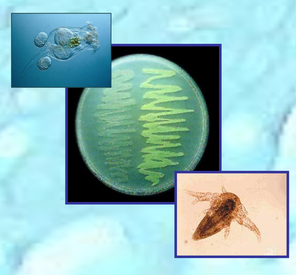
Effects of *Shewanella putrefaciens* Pdp11 on Senegalese sole skeletogenesis

Lobo C.[1*], Gavaia P.[2], García de la Banda I.[1], Gutiérrez J.R.[1], Oria M.[1], Díaz M.[1], Moreno-Ventas X.[3], Moriñigo M.A.[4] & Tapia-Paniagua S.T.[4]

[1] Spanish Institute of Oceanography, C.O. Santander Promontorio S. Martín s/n 39080 Santander, Spain. E-mail*: carmen.lobo@st.ieo.es
[2] CCMAR, Universidade do Algarve, 8005-139 Faro, Portugal
[3] Ecological Area of CYTAMA, University of Cantabria, Avda Castros s/n 39005 Santander, Spain
[4] Department of Microbiology, University of Málaga, Campus Teatinos, 29071 Málaga, Spain



Introduction

Solea senegalensis is a highly valuable commercial fish. Skeletal abnormalities are a serious economical problem in sole aquaculture that may suppose about 15-40% of production discards. Probiotic supplementation has been reported to improve skeletal development in rainbow trout and seabass larviculture (Aubin et al., 2005; Lamari et al., 2013). *Shewanella putrefaciens* Pdp11 bioencapsulated in *Artemia* (10-86 dah) enhanced sole production parameters (Lobo et al., 2014). The aim of this study was to evaluate the effect of two different Pdp11 probiotic pulses using *Artemia* and rotifer (2-21 dah) or only *Artemia* (10-21 dah) as live vectors on the presence and severity of skeletal abnormalities in post-weaned (66 dah) sole juveniles.

Table I: Skeletal areas affected (%) in *S. senegalensis* (66 dah) fed with the three experimental diets. (Mean SEM)

	Cephalic vertebra		Prehemal vertebra		Hemal vertebra		Caudal vertebra		Caudal fin		Dorsal fin		Anal fin	
CCC	0.0	0.0	12.6	0.3	7.87	1.40	58.5	3.8	28.5	3.3	57.8	9.1	61.6	2.9
CPC	18.1	4.5	10.8	2.2	13.3	5.2	38.7	10.0	33.1	5.4	38.2	4.2	33.5	2.7
PPC	0.0	0.0	8.33	1.50	12.5	2.6	38.7	2.2	22.0	4.1	25.6	2.4	60.7	0.6

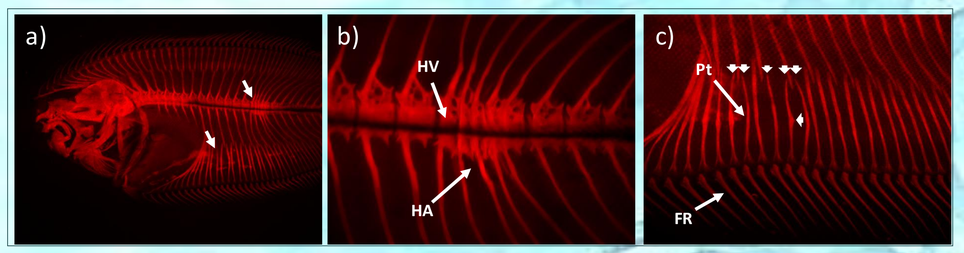


Fig. 1.a) Deformed CCC juvenile presenting a vertebral compression in the haemal vertebrae (HV) (b) and an altered anal fin (c). b) Compression of haemal vertebrae 16-20 with partial fusions affecting haemal (HA) but not neural arches. c) Deformity in the anal fin caused by deformity of pterygiophores (Pt) that altering fin conformation but not fin rays (FR).

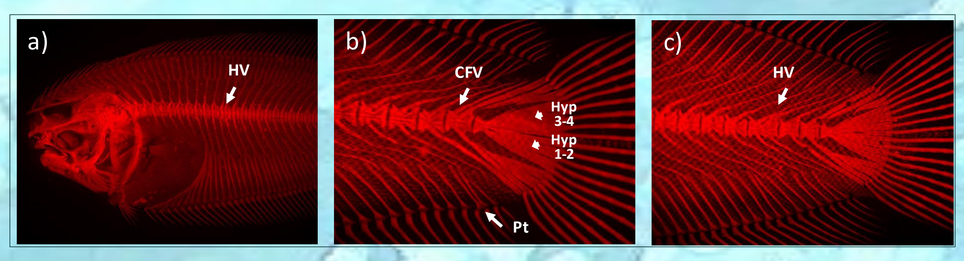


Fig. 2. a) Deformed CPC juvenile presenting a vertebral fusion and compression in haemal vertebrae (HV) 13-14. b) Fusion of caudal fin vertebrae (CFV) 43-44 with fused modified haemal (MHA) but not neural arches. Also present fused anal fin pterygiophores (Pt) and fused hypurals (Hyp). C) Fusion of haemal vertebrae 41-42.

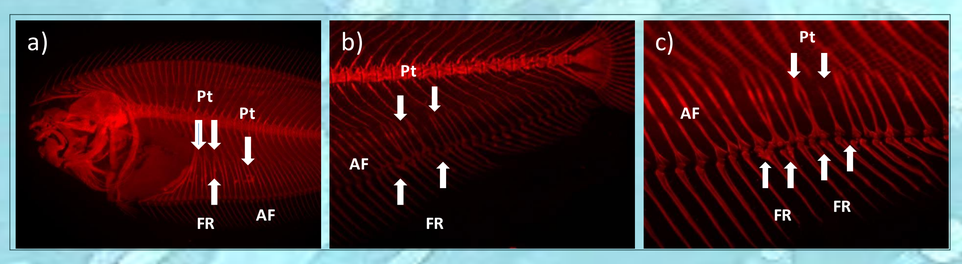


Fig. 3. a) Deformed PPC juvenile presenting a deformity in the anal fin (AF) caused by deformed pterygiophores (Pt) and anal fin rays (FR) caused by b) fusions and malformation of the pterygiophores (Pt) and anal fin rays (FR) c) Magnification of b)

Discussion:

Our results indicate a better skeletal condition in sole probiotic groups at the end of weaning, although the high variability observed recommend a further robuster study. The average rate of deformities was similar to those described by Gavaia et al, (2009) and Boglino et al, (2012), although the level of caudal and anal fin abnormalities was higher than those obtained by these authors. This findings may be due to a slight imbalance of minerals ocurred at larval stages.The severity of the skeletal deformities detected, involving a loss of 12% of the batches, was less than that observed in sole intensive larviculture (Boglino et al, 2012).

Material and Methods

S. 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). To identify and quantify different typologies of skeletal deformities in the 3 experimental groups 27 specimens per treatment were randomly sampled at 66 dah. Fish were anesthetized (clove oil, 40ppm) and fixed (4% formaldehyde in PBS. ph 7.4) for 24h and preserved (70% ethanol). For the visualization of the skeleton, fries were submitted to specific staining procedures (alcian blue for cartilage and alizarin red S for calcified structures) as Gavaia et al (2000). All observations were performed in a fluorescence stereomicroscopy Leica MZ7.5 equipped with a digital camera Olympus F-View. The deformities were separated according to the affected structures: cephalic (1-4), prehemal (5-9), hemal (10-42) and caudal (43-44) vertebra, caudal, dorsal and anal fin and cephalic area.

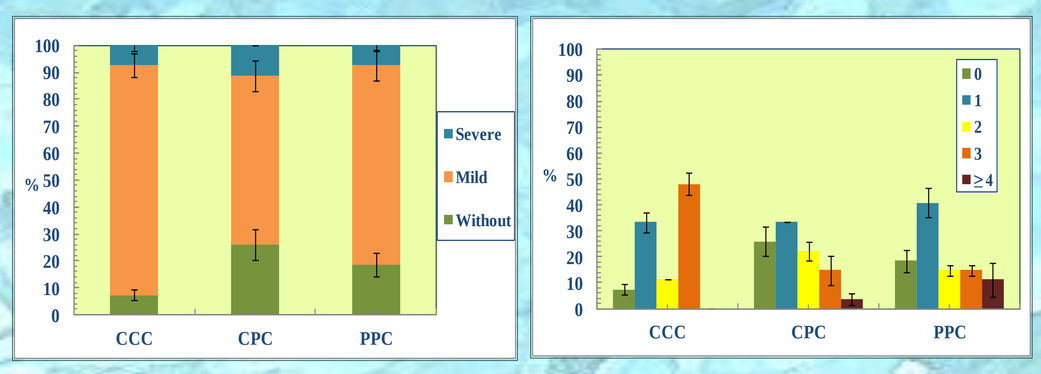


Figure 4. a) Level and b) number of skeletal deformities detected in *S. senegalensis* (66 dah) fed with the three experimental diets. (Mean SEM).

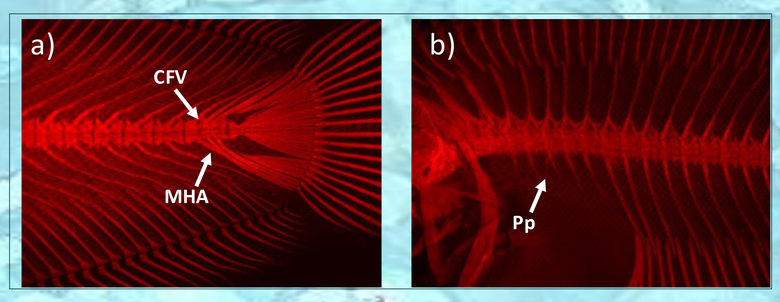


Fig 5. CCC sole juvenile with a) Severe fusion and compression of caudal fin vertebrae (CFV) and fusion of modified haemal arches (MHA) and b) deformed parapophysis (Pp) in pre-haemal vertebrae 7

Results:

Skeletal deformities registered in the three sole experimental groups were shown in Fig.4.a-b. It was observed a great variability intragroups. No significant differences were detected among diets but probiotic groups revealed a decrease in deformed specimens of up to 11%. Severe deformities were below 12% in the 3 groups and were mainly located in the vertebral column (60%) and the anal fin (40%). No deformities were observed in the cephalic area. The most affected area in the vertebral column was the caudal vertebra (38.7-58.5 % specimens affected) (Table I). Skeletal deformities in cephalic, prehemal and hemal vertebra did not exceed 20% in the three studied groups. Caudal, dorsal and anal fins abnormalities in the treatments ranged 22.0-33.1 %, 25.6-57.8 %, and 33.5-61.6 % respectively (Table I). Probiotic groups had a slightly higher incidence of fish without deformities (25.9% and 18.5% for CPC and PPC) compared to Control (7.4%) (Figure 4b). In this way Control group had a higher level of multiple abnormalities (48.2%) than probiotic groups (18.5-25.9%).

In conclusion: *S. putrefaciens* Pdp11 bioencapsulated in live diets seems to decrease skeletal deformities in sole fry. Our rearing regime seems to have a positive effect on skeletogenesis during sole larviculture but further studies are needed providing higher number of samples to better establish the probiotic effects.

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