

EFFECTS OF AXENITY AND DISINFECTION ON GROWTH AND DEFORMITIES OF EARLY LARVAL SEABASS (*Dicentrarchus labrax* L.)

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Introduction

Disinfection of fish eggs in hatcheries is a useful and common practice that reduces their intense bacterial load and improves their survival. Furthermore, new studies of fish larvae reared in axenic and gnotobiotic environments reveal the importance of this procedure as a means to study host–microbial interactions, and eliminating bacterial interference that tends to confound experimental results. However, a quantification of the influence that the disinfection and axenic rearing may have on the growth and shape of the larvae is necessary. To meet this goal, the present study aims to quantify the effects of disinfection and axenic rearing on larval growth and quality in seabass.

Materials and methods

Seabass eggs disinfected with 20 ml l⁻¹ 0.5% active iodine for 10 min 24 h prior to arrival at the Laboratory of Aquaculture & Artemia Reference Center were obtained by the Ecloserie Marine de Gravelines hatchery in France, and transported to the hatching room where they were split in three treatment groups, with hatched larvae reared until day after hatching (DAH) 15. The first treatment involved eggs that were disinfected with glutaraldehyde and subsequently added into axenic seawater (treatment D-A). The second included eggs that were disinfected but added into xenic seawater (D-X), and the third eggs not disinfected with glutaraldehyde and put directly into xenic seawater (N-X). The glutaraldehyde disinfection, axenic rearing and axenity tests were performed according to Dierkens et al. (2008).

The larvae were fed axenic Artemia on days 7, 9, 11, 13 and 15. On DAH 0, 5, 11 and 15 a total of at least 30 specimens per group were collected per treatment, anesthetized with MS222 and fixed in 3% phosphate buffered glutaraldehyde. They were photographed under an Olympus SZX9 stereoscopic microscope equipped with a Colorview 8 digital camera, and analysed with Image J v1.45d and Corel Draw 12 software.

To evaluate the effect of the different treatments on the larvae, an elliptic Fourier analysis was performed on their body outlines using SHAPE v1.3 software. Shape variation was analysed by a principal component analysis on the Elliptic Fourier coefficients. The squared Mahalanobis distance reflects the distance of group means in a multivariate morphospace by taking into account the within group variance, with lower values corresponding to greater group similarity. In the present study, they were determined between treatment shape centroids based on a canonical variate analysis. Difference in mean shape was tested by a MANOVA or non-parametric MANOVA on the principal component scores, using PAST v2.07 software.

Results and discussion

On DAH0, the larvae of treatment N-X had significantly larger ($P < 0.05$) total lengths than the ones from treatment D-A, but not from treatment D-X. However, at DAH5 and DAH11 specimens of treatment D-A were significantly larger than those from D-X, which suggests a faster growth under axenic conditions. Furthermore, on DAH5 larvae

of D-A were also significantly larger than those of N-X. However, on DAH15 all length differences were not significant.

At DAH0, the shape difference between D-A and D-X was not significant, and the squared Mahalanobis distance between their shape centroids was the smallest. At such an early stage immediately after hatching, the larvae have not grown in the axenic medium yet, but have only been exposed to it for the three days of their egg incubation period. Therefore, it might be too soon for any shape differences to manifest themselves as a result of the axenicity alone.

However, the differences observed at DAH0 between the axenic treatment and each of the two xenic ones become significant at DAH5, with an increase in their squared Mahalanobis distances. In contrast to this, at DAH11, four days after their first feeding with axenic Artemia, these differences were simultaneously reduced, suggesting that the larvae can recover from the initial effect induced by the axenic rearing. At DAH15, the differences between the axenic treatment and each of the two xenic ones remain significant and even increasing, hence showing that further axenic rearing continues to induce shape differences.

A significant difference in shape between the two xenic treatments was observed at the beginning of the experiment but remained low, got smaller during its progress, and at the end was no longer significant. Since the only difference in treatment is the application of an additional glutaraldehyde disinfection at the start of the experiment or not, it suggests that shape differences are slowly eliminated during their rearing stage, where larvae reach a degree of shape uniformity in spite of any possible adverse initial disinfection effects.

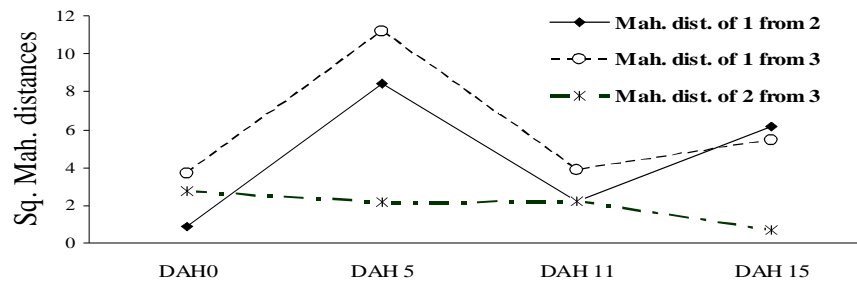


Fig.1: Squared Mahalanobis distances between shape centroids of treatments 1 (D-A), 2 (D-X) and 3 (N-X).

Conclusions

These preliminary results indicate that the growth of seabass larvae does not seem to be influenced by the conditions of secondary disinfection or axenicity. The latter does appear to introduce shape changes in the crucial very early stages of their development, but not on newly hatched larvae that underwent a secondary egg disinfection. Further studies are needed over a wider period in order to explore long term effects, but the difficulty of maintaining axenic larvae remains an issue.

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References

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