Non-Specific Immune Parameters in Some New Candidate Species for Mediterranean Aquaculture: Results of First Studies

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Abstract: The aim of this paper was to describe the distribution of non-specific immune parameters in two fish species considered as new candidates for aquaculture diversification, greater amberjack and blackspot seabream. In particular, haemolysins, haemagglutinins and lysozyme were investigated to monitor their changes induced by two different stressors: 1) an experimental challenge of greater amberjack with the bacterial pathogen *Listonella* (*Vibrio) anguillarum*, which simulated an acute stress condition, and 2) a 14-day starvation period in blackspot seabream, which simulated a chronic stress condition. For each experiment, unstressed fish were kept and sacrificed at the same time intervals to be used as controls. In greater amberjack an increase in lysozyme was detected in challenged fish compared to control ones, while haemolytic activity and haemagglutinating titres showed a depressive effect following to the challenge. In blackspot seabream, starvation resulted in a reduction of lysozyme content in the mucus and plasma, as well as of haemolytic activity. The variations measured in non-specific immune parameters suggest their use as indirect markers of stress or altered health conditions.

Keywords: Non-specific immune parameters, *Seriola dumerilii*, *Pagellus bogaraveo*, acute and chronic stress, lysozyme, haemolysis, haemagglutination.

INTRODUCTION

Fish, likely to other Vertebrates, are provided with an efficient immune system which protect them from substances or agents recognised to be "foreign" to the organism [1-5]. Immune organs of fish are homologues to those of the mammalian immune system.

The innate immune defence system of fish, which is constitutive but also inducible by external molecules, is involved in the mechanisms of non-specific defence of organisms, namely it has the main characteristic of being independent upon previous recognition of the surface structure of the foreign molecules [4]. Among its components are the reactive protein C, interferon, the complement system, lysozyme, lysins and agglutinins [1]. In particular, three parameters such as lysozyme, haemolysins and haemagglutinins, are the most studied innate responses in fish, since they contribute to the natural defence mechanisms against foreign agents and pathogenic bacteria [1, 6].

Lysozyme or muramidase is a glycosidic enzyme produced by neutrophils and macrophages [7, 8]; it is capable of splitting peptidoglycan in bacterial cell walls, causing the lysis especially of Gram-positive bacteria, but also of Gram negative species [9]; therefore it possess antibacterial properties [10]. It has been widely detected in the sera, mucus and in some tissues of marine and freshwater fish [1, 6, 11-16]. The activity in the mucus is connected to the defence function of this enzyme [14] and to the protective barrier formed by mucus [17]. Increased lytic values have been recorded after infections, injections of foreign material and low stressful conditions [18-20]. Lysozyme act as an acute-phase protein, being released not only in response to bacterial antigens, but also to other alarm situations such as after stress [21, 22]. Moreover, a drastic reduction in lysozyme concentration in blood has been observed after prolonged environmental stress [15].

Besides lysozyme, fish serum possess haemolytic activity which is active against a variety of antigens, including heterologous erythrocytes [1]. Two kinds of haemolytic activities can be distinguished, the one specific complement-mediated haemolytic activity, which is antibody-dependent (CH50) and the other one non-specific, natural, antibody-independent activity (SH50)[23]. The natural haemolytic activity is depressed in stress conditions [1, 6] and therefore this parameter may provide information on the physiological conditions of fish.

Bacterial agglutinins or haemagglutinins which react against the erythrocytes of homologous (iso-agglutinins) and heterologous (hetero-agglutinins) species have been reported in many Teleosts, both marine and freshwater, where natural agglutinins play a role in their non-specific defence [1]. The detection of bacterial agglutinins can be used as a suitable indicator of a previous infection or a contact with pathogens [24].

The study of the immune defence mechanisms is of particular significance for evaluating fish welfare, since a

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direct relationship between the functioning of the immune system and the ability to counteract disease outbreaks has been established [25]. Immunodepression consequent to stress conditions may result in increased susceptibility of animals to develop pathologies [3, 26, 27].

Studies performed in recent years at the IAMC- Messina have been devoted at evaluating the immunological response of reared fish to normal, undisturbed or altered conditions acting as acute or chronic stressors [28-30]. This research has aimed at monitoring the evolution of selected parameters (haematological, biochemical and those related to the natural immune response) during different altered conditions in order to identify a battery of those that could be proposed as the most suitable indicators for fish welfare assessment. In particular, the panel of assayed parameters has included measures of serum haemolytic and haemagglutinating activities and the lysozyme content of plasma, mucus and kidney. Until now, in fact, no single indicator of metabolic alterations has been recognised [31].

The aim of the present study was to describe the evolution of some non-specific immune parameters in two different fish species, exposed to different stressors, and in particular: 1) greater yellowtail (*Seriola dumerilii*) exposed to an experimental challenge with a bacterial pathogen [*Listonella* (formerly *Vibrio*) *anguillarum*], which simulated an acute stress condition, and 2) blackspot seabream (*Pagellus bogaraveo*) exposed to a 14-day starvation period, which simulated a chronic stress condition. The patterns of the examined parameters were also compared with those displayed by another fish species commonly reared in Mediterranean aquaculture, such as seabass (*Dicentrarchus labrax)*, kept under similar stress conditions.

MATERIALS AND METHODOLOGY

Biology of the Study Species

The Mediterranean greater amberjack (*Seriola dumerilii*, Risso 1810) is a Carangid species coming from the Atlantic Ocean, which is spread in numerous stocks during the early life stage and during the reproductive period. This species is distributed in temperate and sub-tropical areas of pelagic environments. It is a gonocoric species which reproduces between the end of spring and the beginning of summer; it lives between 20 and 70 m depth, although it has been found at 360 m depth in the Atlantic off Morocco. It can reach a large size: over 50 Kg of weight and 2 m of length [32]. The interest towards this species is related to the ease of its rearing and to its high commercial value in the Mediterranean countries. Research aimed at improving knowledge towards many different physiological aspects of greater amberjack (reproduction, digestion, spreading and breeding area, metabolism) were carried out at the IAMC Institute-Messina since 1995 within the II Three-Years Plan of Research funded by the Ministry for Agriculture, Food and Forestry Resources (MRAAF) [33].

The blackspot seabream *Pagellus bogaraveo* (Brünnich, 1768) is a fish species belonging to Sparidae; it is largely consumed and appreciated for commercial fisheries in the Mediterranean Sea [34, 35]. This justifies the interest towards this species, considered as a potential novel candidate to diversify Mediterranean aquaculture production. Blackspot seabream has been investigated for the physiological and biochemical features related to its digestive system [36], as well as to its reproductive biology [37], but there is no information about its response to stress. Some studies have been undertaken in recent years only [38].

The experiment reported here is a part of a wide research project focused on the blackspot seabream as a novel species.

Experimental Design and Samples Collection

The two experiments were carried out using the facilities of the Institute for Coastal Marine Environment of Messina (Italy).

For the experimental challenge, 300 juveniles of greater amberjack were caught during a survey performed in the Southern Tyrrhenian Sea in July 2003. They were acclimated for one month in a fibreglass tank (40 L volume), kept under natural photoperiod and fed daily with *Trachurus trachurus* and *Loligo vulgaris* administered "ad libitum".

After acclimation, fish were randomly distributed into five groups for the challenge with *Listonella anguillarum*. Each fish group consisted of 25 specimens (average weight = 100 g) kept in a plastic tank having a 100-L volume: Group 1 (Control) and Groups 2 to 5 (Challenged). The replicates of the challenged groups were set up in order to avoid variability due to rearing in a single tank.

During the experiment, water temperature was maintained stable between 20 and 22°C, while salinity (~38) and pH (~8.15) were monitored.

The strain used for the experimental challenge of greater amberjack was *Listonella anguillarum* ATCC 43305 HIB. It was grown in Brain Heart Infusion Broth supplemented with 1.5% NaCl and incubated at 24°C for 24-48 h. Bacteria were collected by centrifugation and suspended in sterile physiological solution to obtain a final concentration of 1.5 x 10^6 cells ml^{-1} (which was previously determined as the challenge dose through a LD_{50} experiment). On day 0, one ml of the challenge dose was injected by intraperitoneal inoculation in each fish of challenged groups, while control fish were injected with an equal volume of sterile saline solution.

At prefixed times, namely 1, 5, 11, 15, 20 days after antigen injection, five fish from both the experimental groups were sacrificed.

For the experiment of starvation, blackspot seabream were caught from the Straits of Messina and kept in tanks having a volume of 1.9 m^3. Each tank was supplied with a continuous aerated flow of seawater, with three daily changes. Fish were maintained in these conditions for 2 weeks to allow acclimation. The water temperature was 24-25°C, salinity ~38, pH 8.2 and dissolved oxygen 7-8 mg L^{-1}. Photoperiod was kept natural. During this adaptation period fish were fed a commercial pellet for carnivorous fish administered until satiation.

After acclimation, fish were divided in two different groups, one maintained at starvation and another one fed daily "ad libitum" a commercial feed, taken as a control. The

experiment lasted 20 days, during which five specimens from both the control and fasted groups were sampled and sacrificed at time zero (before starvation) and on Day 20 after starvation, for the measurement of selected physiological parameters.

In both the study cases (experimental challenge and starvation), before their killing, fish were anesthetised in MS-222 (tricaine methanesulfonate, Sigma-Aldrich), which was added at a final concentration of 0.1 g L^{-1}. The spiking method (i.e. destruction of the brain by a sharp spike) was used to kill the fish.

To avoid a sampling-induced stress response the time between dipnet introduction and withdrawal lasted less than 3-4 minutes for group, as the withdrawal was carried out simultaneously by two operators.

Sample Treatment and Determination of Non-Specific Immune Parameters

Samples of skin mucus, anterior kidney and blood were collected and differently treated according to each parameter to be determined. From each fish, skin mucus and kidney samples were separately removed and stored at -80°C for lysozyme determinations.

Blood samples were drawn separately from the caudal vein of each individual and put into a tube containing heparin (final concentration 14 International Units mL^{-1}). Small volumes of heparinised blood were centrifuged and the obtained plasma was stored as above described for further lysozyme assay.

For the determination of haemolysins and haemagglutinins, serum was extracted from blood samples untreated with heparin, which were left to clot at 4°C and centrifuged at 1500 g for 10 minutes; the obtained serum was stored at -80°C until analysis.

The lysozyme content was determined on plasma, mucus and kidney. The assay was performed using the radial diffusion method in agarose plates containing 1% agar added with 0.05% lyophilised *Micrococcus lysodeikticus* (Sigma-Aldrich, Italy) as the substrate, dissolved in pH 5.75 phosphate buffer.

The diameter of lysis produced after incubation at 32°C for 24 h was measured and converted into Units of lysozyme per mL of sample (U mL^{-1}) through calibration with known amounts of egg-white lysozyme (Sigma-Aldrich) used as the standard [39, 40].

Spontaneous haemolytic activity was determined after incubation of serial dilutions of serum samples with a 2.5% suspension of sheep red blood cells in phosphate buffer (PBS); incubation was carried out at 37°C for 1 h and the absorbance of the supernatants was measured at 540 nm.

The haemagglutinating activity of the serum was determined in 96-well microtiter U plates (NUNC INC., Denmark) according to the two-fold serial dilution procedure.

After addition with a 2% (v/v) sheep erythrocyte suspension in PBS, serial dilutions of the serum were incubated at 35°C for 1 h and at +4°C overnight [24]. Results are reported in terms of haemagglutinating titre, defined as the log 2 value of the final serum dilution showing visible agglutination.

Statistical Analysis

All results are expressed as means ± SE. Statistical analysis was performed by ANOVA to evaluate differences between the experimental and control groups. Differences were considered statistically significant when $P \leq 0.05$.

RESULTS

The effects produced on the analysed immunological parameters by the two stress conditions are shown in Figs. (**1-9**).

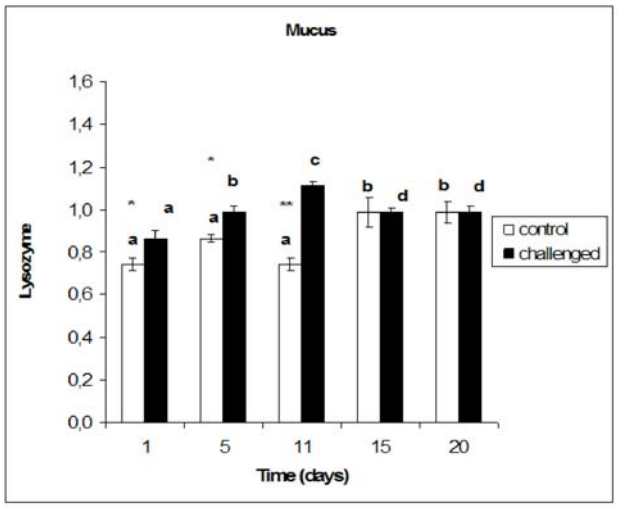

Fig. (1). Time course of lysozyme content (in Units mL^{-1}) in the mucus of control and challenged greater amberjack. Values are means ± SEM (n = 5). Asterisks indicate statistical differences from the control (*P<0.05, ** P<0.01). Different letters indicate significant variations within each group.

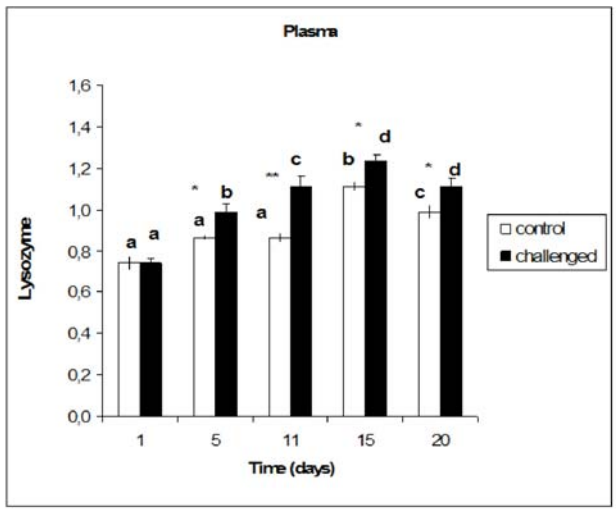

Fig. (2). Time course of lysozyme content (in Units mL^{-1}) in the plasma of control and challenged greater amberjack. Values are means ± SEM (n = 5). Asterisks indicate statistical differences from the control (*P<0.05, **P<0.01). Different letters indicate significant variations within each group.

In greater amberjack, minimum values of lysozyme activity, ranging from 0.74 to 0.99 U mL^{-1} in the control specimens and from 0.87 to 1.11 U mL^{-1} in the challenged ones, were measured in the mucus (Fig. **1**). In the plasma, values ranged from 0.74 to 1.11 U mL^{-1} and from 0.74 to 1.24 U mL^{-1} in control and challenged fish, respectively (Fig. **2**).

Lysozyme prevailed in the kidney, where values ranged from 0.99 to 1.36 U mL^{-1} in the challenged fish and from 0.99 to 1.24 U mL^{-1} in control ones (Fig. **3**). The challenge with *L. anguillarum* resulted in an increase in lysozyme content, which showed a peak 11 days after infection in the skin mucus and kidney, and after 15 days in the plasma.

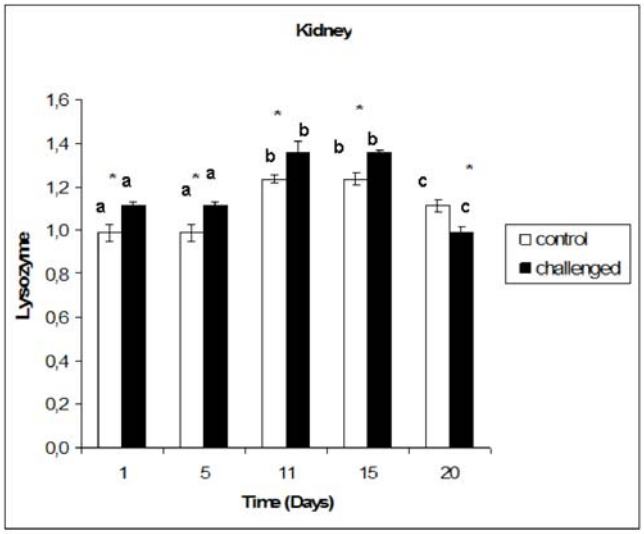

Fig. (3). Time course of lysozyme content (in Units mL^{-1}) in the kidney of control and challenged greater amberjack. Values are means ± SEM (n = 5). Asterisks indicate statistical differences from the control (*P<0.05). Different letters indicate significant variations within each group.

In all the organs, a progressive decrease in enzyme levels occurred later (on Day 20), when values similar to those recorded on Day 1 were measured. In correspondence to the minimum lysozyme content in serum and skin mucus a peak of mortality occurred (on Day 1) (Data not shown in figure), that decreased further.

ANOVA revealed the occurrence of the most significant differences between control and challenged groups on Day 11 both in the mucus and plasma (F= 528.9, 105.4, P<0.01, respectively).

The haemolytic activity (Fig. **4**) showed a reduction in challenged groups with respect to the control one (range values SH50: controls, 29.06-21.16; challenged: 21.16-6.48), which was already detectable on Day 1 (F= 273.5, P<0.01).

A similar reduction was recorded for haemagglutinating titres (range values log 2: controls, 3-4; challenged: 2-3), especially on Day 15 (F = 153.8, P<0.01) (Fig. **5**).

ANOVA confirmed that both for haemagglutinins and haemolysins, averaged values measured in challenged specimens differed significantly from those of control ones (F =10.7 and F= 20.3 respectively, P<0.05).

With respect to the temporal distribution of non-specific immune parameters, except for the haemolytic activity, which remained unchanged after Day 5, significant differences were observed both for lysozyme activity values and haemagglutinating titres.

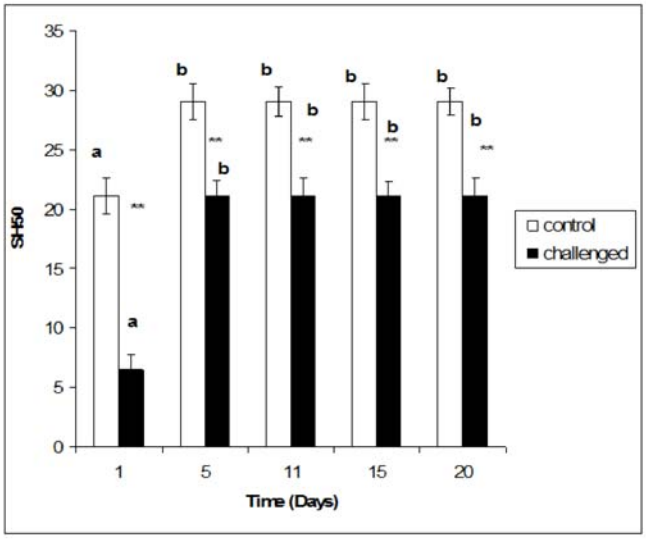

Fig. (4). Time course of haemolytic activity (SH50) in the serum of control and challenged greater amberjack. Values are means ± SEM (n = 5). Asterisks indicate statistical differences from the control (** P<0.01). Different letters indicate significant variations within each group.

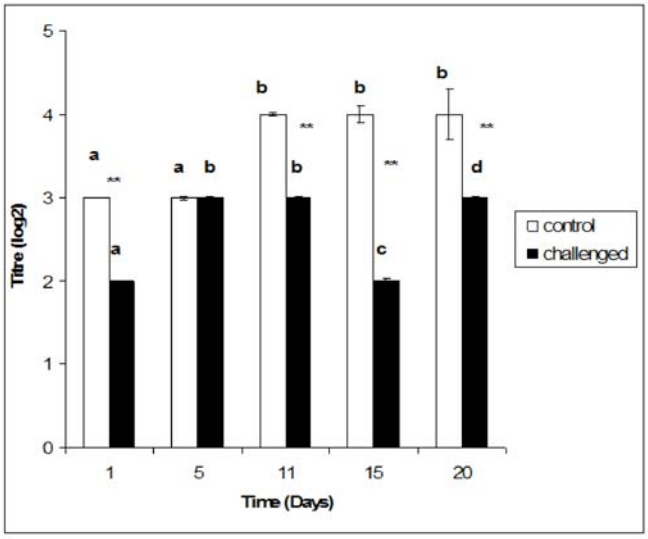

Fig. (5). Time course of haemagglutinating titres (reported as Log 2 values of the final serum dilution showing visible agglutination) in the serum of control and challenged greater amberjack. Values are means ± SEM (n = 5). Asterisks indicate statistical differences from the control (**P<0.01). Different letters indicate significant variations within each group.

Concerning the experiment of starvation performed in blackspot seabream, the lysozyme content of the plasma (Fig. **6**) ranged from 1.11 to 1.15 U mL^{-1} in fasted fish and from 1.19 to 1.24 U mL^{-1} in control ones. From averaged values, a significant difference between fasted and control fish was detected (F=27.0, P<0.01).

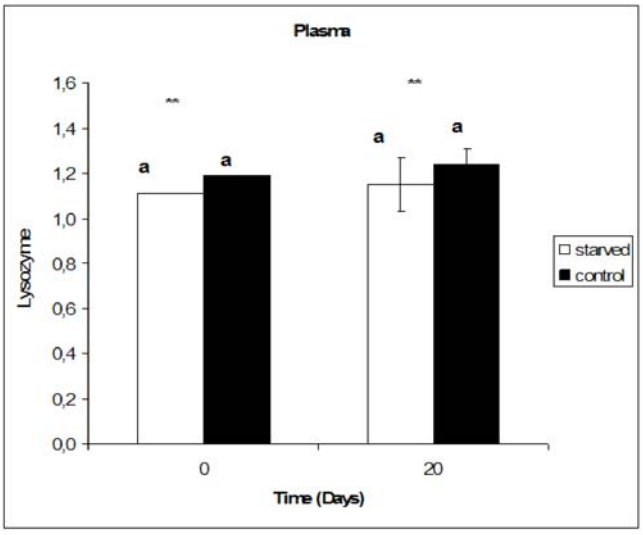

Fig. (6). Time course of lysozyme content (in Units mL^{-1}) in the plasma of control and starved blackspot seabream. Values are means ± SEM (n = 5). Asterisks indicate statistical differences from the control (** P<0.01). Different letters indicate significant variations within each group.

In the mucus (Fig. **7**), lysozyme levels remained unchanged over the experiment and were 1.03 and 1.19 U mL^{-1} in fasted and fed fish, respectively. Averaged values detected in fasted fish were significantly different from those measured in control ones (F = 7.836, P<0.05).

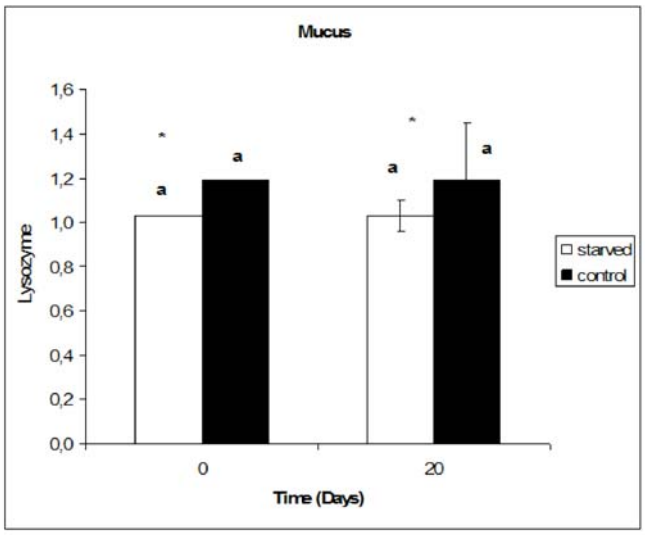

Fig. (7). Time course of lysozyme content (in Units mL^{-1}) in the mucus of control and starved blackspot seabream. Values are means ± SEM (n = 5). Asterisks indicate statistical differences from the control (*P<0.05). Different letters indicate significant variations within each group.

Spontaneous haemolytic activity values varied between 0.84 and 64.06 SH50 units in the fasted group, while in fed fish higher values were measured during the experiment (range: 1.71-79.62 SH50 Units) (Fig. **8**).

ANOVA of the haemolytic activity values revealed that at the end of the experiment, SH 50 values were significantly different from those recorded at the beginning for both the groups (F= 70.88 and 80.83, P<0.01 in starved and control fish, respectively). Haemagglutinating titres in blackspot seabream did not change between fasted and fed fish, as log 2 values ranged from 2 to 3 in both the groups (Fig. **9**).

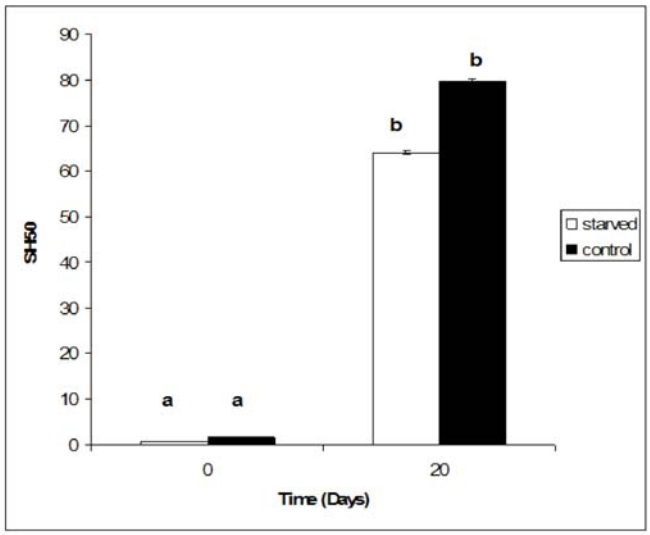

Fig. (8). Time course of haemolytic activity (SH 50) in the serum of control and starved blackspot seabream. Values are means ± SEM (n = 5). Different letters indicate significant variations within each group.

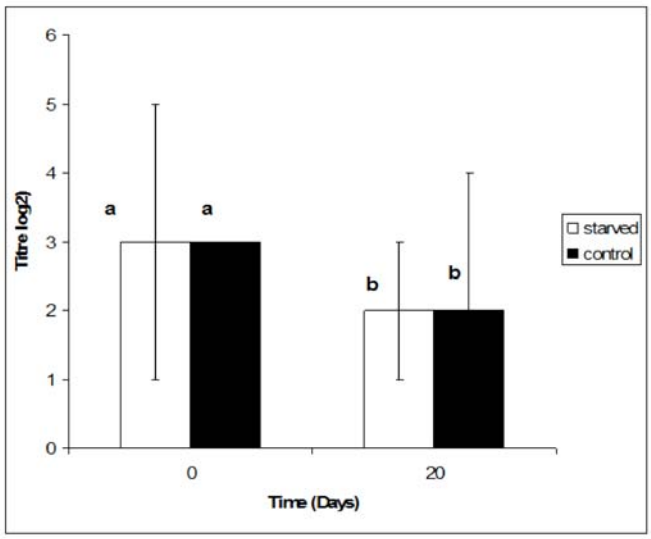

Fig. (9). Time course of haemagglutinating titres in the serum of control and starved blackspot seabream. Values are means ± SEM (n = 5). Different letters indicate significant variations within each group.

DISCUSSION

This study represents a first contribution to knowledge of the main parameters (lysozyme content, haemolytic and haemagglutinating activities) related to the non-specific immune response in two fish species, such as greater amberjack and blackspot seabream. Both of them have been indicated as promising, new candidate species for aquaculture diversification. Despite the growing attention addressed to the culture of these two fish species, to our knowledge, no data are currently available on their humoral immune sys-

tem; particularly, studies concerning the effects of stressors on this important aspect of fish physiology are still lacking.

Understanding of physiological response to stress is needed to improve fish welfare. In fish, as well as in other Vertebrates, stressors are known to cause a set of physiological reactions. Generally, increased levels of cortisol and hyperglycemia are the first and most known responses to stress conditions [27, 41], but also non-specific immune parameters have been reported to be affected by stress [31, 42-45]. In fact, among the tertiary responses to stress, severe behavioural, fish growth and reproduction modifications are included, together with an increased susceptibility to diseases [46-48]. Physiological responses of fish to acute and chronic stressors and the relationship between stress and fish welfare have been the subject of several studies [21, 28-30, 49, 50] performed since the last decades. The most significant result highlighted by this research is that the magnitude and duration of biochemical stress response varies largely between species. Furthermore, in the same species, the strength of the stressor and the health status of each individual may differently modulate the response of non-specific immune system to stress, resulting in its activation or suppression [15, 21, 51].

Acute stressors can have different effect in fish; severe stress can have lethal consequences, but they can also lead to an enhancement of fish immune response (increased concentrations of specific plasma protein, such as lysozyme or complement) and to a better protection against any possible damage [30]. Conversely, chronic stressors are usually associated with reduced growth, changes in reproductive function and behaviour and increased susceptibility to disease [52].

Elucidating the response of the immune system to stress is especially important when the suitability of one fish species to intensive rearing is explored. In this case, preliminary investigations are needed to obtain information on the susceptibility of fish to the eventual outbreak of diseases.

In this study, we selected two different kinds of stressors to simulate an acute stress, such as that caused by the intraperitoneal injection, and a chronic stress, such as that produced by a short-term starvation period. Moreover, the particular stressor applied to greater amberjack (experimental challenge with *L. anguillarum*) was chosen to investigate the response of this fish species against vibriosis. In fact, the outbreak of diseases caused by *L. anguillarum* is recognised to be one of the major threats in marine fish farming in the Mediterranean area (see [53] for a review). Moreover, in fish it is assumed that the response to immune challenge is mainly based on the innate immune response [4]. Preliminary results obtained during the challenge of greater amberjack were reported by Caruso *et al.* [54] and are here described in detail. In this species, an increase in lysozyme was detected in challenged fish compared to control ones, while haemolytic activity and haemagglutinating titres showed a depressive effect following to the challenge.

In greater amberjack, the response of the parameters related to non-specific immune defence against *L. anguillarum* infection followed a different time scale, being earlier for haemolysins and haemagglutinins and delayed for plasma lysozyme. A similar increase in plasma lysozyme level over time was observed in *Silurus glanis* experimentally infected with *Edwardsiella tarda* [55], although the peak in the non-specific immune response was recorded earlier than in our study (i.e. 3 days after challenge). The occurrence of variations not only in challenged groups but also in control one, suggested that handling due to the experimental infection could affect the non-specific immune response, particularly for lysozyme. Conversely, variations in the haemagglutinating titres and haemolytic values recorded in challenged fish, seemed more strictly dependent on the applied stressor. Both these considerations supported our hypothesis that haemolytic and haemagglutinating activities were the parameters most suitable, among those assayed, to monitor changes following experimental infection. On the other hand, lysozyme levels could exhibit variability in their behaviour in response to different stressors. Mock and Peters [15] found that they were significantly affected in rainbow trout exposed to different stressors such as handling, transport and poor water quality. Moderate or heavy stress result in an increase in lysozyme levels in the blood in rainbow trout [15, 56-58]. Conversely, a reduction of lysozyme content was found in rainbow trout after prolonged stressors such as that following to handling [15]; in this case, changes detected in lysozyme activity were assumed to reflect the modulation of the defence system played by the applied stressors.

Comparing the immunological response of greater amberjack to that of seabass specimens exposed to experimental challenge with the same bacterium, a previous study (Caruso G., unpublished) showed that in seabass, similarly to greater amberjack, the highest lysozyme levels were measured in the kidney while the lowest ones were detected in the serum. Independently of the examined organ, lysozyme content was higher in the challenged fish than in the control ones. The peak of lytic activity occurred 11 days after the challenge, indicating the response of seabass to *L. anguillarum* infection.

Haemolytic activity values decreased in challenged seabass compared to control fish (min-max: 21.06-41.00 *versus* 41.00-62.30 SH50 units, respectively). Challenge caused a depressive effect on the haemagglutinating titres similar to those observed in haemolytic activity; haemagglutinins decreased in infected fish (range log2: 3-6) with respect to control ones (range log2: 6-9). Both haemolytic and haemagglutinating activities decreased significantly 15 days after challenge; the decreasing trend continued to be evident also in the successive sampling.

Therefore, in challenged seabass and greater amberjack, a similar behaviour was found in the lysozyme levels of the kidney and mucus, with a peak 11 days after infection. Conversely, in greater amberjack haemolysins and haemagglutinins concentrations were lower than those measured in seabass, and although being less intense, the immune response was earlier, occurring already 5 days after challenge. These characteristics led us to suppose that in greater amberjack the humoral system was involved in the defence against *L. anguillarum* at a lesser extent than in seabass.

Following another acute stress condition, such as that caused by intraperitoneal vaccination, Maricchiolo *et al.* [30] found in seabass significant changes in cortisol and glucose,

whereas no significant differences between stressed and control fish were observed for lysozyme content and haemagglutinating activity throughout the experiment.

A reduction of haemolytic activity was observed by Sakai [6] in the serum of salmonids infected with *Aeromonas salmonicida*; in contrast, a significant increase of the spontaneous haemolytic activity and lysozyme was found by Roed *et al.* [58] in specimens of *Salmo salar* subjected to experimental challenge with *Vibrio salmonicida* and *Aeromonas salmonicida*. In *Salmo gairdneri* immunised against vibriosis through intraperitoneal injection of *L. anguillarum* antiserum, agglutinins were found in the serum 4 days after infection [24].

The second typology of stress investigated in this study concerned starvation, which may occur during life in natural environments, in relation to temperature changes or migration; although it is not frequent in well-managed aquaculture conditions, starvation has been adopted by farmers for the cultured fish to avoid risks of overproduction [59]. The study of physiological responses to starvation is interesting in order to set up proper management protocols [29].

Starvation is reported to negatively affect growth and development [60, 61]; also biochemical [62, 63] and haematological parameters [64, 65] are significantly affected. Most research focuses on metabolic changes [66-68], as well as on changes in the cardio-respiratory system [63], and in body composition and energy consumption [69-71] in response to food deprivation. In contrast, limited knowledge of the effects of starvation on the immune response is available [72-74].

In the specimens of blackspot seabream examined in this study, starvation resulted in a reduction of lysozyme content in the mucus and plasma, as well as of haemolytic activity. A similar decrease in the serum haemolytic activity after starvation was observed in salmonids [6].

Comparing the effects induced by starvation in seabream with seabass [75], in sea bass, a lower lysozyme concentration was measured in the plasma of the fasted specimens compared to the control ones. The opposite result was recorded in the kidney, as fasted fish displayed higher lysozyme values than that of fed fish, where lytic activity increased 11 days after starvation. Spontaneous haemolytic activity values ranged from 19.12 to 96.87 SH50 units in the fasted group, while in fed fish lower and almost constant values were measured during the experiment (range: 22.51-25.30 SH50 Units). Haemagglutinating titres in fasted seabass (range: 4-8) were slightly lower than those measured in fed fish (range: 8-32); therefore, similarly to amberjack, starvation was supposed do not affect significantly haemagglutinating activity.

In seabass reared in off-shore cages, lysozyme increased significantly, while a reduction in haemolytic and haemagglutinating titres was observed [28].

CONCLUSIONS

Those reported in this study are only preliminary results obtained in some novel species for aquaculture. The response of immunological parameters is different for each examined parameter and for the kind of applied stressor. This reinforces the need to monitor a panel of parameters to better assess the welfare status of the organisms under rearing. The non-specific immune response parameters proved to be sensitive enough to detect physiological alterations, so their use as early warning signal of metabolic changes can be proposed. Future studies, including also biochemical parameters such as plasmatic glucose and cortisol levels, are further needed to improve the comprehension of the physiological response of fish to rearing conditions.

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