Effect of the application of β-glucans and mannan-oligosaccharides (βG MOS) in an intensive larval rearing system of *Paralichthys adspersus* (Paralichthydae)

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ABSTRACT. For successful rearing of the flounder *Paralichthys adspersus*, it is important to optimize growth and survival in the early larval stages. Several authors indicate that the application of β-glucans and mannan-oligosaccharides (βG MOS) in rearing water should improve the larval health, diminishing the effects of physiological stress and physical damage that the aquaculture activities cause to the individuals. In order to evaluate the effect of βG MOS on *P. adspersus* incorporation on *P. adspersus* larval survival and growth in intensive culture, experiments were carried out with six-days post-hatch larvae, which had only just begun to feed on live prey (rotifers), and fifteen-day post-hatch larvae. Three treatments were used, applying 5 mg·L^{-1}, 10 mg·L^{-1}, and 15 mg·L^{-1} of βG MOS to the rearing water during the first five days of the experiment and then comparing the results with a control. The results indicate that applications of 5 mg·L^{-1} of βG MOS in the rearing water enhance larval survival and growth with respect to the control, whereas additions of 15 mg·L^{-1} of βG MOS suppressed both of these parameters. This effect increases for larvae that have recently absorbed the yolk sac. An histological analysis of the intestinal epithelium of the larvae suggests that βG MOS promotes the expression of monocytes (forerunner cells of macrophages) associated with the non-specific immune system of the fish.

Key words: *Paralichthys adspersus*, flounder, larval rearing, growth rate, survival, β-glucan and mannan-oligosaccharides, immunestimulants.

Efecto de la aplicación de β-glucanos y manano-oligosacáridos (βG MOS) en un sistema de cultivo intensivo de larvas de *Paralichthys adspersus* (Paralichthydae)

RESUMEN. Para el éxito del cultivo del lenguado *Paralichthys adspersus* es importante optimizar el crecimiento y la supervivencia en los primeros estadíos de desarrollo larval. Diversos autores señalan que la aplicación de β-glucanos y manano-oligosacáridos (βG MOS) en el agua de cultivo debería mejorar la salud de las larvas, disminuyendo los efectos del estrés fisiológico y el daño físico de los individuos causado por las actividades propias de la acuicultura. Con el objetivo de evaluar el efecto de la incorporación del βG MOS en la supervivencia y el crecimiento de larvas de *P. adspersus* en cultivos intensivos, se realizaron experimentos utilizando larvas de seis días post-eclosión que recién han comenzado la alimentación con presas vivas (rotíferos) y larvas de quince días post-eclosión. Tres tratamientos aplicando 5 mg·L^{-1}, 10 mg·L^{-1} y 15 mg·L^{-1} de βG MOS al agua de cultivo fueron contrastados durante los primeros cinco días de experimentación con una condición control. Los resultados indican que aplicar 5 mg·L^{-1} de βG MOS en el agua de cultivo aumenta la supervivencia y el crecimiento de las larvas con respecto al control, mientras que 15 mg·L^{-1} de βG MOS tiene un efecto supresor en ambos parámetros poblacionales. Este efecto aumenta si se aplica en larvas que recién han absorbido el saco vitelino. El análisis histológico del epitelio intestinal de las larvas sugiere que el βG MOS promueve la manifestación de monocitos (células precursoras de macrófagos) asociados al sistema inmune no específico de los peces.

Palabras clave: *Paralichthys adspersus*, lenguado, cultivo larval, tasa de crecimiento, supervivencia, β-glucanos y manano-oligosacáridos, inmunoestimulante.

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INTRODUCTION

Under intensive culture, the manipulation of individuals specimens and the chemical and physical conditions of the system cause physiological stress and physical damage to the fish (Rottmann *et al.*, 1992). Stress during fish larval rearing is intermittent and synergic, which amplifies its negative effect and increases the risk of mortality caused by opportunistic microorganisms or pathogenic agents (Skjermo & Vadstein, 1999; Ellis, 2001). The application of prophylactic compounds such as β-glucans and mannan-oligosaccharides (βG MOS) improves the health of fish during the early development stages (pre-larva, larva, post-larva; *sensu* Silva, 2000), mainly by stimulating the non-specific immune system to create defenses against viral, bacterial, and fungal attacks (Sakai, 1999; Raa, 2000). Moreover, applications of compounds containing β-glucans (βG) are coadjuvant, increasing the fish's resistance to parasites and improving the effectiveness of vaccines (Anderson, 1992; Robertsen *et al.*, 1994). The action of βG in the intestine is similar to that of probiotics, stimulating the proliferation of beneficial bacteria that assist the immunological system, permitting decreased the use of exogenous or greater effects from exogenous antibiotics. The mannan-oligosaccharides (MOS), on the other hand, absorb the mycotoxins found in the nutrients commonly used in formulated diets (Vadstein, 1997; Raa, 2000; Bergh *et al.*, 2001; Pryor *et al.*, 2003). Other compounds besides βG MOS also activate macrophages, including γ-interferon, peptides, proteins, and lipopolysaccharides (Sakai, 1999; Raa, 2000; Jin & Xiao-ling, 2004; Bricknell & Dalmo, 2005; Kumari & Sahoo, 2006).

The application of βG for prophylactic purposes in juvenile and adult farmed fish is done through injections (intraperitoneal, pre-anal, or intravenous), or by incorporating these compounds into formulated diets (Robertsen *et al.*, 1994; Sakai, 1999). On the other hand, these compounds can be incorporated through live prey or by applying them directly to the rearing water as immersion baths during the early stages of fish development (Skjermo & Vadstein, 1999; Bergh *et al.*, 2001; Skjermo & Bergh, 2004). The compounds that contain βG act at the basal level of the development of the immunological system and are soluble in sea water, facilitating their absorption through the skin, gills, and mouth (Strand & Dalmo, 1997; Dalmo *et al.*, 2000; Raa, 2000). Nonetheless, although the protocols for administering the prophylactic compounds are effective transfer mechanisms to strengthen the immunological system of the fish, the application times for activating and maintaining antibody levels during early development seems to be species-specific (Bricknell & Dalmo, 2005).

Under larval rearing conditions, the individuals must combat infectious diseases through the non-specific immune system (Ellis, 2001). This is because the fish larvae have not developed a specific immunological defense system, that is, the capacity to offer immune protection against pathogenic agents (Esteban *et al.*, 1994; Bricknell & Dalmo, 2005). Thus, a preventive treatment (prophylactic) such as the application of βG MOS to the rearing water should increase the survival of the larvae, strengthening the non-specific immune system and, moreover, minimizing the environmental problems associated with other alternative protocol such as vaccines and/or drug therapy (Anderson, 1992; Raa, 2000). The constant application of such protocols (*e.g.* vaccines, drugs) over time produces residuals that persist in the environment and are transmitted to other organisms, possibly reaching toxic levels. Furthermore, such protocols favor antibiotic resistance in the pathogens and affect the microbial activity responsible for the breakdown of organic matter in the marine sediments (SERNAPESCA, 2005).

Different techniques have been used to determine whether applied organic compounds improve larval health, most noticeably biochemical analysis, assays with pathogenic bacteria, histological cuts and, recently, immunofluorescence markers. The biochemical analysis are based on blood and/or serum samples (Kumari & Sahoo, 2006), which are very difficult to obtain from small individual larvae (Bricknell & Dalmo, 2005). As well, the assays with pathogenic bacteria have shown that the intrinsic factors of the fish (fitness, developmental stage, age) produce greater variability between replicates than between treatments, obscuring the effect of the application of the prophylactic compound (Bricknell & Dalmo, 2005). Classic histological stains (nuclear, cellular), however, are similar in effectiveness and less costly than immunoflorescent markers for confirming the absorption of these organic compounds in the fish larvae (Cousin *et al.*, 1986; Esteban *et al.*, 1994; Strand & Dalmo, 1997; Luizi *et al.*, 1999; Ribeiro *et al.*, 1999; Dalmo *et al.,* 2000; Bergh *et al.*, 2001).

In Peru and Chile, the flounder *Paralichthys adspersus* Steindachner, 1867 is an attractive species for intensive culture (Silva, 2001; Ángeles & Mendo, 2005). Nevertheless, one of the obstacles for its

culture, as with most potentially farmed fish species, is its high mortality during the first larval stage. In fact, according to Silva (2000), the first 21 days after eclosion of the egg in *P. adspersus* rearing are critical; larval mortality nears 80% during this period. Such mortality rates are frequently reported for larval rearing of other *Paralichthys* species (Kuronuma & Fukusho, 1984; Bisbal & Bengtson, 1995; Silva, 2001; Silva & Castelló, 2005). Mortality in early and more advanced fish development stages is recurrently associated with diseases produced by infections from opportunistic bacteria and the stress caused by manipulation during culture and the culture system itself (Miranda & Rojas, 1993; Skjermo & Vadstein, 1999; Ellis, 2001). For example, in *P. adspersus*, vibriosis is manifested when the fish present stress-related immunedepression (Miranda & Rojas, 1996). Thus, we propose the hypothesis that the addition of βG MOS should decrease mortality in intensive culture of *P. adspersus* larvae. Accordingly, our objective is to evaluate survival and growth in the first larval stage of the farmed flounder given treatments with different concentrations of βG MOS in the rearing water. Furthermore, we documented whether the βG MOS compound strengthened the health of the larvae, attempting to detect cells that characterized the non-specific immune system in the intestine.

MATERIALS AND METHODS

In order to evaluate the effect of βG MOS in the rearing water had on *P. adspersus* larvae, we carried out two experiments in different time periods of the first feeding (live prey) stage. This stage begins when the larvae have absorbed the yolk sac (4-5 days post-hatch) and begin to feed on rotifers (*Brachionus plicatilis*); it ends between 20 and 25 days post-hatch, when the second stage of feeding (on *Artemia*) begins (Silva, 2001). In Experiment 1, 6-day post-eclosion larvae were used and, whereas in Experiment 2, 15-day post-hatch larvae were used. The β-glucan and mannan-oligosaccharide compound used in the experiments (called DP MOS βG) was provided by DESPRO S.A. company (Desarrollo de Proteínas de Chile S.A.). Used as a complement in the diet of farmed fish, this compound is an association of β-glucans (46%), mannan-oligosaccharides (53%), and cytoplasmatic content (1%). The βG MOS was obtained from yeast (*Saccharomyces cerevisiae*) through lysis and fractionation of the cell wall, with a particle size that fluctuated between 0.5 and 10 μ.

Obtaining the larvae

The larvae were obtained from two spontaneous spawnings of the *P. adspersus* reproductive stock at the Laboratorio de Peces, Universidad Católica del Norte, in September and October 2002. The fertilized eggs were collected with a 400-μm sieve placed in the exterior drain tube of the tanks. The eggs were filtered, washed, and left to rest for approximately 20 min in a 10-L recipient in order to select the viable eggs according to the protocol described by Silva & Castelló (2005). The viable eggs, previously quantified and disinfected, were deposited in 100-L tanks and incubated for 54 to 62 h in filtered, sterilized sea water with ultraviolet light (Silva & Castelló, 2005). The eclosed larvae were moved to a black, cylindrical-conical 500-L tank with filtered sea water (salinity 34 ± 0.5), aerated continuously, and kept at room temperature (16-17°C) until their extraction for the experiments.

Experimental design

In Experiment 1, larvae of 4.0 ± 0.3 mm standard length (six-day post-hatch) were used and, in Experiment 2, larvae of 5.4 ± 0.2 mm standard length (15-day post-hatch) were used. Both experiments were carried out during the first stage of feeding of the farmed larvae. In this stage, the larvae are fed with rotifers (*Brachionus plicatilis*; 5-10 rotifers·mL^{-1}) (Silva & Vélez, 2005). The rotifers, farmed in batch systems, were fed yeast (*Saccharomyces cerevisiae*) and enriched with a mixture of microalgae (*e.g.* *Isochrysis* sp., *Nannochloropsis* sp.). Each experiment lasted ten days and, in both cases, the larvae were exposed to treatments with concentrations of 5 mg·L^{-1}, 10 mg·L^{-1}, and 15 mg·L^{-1} βG MOS through immersion (Tytler & Blaxter, 1988); a control group was not treated with the compound. This concentration range was previously used in other experiments that evaluated the effect of βG-based prophylactic compounds on fish larval survival and growth (Dalmo *et al.*, 2000; Bergh *et al.*, 2001; Skjermo & Bergh, 2004).

The treatments, with three replicates, were distributed at random in 12 black, cylindrical, 30-L tanks at a density of 30 larvae·L^{-1}. The larval groups were treated with βG MOS dissolved in sea water at concentrations of 5 mg·L^{-1}, 10 mg·L^{-1}, and 15 mg·L^{-1}, during the first five days of each experiment. The βG MOS concentration for each treatment was maintained daily in experimental tank by absorbing the water with a siphon and replacing it with the

diluted compound in sea water. The sea water used for the βG MOS dilutions was filtered at 1 μm and sterilized with ultraviolet light (UV). On days 2, 4, 6, 8, and 10 of the experiments, 30 larvae were extracted from the experimental tanks with a siphon. The sampled larvae were excluded from the survival analyses. After each sampling, the volume of water was decreased proportionally in each tank in order to maintain a constant density (30 larvae·L^{-1}). In both experiments, all the utensils were duly washed and disinfected.

In order to determine the effect of the βG MOS on the larvae, the percentage of survival was evaluated along with the growth rate and the manifestation of monocytes or macrophage precursor cells in the larval intestines. The percentage of survival (S) was calculated for each treatment using the equation $S = (n_f/n_i) \cdot 100$ (Downing & Litvak, 1999), where n_i and n_f are the initial and final number of larvae. The growth specific rate (G_s) was calculated for each replicate of each treatment during the experimental period with the equation $G_s = ([\ln \omega_2 - \ln \omega_1]/T_2 - T_1) \cdot 100$, where ω_1 is the standard length (mm) at time T_1 (Downing & Litvak, 1999). The standard length (ω) was measured from the extreme anterior point of the upper mandible to the extreme posterior point of the notochord (Downing & Litvak, 1999) using a profile projector (Nikon V12). All the sampled larvae were preserved in formaline diluted in sea water at 10% in black plastic bottles with labels for later histological analyses (Muñetón-Gómez *et al.*, 2000; K. Lohrmann, pers. comm.).

The histological cuts of *P. adspersus* larvae were done at the intestinal level since the larvae had not yet developed a functional stomach. In this stage of development, the anterior intestine is responsible for the digestion processes and the posterior intestine for the absorption processes (Ribeiro *et al.*, 1999). The larvae collected for each treatment per sampling time were dehydrated in an increasing battery of ethanol for their later inclusion in paraffin (Muñetón-Gómez *et al.*, 2000). The cuts of the anterior and posterior section of the larval intestines were 5 μ, using a rotory microtome (Leitz 1512). The tissues were stained with Hematoxiline-eosine using the Entellan mounting medium and Hemacolor (Muñetón-Gómez *et al.*, 2000). All the cuts were checked under a light microscope (maximum magnification 1000X) using immersion oil to evaluate the presence of monocytic cells that characterize the non-specific immune system in the larval intestines. The monocytic cells in both intestinal sections were evaluated.

The survival (S) and growth (G_s) data for the larvae in the experimental treatments were compared using analysis of varianza (ANOVA), prior to their transformation to the arc-sine of the square root (Sokal & Rohlf, 1981). When the ANOVA showed significant differences, an *a posteriori* Tukey test was performed to detect differences between the treatment pairs. The effect of the application of βG MOS on the temporal increase in the standard length was evaluated with an analysis of covariance (ANCOVA). Before carrying out the ANOVA and ANCOVA, the normality of the data, the out-of-range data, and the homocedasticity of the variances were proven through the Barlett test, the Lilliefors test, and visual observation (Sokal & Rohlf, 1981) using the computer software SYSTAT 8.0®.

RESULTS

Larval survival

The effect of βG MOS on the survival of *P. adspersus* larvae was dependent on the concentration of dilution applied to the rearing water. No significant differences were found between the survival percentages of six-day post-hatch individuals that received 5 mg·L^{-1} βG MOS in the larval rearing water (Experiment 1) and the control group (0 mg·L^{-1} βG MOS; Fig. 1). However, survival decreased significantly ($F_{(3.8)}$ = 9.018, p < 0.05; Fig. 1) with the use of greater concentrations (10 and 15 mg·L^{-1}) of βG MOS with respect to the control group and the other treatment. For the 15-day post-hatch larvae (Experiment 2), the application of 5 mg·L^{-1} of βG MOS in the rearing water significantly improved ($F_{(3.8)}$ = 5.68, p < 0.05) the survival rates as compared with the control situation (0 mg·L^{-1} βG MOS) and the other treatments (Fig. 1). Concentrations of 10 and 15 mg·L^{-1} of βG MOS significantly decreased ($F_{(3.8)}$ = 5.68, p < 0.05) the survival of the larvae with respect to the control situation (Fig. 1). Larval survival was also affected by the age of the larvae when the compound was applied. So, although a similar tendency was observed in both experiments, the application of βG MOS to the six-day post-hatch larvae resulted in 13% more survival than when applied to 15-day post-hatch larvae (Fig. 1). In this context, the partial increments in the survival percentage of the 6-day post-hatch larvae as compared with the 15-day post-hatch larvae were 12% (5 mg·L^{-1}), 18% (10 mg·L^{-1}), and 10% (15 mg·L^{-1} βG MOS).

Larval growth

The standard length of both the larvae treated with βG MOS and those in the control groups (0 mg·L^{-1} βG MOS) of both experiments showed a significant increase over time (Experiment 1: $F_{(4.52)} = 43.806$, $p < 0.05$; Experiment 2: $F_{(4.52)} = 133.283$, $p < 0.05$). Nonetheless, the effect of the concentration of the βG MOS concentration depends on the age of the larvae (6-day *versus* 15-day post-hatch). In the 6-day post-hatch larvae (Experiment 1), the increment in standard length during the time of the experiment was significantly greater in the individuals treated with 5 mg·L^{-1} βG MOS (approximately 10% larger) than in the other treatments; concentrations of 15 mg·L^{-1} of the compound significantly affected the standard length of the individuals, resulting in smaller final sizes ($F_{(3.52)} = 3.655$, $p < 0.05$; Tukey test). In 15-day post-hatch larvae (10 days feeding rotifers; Experiment 2), the increment in the standard length showed a similar temporal tendency in all the treatments, including the control (Fig. 2).

The concentration of βG MOS applied to the rearing water affected the growth rates of the *P. adspersus* larvae. The growth rate of the 6-day post-hatch larvae (Experiment 1) treated with 5 mg·L^{-1} βG MOS was significantly higher than the rate observed for the control larvae ($F_{(3.8)} = 91.040$, $p < 0.05$) and the larvae treated with 10 and 15 mg·L^{-1} βG MOS (Fig. 3). The growth rate of the 15-day post-eclosion larvae (Experiment 2) treated with 5 mg·L^{-1} βG MOS was similar to the larvae maintained in the control condition ($F_{(3.8)} = 91.040$, $p > 0.05$) and significantly higher ($F_{(3.8)} = 6.231$, $p < 0.05$) than that observed for the larvae treated with 10 and 15 mg·L^{-1} βG MOS (Fig. 3). Although the larvae treated with 5 mg·L^{-1} βG MOS presented the greatest growth rates in both experiments, the compound was most effective for growth in the 6-day post-hatch larvae (Fig. 3).

Histological analysis

The histological analyses of the anterior and posterior intestines of the larvae stained with Hematoxiline-eosine and Hemacolor showed clearly differentiated monocytic cells, characterized by well-defined nuclei (Fig. 4). The temporal follow-up of the treatments through histological analyses showed a greater relative frequency of monocytes in the groups treated with βG MOS on the tenth day of the experiment than in the normal or control group. Likewise, a high variability was detected between the histological cuts of the larval intestines given the same treatment, making it impossible to statistically quantify and evaluate in which βG MOS treatment the monocytes were more frequent. The presence of monocytic cell as a macrophage precursor is an evidence that complements the results of the effect of the βG MOS on the survival and growth of *P. adspersus* the larvae in intense culture systems.
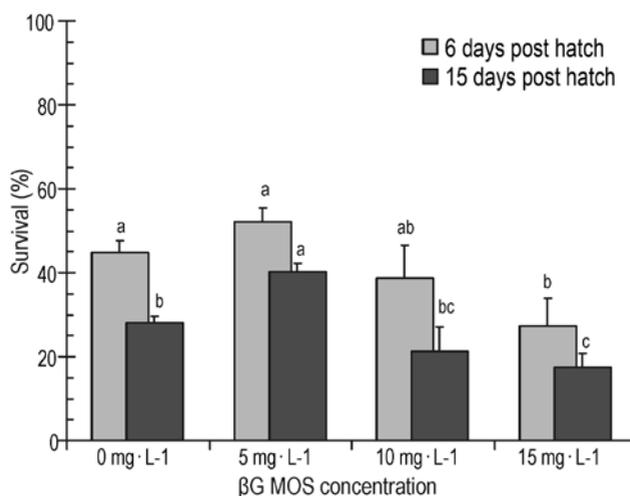

Figure 1. Survival (%) of *P. adspersus* larvae of six day post hatch (Experiment Nº1-clear bars) and fifteen days post hatch (Experiment Nº2-dark bars) treated with βG MOS at different concentrations. The 0 mg·L^{-1} βG MOS concentration corresponds to the control larval group. The letters indicate homogenous groups obtained from Tukey test ($p < 0.05$). Mean ± Standard deviation.
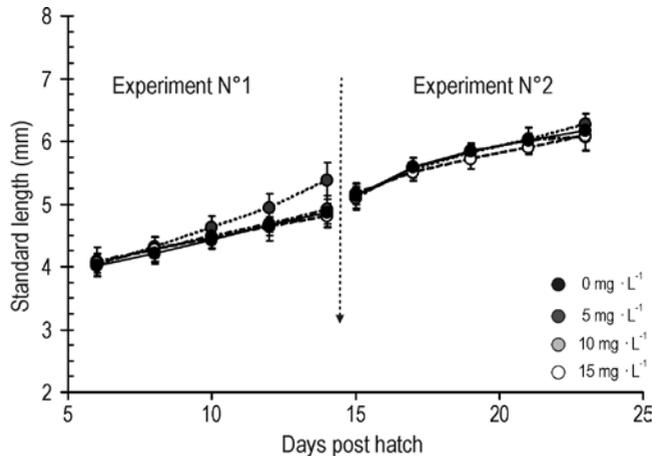
Figure 2. Standard length increment of *P. adspersus* larvae of six days post hatch (Experiment Nº1) and fifteen days post hatch (Experiment Nº2) treated with βG MOS at different concentrations. The 0 mg·L⁻¹ βG MOS concentration corresponds to the control larval group. Mean ± Standard deviation.
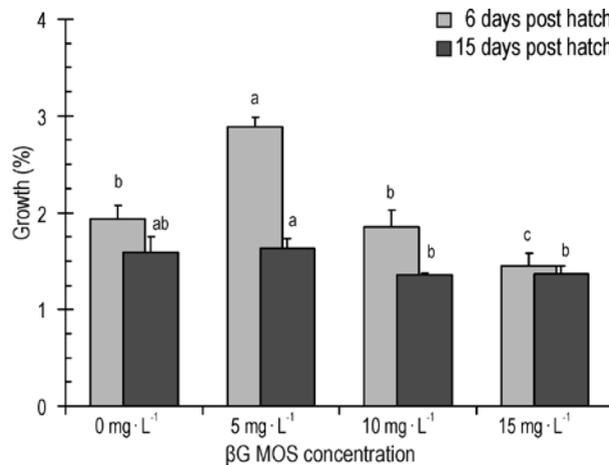

Figure 3. Growth (%) of *P. adspersus* larvae of six day post hatch (Experiment Nº1-clear bars) and fifteen days post hatch (Experiment Nº2-dark bars) treated with βG MOS at different concentrations. The 0 mg·L⁻¹ βG MOS concentration corresponds to the control larval group. The letters indicate homogenous groups obtained from Tukey test (p < 0.05). Mean ± Standard deviation.
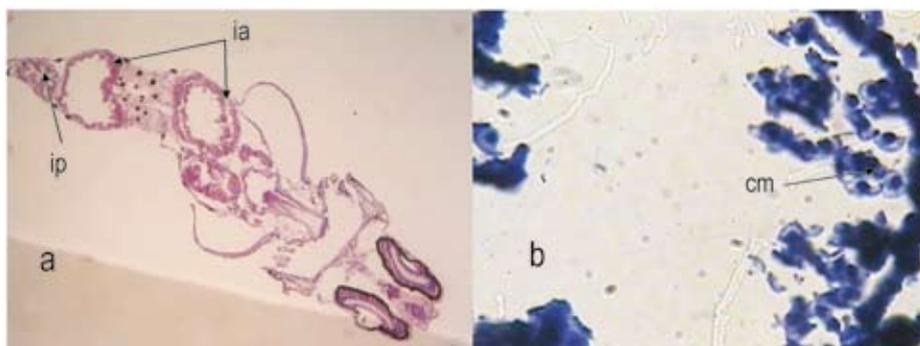

Figure 4. a) Histological slide of *P. adspersus* larvae of 23 days post hatch stain with Hematoxilin-Eosin (10x); there are observed anterior intestine (ia) and posterior intestine (ip). b) Histological slide of posterior intestine (1000x) stain with Hemacolor; are observed group of monocytic cells in intestinal tissue.

DISCUSSION

Among the administration protocols of β-glucan-based compounds were successful in improving the health of larvae reared in captivity, oral administration was noteworthy. This administration protocol is useful for rearing *P. adspersus* larvae when is given during the first feeding stage with live prey depends on the concentration of βG MOS applicated. Similar results, using the same protocol, have been obtained for *Hippoglossus hippoglossus* and *Scophthalmus maximus* larvae, as well as for other fish species raised in captivity (Dalmo *et al*., 2000; Bergh *et al*., 2001; Skjermo & Bergh, 2004). In our study, the application of 5 mg·L^{-1} βG MOS significantly increased survival and growth of *P. adspersus* larvae, whereas concentrations of 15 mg·L^{-1} βG MOS suppressed these population parameters. An excess of prophylactic compounds applied to *Sparus aurata* suppressed the non-specific immune response and had negative consequences for the survival and development of the individuals (Mulero *et al*., 1998). This explanation could respond to the results found in this study, as an excess of this type of prophylactic compound could interfere in the optimal or posterior development of the larval immune systems (Bricknell & Dalmo, 2005).

The age at which the βG MOS is applied also affects the farming of *P. adspersus* larvae. The application of βG MOS at the beginning of the first feeding (live prey) stage increases survival and growth as compared with the treatments given after ten days of feeding on live prey. This result challenges the expected results, that is that older *P. adspersus* larvae should have greater life expectancies (Silva, 2000, 2001). Some as yet unidentified compounds of maternal origins might provide innate protection against opportunistic bacteria. Eggs are rich in some non-specific defense compounds such as lectins and lysosomes (that probably come from the ovary) that differentiate macrophages early in the embryonic development (Ellis, 2001). Nevertheless, these compounds decrease with larval age (Browman *et al*., 2003). Thus, the application of βG MOS should complement and strengthen the immunity of the *P. adspersus* larvae that is transmitted by the mother during the early stages of development, mainly after the period in which the larvae have a yolk sac. However, this innate defense mechanism of the eggs and larvae seems to depend on the mother's state of health (Ellis, 2001; Browman *et al*., 2003).

The temporal increase in the standard length of the *P. adspersus* larvae in the experimental groups presented a similar tendency as that observed in prior studies (Silva & Flores, 1989; Silva 2000, 2001). However, when compared with these studies, the larvae treated with βG MOS had higher growth rates, particularly when treated with 5 mg·L^{-1} βG MOS. Nevertheless, this result cannot be completely attributed to the effect of the βG MOS application since these differences could be related to extrinsic factors such as temperature, light, and other environmental factors (Silva & Flores, 1989; Downing & Litvak, 1999; Silva 2000, 2001; Skjermo & Bergh, 2004) that could have a synergic effect with the concentration of βG MOS applied to the rearing water.

In our experiment, the tissues of the intestinal epithelium revealed cells with circular, well-defined, mid-sized nuclei; these characteristics, according to the literature, define the monocytes (Esteban *et al*., 1994). The monocytes that are macrophage precursors are key cells in the non-specific defense system of the fish (Dalmo *et al., 1997). Such cells may have one or several nuclei and are able to endocyte and to phagocyte foreign bodies that enter to the organism (1-10 μm in size) due to the presence of glycoprotein receptors in their plasmatic membrane that modify their means for secretions and marker substances (Esteban *et al*., 1994; Dalmo *et al*., 1997). When the macrophages are stimulated, they produce an intermediate reaction of oxygen, nitric oxides, enzymes, lysozymes, cytokines, and other marker molecules (Bricknell & Dalmo, 2005). The high frequency of monocytes in the intestinal tissues and the greater growth and survival of the individuals treated with βG MOS suggest a positive effect on the health of the *P. adspersus* larvae.

For *P. adspersus* individuals living in captivity, vibriosis is an opportunistic pathological process associated with situations of stress such as changes in the diet and/or increased temperature; it can be treated with wide-spectrum antimicrobial agents (Miranda & Rojas, 1993, 1996). Further experiments with this type of opportunistic pathogenic strain and *P. adspersus* larvae previously treated with βG MOS baths could complement our results, also considering the intrinsic variability of the responses of individuals from a single cohort.

This study is the first to indicate that the application of βG MOS (5 mg·L^{-1} βG MOS) diluted in the rearing water for five days significantly increases the percentage of survival as compared with the control

situation, or as compared to other previous studies carried out with *P. adspersus* larvae (Silva & Flores, 1989; Silva, 2000, 2001). The degree of protection obtained by administering this compound is probably related to the stimulation of the non-specific components of the immune system that have antibacterial activity, as shown by the presence of macrophage precursor cells. Other studies carried out on flat fish (*e.g.*, *Hippoglossus hippoglossus*, *Scophthalmus maximus*, *Paralichthys olivaceus*) have suggested that the administration of compounds containing β-glucans improves resistance against opportunistic bacteria (Robertsen *et al.*, 1994; Dehasque *et al.*, 1997; Strand & Dalmo, 1997; Sakai, 1999; Dalmo *et al.*, 1997, 2000; Bergh *et al.*, 2001; Bricknell & Dalmo, 2005). It is in this context that βG MOS, or other products acting as prophylactic compounds, should be considered as a tools for prevention when planning and developing management schemes for the intensive production of *P. adspersus* juveniles, particularly during the first feeding (live prey) stage of the larvae.

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