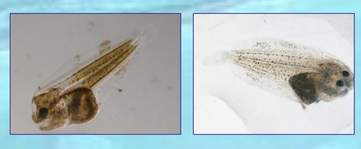



## Introduction

*Solea senegalensis* is a promising flatfish species for intensive farming. Appropriate nutrition at first feeding in marine fish larvae is a key factor for successful larval and juvenile rearing. Numerous studies have demonstrated that probiotics increased both feed conversion ratio and efficiency, and also digestive enzyme activities. In this way a tailormade probiotic protocol is essential to achieve the desired result. It is commonly accepted that digestive enzyme activity levels could be used as a physiological indicator to estimate growth and digestive capacity of fish larvae. In this context leucine aminopeptidase, trypsin and α-amylase activity levels could be used to assess the intestine epithelial functionality, the digestive ability and the inert diet adaptation at weaning in marine fish species. The aim of this study is to compare two different *S. putrefaciens* Pdp11 pulses (2-21 *vs* 10-21dah) in terms of growth, digestive enzymatic capacity and body composition along larval and postlarval culture of Senegalese sole.

## Material and methods

*Solea senegalensis* larvae were distributed into 280 l tanks by triplicate. (19.3 ± 0.5 ºC). Illumination and feeding regime was based on Lobo et al. (2014). Phytoplankton and rotifers were supplied (2-9 dah) and cofeeding was carried out with *Artemia* metanauplii (Origreen, Skretting) and dry feed (Larviva, Biomar) since 10 dah. *S. putrefaciens* Pdp11 was daily incubated in TSA (1.5%NaCl) at 22ºC, collected and suspended in a PBS solution (pH 7.2) and finally supplied to live feed ($2.5*10^7$ cfu $mL^{-1}$) 3 hours prior to larval feeding. Three experimental groups were established: Control fish (CCC), PPC fish fed with Pdp11 (2-21 dah) and CPC fish fed with Pdp11 (10-21 dah). Growth in length and weight was weekly determined. Size heterogeneity and fish survival were also checked. Live diets and sole postlarvae (48 dah) were sampled for biochemical purposes. Total protein and total lipid content were determined (Bradford, 1976; Blight and Dyer, 1959; Fernández-Reiriz *et al*. 1989). Fish samples were collected (10, 16, 32, 48, 66 dah) for digestive enzymatic studies. Trypsin, α-amylase and leucine aminopeptidase activities were determined (Jiménez-Martínez *et al*.,2011) and a zymogram of alkaline protease activities was also obtained (García-Carreño *et al*, 1993; Alarcón *et al*., 1998).

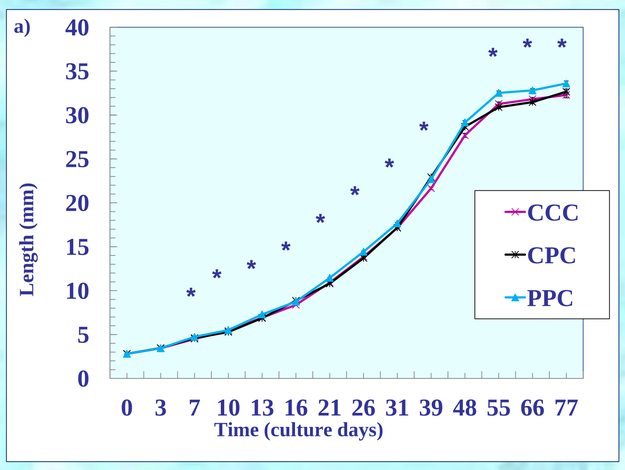


Fig. 1. Larval growth in (a) length (mm) and (b) dry weight (mg) of sole fed the three experimental diets. (Mean ± SEM). Asterisk (*) denotes significant differences among treatments ($P<0.05$)

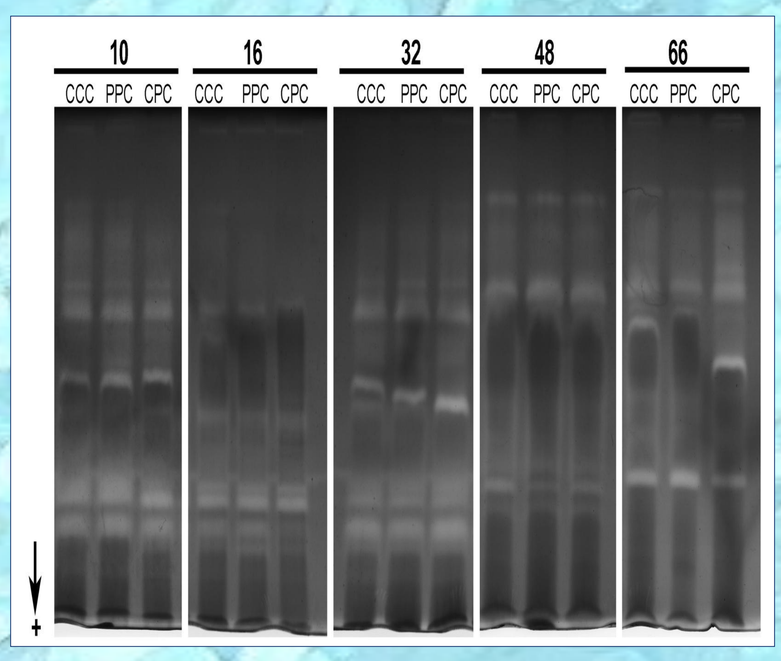


Fig 2. Zymogram on total proteolytic activity from pooled sole larval gut extracts of the three experimental groups at 10, 16, 32, 48 and 66 dah

Table I. Protein and lipids (% dry weight) of sole (48 dah) fed the three experimental diets. (Mean ± SEM). Different letters denote significant differences among treatments ($P<0.05$)

| | CCC | CPC | PPC |
|---|---|---|---|
| Protein | 53.3 2.3[a] | 47.9 0.7[ab] | 46.9 1.3[b] |
| Lipids | 14.7 0.8[ab] | 14.1 0.6[b] | 17.1 0.7[a] |

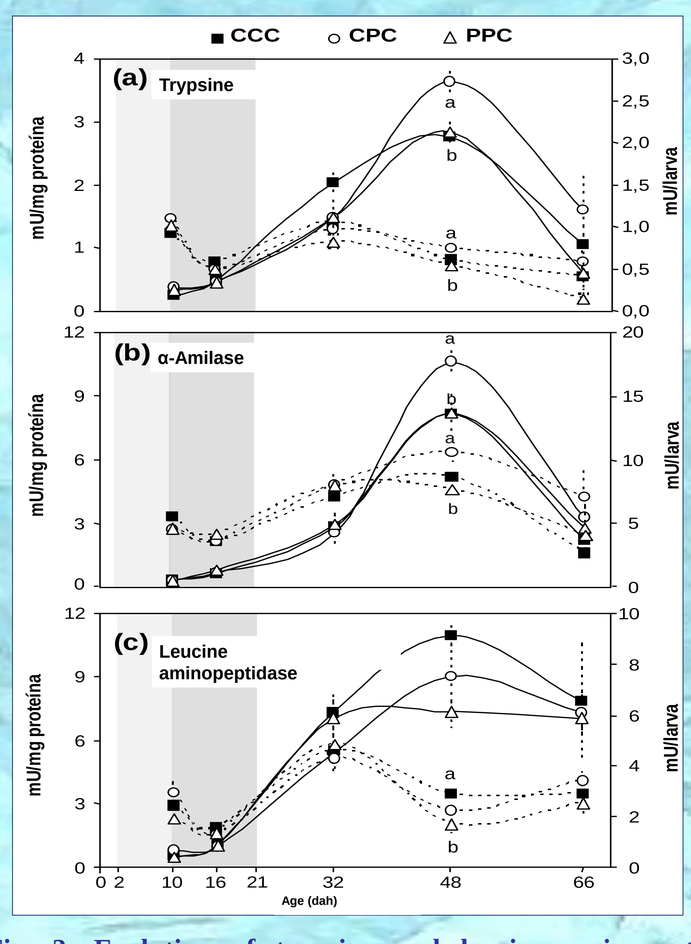


Fig. 3. Evolution of trypsine and leucine aminopeptidase activities in sole. Experimental groups are CCC ■,CPC ○ and PPC △. (Mean ± SEM). Dashed and continuous lines represent specific (U mg $protein^{-1}$) and individual (U $larva^{-1}$) enzyme activities, respectively. Different letters denote significant differences among dietary treatments ($P<0.05$)

## Results and discussion

*S. putrefaciens* Pdp11 bioencapsulated in live prey (2-21 dah) promoted a significantly higher growth in length and dry weight from 31 dah onwards (Fig. 1). A shorter pulse (10-21 dah) increased growth in dry weight since 39 dah. A less heterogeneous fish size was also related to Pdp11 administration at weaning (5.87 in PPC and 6.34 in CPC *vs* 8.16 in CCC). No differences were detected in biochemical composition among live diets Nevertheless Pdp11 pulse (2-21 dah) contributed to an increase in lipid levels ($P<0.05$) and a reduced protein content ($P<0.05$) compared to CPC and CCC (Table I). A better growth linked to higher lipid content was previously described (Plante *et al*.,2007) related to probiotic digestive microbiota modulation. Both probiotic groups showed lower leucine aminopeptidase activity than Control (48 dah) CPC specimens had significantly higher α-amylase and trypsin activity levels (48 dah) showing a proteolytic profile quite close to that sole juveniles at the end of weaning. The earlier digestive maturation together with the higher digestive capacity and a suitable potential inert diet adaptation detected in CPC group seem not to produce a growth enhancement. In this context the increase of proteolytic enzyme activities in CPC and CCC groups might be due to a compensatory mechanism to increase the energy reserves for growth performance. Further research should be done mainly focused to know how the interaction host-digestive microbiota affects lipid and protein digestion, body composition and rearing performance in *S. senegalensis* larviculture.


**References:**

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