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Haematological, biochemical and immunological parameters as stress indicators in *Dicentrarchus labrax* and *Sparus aurata* farmed in off-shore cages

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Abstract. In 2000–2001, an investigation was performed in two Sicilian mariculture sites (Pachino and Castellammare) to monitor physiological and biochemical parameters in sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) specimens farmed in off-shore sea cages. Plasma cortisol and glucose levels, tissue lactate concentration, haematocrit and haemoglobin content, lysozyme, haemolytic and haemagglutinating activities were determined. During the experiment, an increase in tissue lactate and plasma cortisol levels and a reduction in haemolytic and haemagglutinating titers, were recorded. Changes occurring in these haematological, immunological and biochemical values suggest that these parameters can be useful indicators in assessing the condition of chronic stress induced by mariculture operations.

Key words: Stress indicators, *Dicentrarchus labrax*, *Sparus aurata*, Mariculture

Introduction

Exposure of farmed fish to acute or chronic stress, due to crowding or handling, cannot be excluded in common rearing conditions. Studies on this issue have recognised that stress has significant implications for fish reproduction and growth (Pankhurst and Van der Kraak 1997); it is known to induce changes in physiological parameters, selected as indicators, such as haemoglobin content, plasma cortisol, glucose and lactate (Hady Kacem et al. 1986; Wells and Pankhurst 1999). Stressful conditions negatively affect both specific and nonspecific immunity, making fish more susceptible to disease (Pickering and Pottinger 1989). Therefore, haemolytic and agglutinating activities have also been regarded as suitable indicators for immunocompetence in stress studies in fish

(Sunyer et al. 1995; Tort et al. 1996a). To assess the effect of sea-cage farming on the metabolism of two marine finfish species, sea bass (*Dicentrarchus labrax)* and sea bream (*Sparus aurata*), until now scarcely understood, an investigation was performed on specimens reared in two Sicilian mariculture sites (Pachino and Castellammare). We aimed at evaluating changes occurring in the physiological parameters involved in the primary, secondary and tertiary response of fish to stress, and thus determine their use as indicators in assessing the condition of chronic stress induced by mariculture.

Materials and methods

The experiment was carried out from March 2000 to July 2001. In Castellammare, the final fish density was 7.99 and 12.62 kg m^{-3} for sea bass and bream, respectively; in Pachino, the final fish density was 40.80 and 21.98 kg m^{-3} for sea bass and bream, respectively. Fish mortality was 5.21 and 4.45% for sea bass and sea bream, respectively, in Castellammare, while rates of 20.46 and 6.22% were recorded in Pachino. At three time intervals (phase I, at the beginning, a no-stress condition, phase II, in the middle, and phase III, at the end of the experiment), 30 specimens for each fish species were captured from each site. Blood samples were drawn from the caudal vein and treated without or with heparin (14 International Units mL^{-1}) according to the analysis to be performed. The following physiological parameters were tested: haematocrit, haemoglobin and plasma glucose (Sigma Diagnostics kit); plasma cortisol (by HPLC, according to Visalli et al. 2000), tissue lactate (Sigma Diagnostics kit, as modified by Santulli et al. 1999); lysozyme content (Ossermann and Lawlor 1966); nonspecific haemolytic (SH50) and haemagglutinating activities against sheep erythrocytes (Sunyer et al. 1995). Analysis of variance (ANOVA) tests were applied on data averaged for each sampling and log transformed. Unfortunately, no sea bream specimens were sampled during phases I and II from Pachino. After capture, fish were acclimated in order to measure cortisol levels in chronic stress and exclude interference on biochemical parameters from acute stress from handling or from other fish in a dipnetted cage (Pickering et al. 1982); all samplings were also carried out at the same time of day to avoid changes related to sampling time.

Results

In Figures 1 and 2 the means of the haematological, biochemical and immunological parameters monitored during the three sampling times are reported. Compared to sea bass (average 36–37%), sea bream displayed the highest haematocrit values both in Castellammare (45%, $F_{1,47} = 71.19$, $P < 0.01$) and Pachino (46%, $F_{1,62} = 45.56$, $P < 0.01$). Haemoglobin content increased throughout the experiment in Castellammare, with significantly higher values in sea bream than in sea bass ($F_{1,47} = 10.10$, $P < 0.01$) in the last sampling; in contrast, fluctuating levels of this parameter were recorded in Pachino, where they were significantly higher in sea bream (13 g^{-1} dL, $F_{1,68} = 12.68$, $P < 0.01$) than in sea bass (7.5 g dL^{-1}). In Castellammare, plasma cortisol values were also significantly higher in sea bream (26 ng mL^{-1} on average) than in sea bass ($F_{1,31} = 33.63$, $P < 0.01$), while no significant differences between the two species were found in Pachino (20 ng mL^{-1} on average). Tissue lactate concentrations increased everywhere during this study and were significantly higher in sea bream from Castellammare (in the 1st and 3rd sampling) than in sea bass (2.6 and 2.9 mg g^{-1} respectively), while in Pachino sea bream and sea bass showed similar values (average = 2.6 mg g^{-1}). An opposite pattern was observed for glucose values. Sea bass reared in Castellammare showed an average value of 240 mg dL^{-1}, which was significantly higher than that measured in sea bream reared at the same site (160 mg dL^{-1}, $F_{1,23} = 8.49$, $P < 0.01$). In Pachino, similar glucose values (average = 190 mg dL^{-1}) were detected for both species.

Immunological parameter values were higher in sea bass than in sea bream. In sea bass reared in Pachino, lysozyme significantly increased from the beginning to the end of the experiment (effect of time: $F_{1,9} = 22.26$, $P < 0.01$); significantly lower and more constant lysozyme values were measured in sea bream (0.99 and 0.74 U mL^{-1}, in Pachino and Castellammare, respectively). In the specimens of sea bass, haemolytic activity decreased significantly in Pachino and Castellammare ($F_{1,9} = 39.37$ and 57.76, $P < 0.01$, respectively). A significant ($F_{1,9} = 12.31$, $P < 0.01$) decreasing trend was also observed in the serum of sea bream reared in Castellammare. A decrease in the haemagglutinating titer was also recorded in sea bass reared in Pachino and Castellammare, and in sea bream from Castellammare.

Discussion

Our research provides the first data on stress in fish farmed in off-shore cages, where the main negative factor that may influence the specimens reared is chronic disturbance due to growth itself, and the subsequent increase in specimen size during the experiment. Haematological values

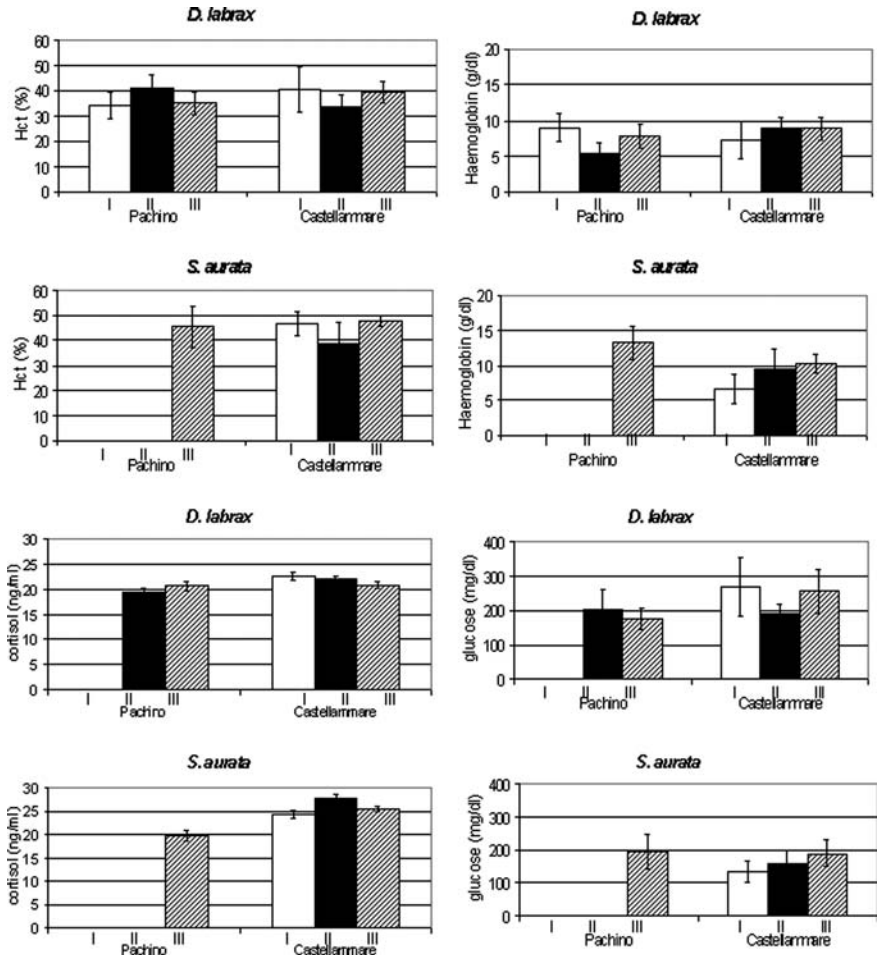

Figure 1. Haematocrit (Hct), haemoglobin, plasma cortisol and glucose values measured in *D. labrax* and *S. aurata* at the three sampling times.

measured were similar to those reported by Pavlidis et al. (1997) in unstressed sea bass (37–43%) and sea bream (37–39%) specimens. Due to their inconsistency over time, they did not appear to be adequate indicators of stress. Changes in haematocrit and haemoglobin, as well as in tissue lactate concentrations, are explained as a strategy to increase oxygen carrying capacity of blood or a consequence of increased swimming activity during periods of high energy demand (Ortuño et al. 2001). Tissue lactate, an anaerobic metabolite, is a good indicator of chronic stress and of fillet quality (Santulli et al. 1999). Stress hormone cortisol and glucose concentrations increased transitorily in both species, thus indicating an immediate response of fish, which progressively

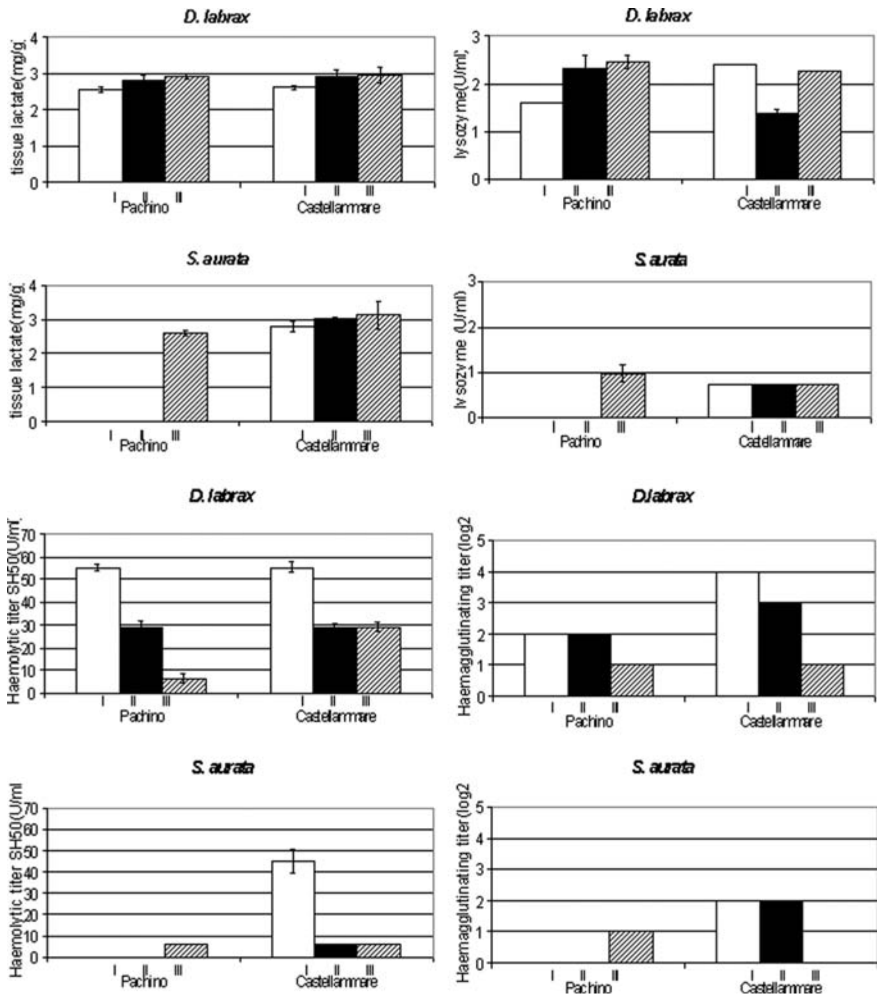

Figure 2. Tissue lactate and serum lysozyme values, haemolytic and haemagglutinating titers measured in *D. labrax* and *S. aurata* at the three sampling times.

become adapted and resistant to the new situation (Montero et al. 1999). The fluctuating trend recorded suggests that the physiological response to mariculture-induced stress had the characteristics of chronic stress. Increased glucose concentrations, observed in *S. aurata* reared in Castellammare and corresponding to high cortisol levels, are commonly explained by the increased requirement of energy occurring during stress conditions. Moreover, the low percentages of mortality recorded in the present study suggest that farmed fish underwent a normal, not harmful, stress condition, overcome by physiological mechanisms (Barton et al. 2002), or that fish size or density were not critical enough to have negative consequences on fish metabolism. The significant changes detected in humoral non-specific immune parameters indicate that mariculture conditions also had an effect on the substances involved in the natural defence mechanisms in fish, as observed in intensive rearing by Sunyer et al. (1995), Tort et al. (1996b) and Montero et al. (1999). The patterns observed for each parameter tested were in agreement with those reported in the literature, although a direct comparison with other reference values was not possible due to the different analytical methods and experimental design used. Sea bream seemed to be more susceptible to stress than sea bass with respect to both the primary (cortisol release) and secondary (changes in haematological parameters) responses. Conversely the tertiary response (change in disease resistance) to stress was higher in sea bass than in sea bream, as shown by the enhanced values of immunological parameters examined. Among all the parameters monitored in our study, cortisol and glucose proved to be the most sensitive for the detection of significant physiological changes occurring in sea bass and sea bream, and therefore their detection is suggested as an early warning signal of metabolic alterations from sea-cage farming.

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