# Welfare Status of Cage Farmed European Sea Bass *Dicentrarchus labrax*: a Comparison Between Submerged and Surface Cages

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**Abstract**

As alternative farming methods, we investigate growth, haematological, biochemical and immunological parameters of European sea bass (*Dicentrarchus labrax*) farmed in submersible cages in order to compare physiological status under different rearing conditions and to validate the efficacy of submersible technology in solving several of the substantial problems that exist in surface-based fish farming as heavy storms, algal and jellyfish blooms and attacks by predators.
The study was conducted in 2008 in the Gulf of Castellammare (NW Sicily, Mediterranean Sea) in two submerged and two surface cages filled with 75.000 *D. labrax* (initial weight: 28.2 $\pm$ 4.3 gr), for each cage.
No significant differences were shown in specific growth rate between the two groups, even if, fish cultured in submerged cage reached the largest size.
Results from biochemical and haematological parameters examined indicated higher welfare state in fish cultured in submerged cages. Fish reared in surface cages showed a significantly higher blood cortisol and glucose levels and haematocrit value than those of submerged cages.
Also lysozme and haemolytic activity, used as indicators of immunocompetence in fish exposed to stress, lead us to suppose that submergence could have a positive effect on some components of innate immune system of cultured fish.
Results of this study suggest mariculture in submerged net cages a promising system that allows to minimize stress and therefore that favours animal welfare.

## 1 Introduction

Offshore submersible fish farms may play an important part in the expanding fish farming industry as inshore sites reach full capacity and offshore farming will open an unknown potential for aquaculture [1].
Submergence may solve several of the substantial operational challenges that exist in surface-based fish farming, including those related to heavy storms, algal and jellyfish blooms, unsuitable temperatures, and biofouling of net cages [2, 3].
Moreover, the high concentration of fish confined in surface net cages systems attracts predators [4], primarily sea birds (i.e., cormorants) and aquatic mammals (i.e., dolphins).
Predation on fish farms results in death and injury to fish, resulting in a economic damage to the farmers [5, 4].
The stress on cultured fish subjected to repeated attacks by predators shows itself in poor feed conversion efficiency and hence

the weight at harvest is not maximized [5]. The negative effects of stress in fish do not occur only on growth but affect many body functions.

Fish respond to stressful conditions with a typical stress response consisting in the release of catecholamines and activation of the hypothalamus-pituitary-interenal (HPI) axis which implies the synthesis of cortisol hormone [6]. Both catecholamines and cortisol initiate secondary and terziary stress responses which translate in maladaptive effects of the organism.

Where fish cannot escape a stressor, or where the stressful stimulus is episodic or intermittent, prolonged activation of the stress response has deleterious consequences. These include loss of appetite, impaired growth and muscle wasting, immunosuppression and suppressed reproduction.

In particular, prolonged activation of HPI axis, reduces growth, indirectly, through a negative effect on energy balance and, directly, through a reduced secretion of growth hormone [7, 8]. Since growth and reproduction are functionally linked, [9] stress-induced impairment of growth may indirectly interfere with maturation. Additionally, reproductive activity is suppressed directly during period of stress *via* a wide range of mechanisms [10]. Finally chronic stress has a generally immunosuppressive effect in fish, mediated in particular by the action of cortisol [11].

On the basis of the above scientific evidences, aquatic producers known that controlling animal welfare is absolutely essential for their economic success and that the development of specific stress management protocols is essential for animal health and survival.

Moreover severe stress conditions can heavily influence the expression of cultured fish quality because of changes during storage of the final product [12].

Reducing stress and maintaining fish welfare is, therefore, an important issue for aquaculture industry for production efficiency, quantity and product quality and recently is also becoming important for both consumer perception and marketing [13, 14, 15].

Submersible cages, also for Mediterranean aquaculture, could improve production conditions and reduce environmental effects as escapes due to storms, sea lice infestations, visual impacts sea birds attacks [16] and may also have a positive effect on fish welfare.

Subsurface technologies have been tested in several production experiments e.g., in farming of yellowtail (*Seriola spp.*) in Japan [17] and Italy [18], Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) in the U.S.A [19], cobia (*Rachycentron canadum*) in the Caribbean [20] and recently Atlantic salmon (*Salmo salar*) in Norway [21].

The growth and behaviour of fish in submerged cages relative to standard surface systems is however largely unknown; objective comparisons of fish performance in commercial-scale submerged cages vs. surface cages have only been undertaken for short-term, shallow submergences [16, 3], or for long-term in Atlantic salmon [21].

The aim of this study is to investigate haematological, biochemical and immunological parameters of European sea bass (*Dicentrarchus labrax*) farmed in submersible cages in order to compare physiological status under different rearing conditions and to validate the efficacy of submersible technology.

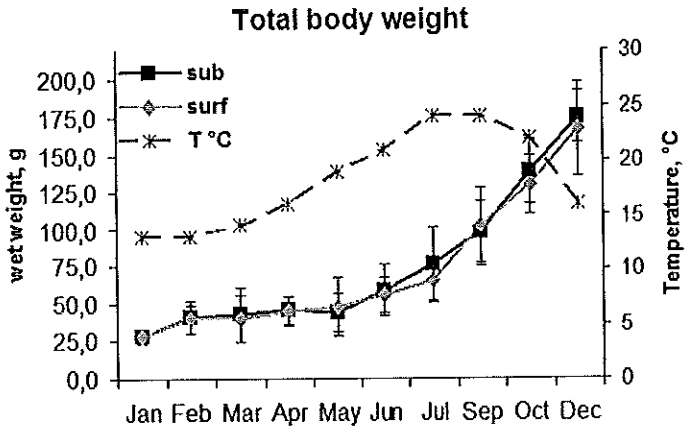


Figure 1: Monthly trends of wet weight of *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages, relative to water temperature changes during farming.

# 2 Materials and Methods

The study was conducted from January to December 2008 in the Castellammare Gulf (NW-Mediterranean Sea) in four fish cages (two submerged and two surface cage, respectively). The fish cages under investigation (2000 $m^3$) were filled on January 2008 with 75.000 specimens of European sea bass (*Dicentrarchus labrax* initial weight: 28.2 $\pm$ 4.3 gr), for each cage.
The fish were manually fed with a commercial diet twice a day according to the estimated live weight and water temperature.
During the trial, fish were randomly collected on a monthly basis, and total and standard length and wet weight were immediately recorded for each specimen, to test growth performance, by calculation of the daily specific growth rate (SGRW %).
The relative condition factor (Kn, [22]) was also calculated by using the follow relationship: $Kn = W_{means}|\overline{aL^b}|$ where W represented our measured weights and $|aL_b|$ represented weight estimated by means allometric equations.

## 2.1 Experimental design and sample collection

At 3 samples time (February, June and September), samples of fish (n=40) were taken to investigate haematological, biochemical and immunological parameters.
Individuals were taken from the cages and immediately anaesthetized using tricaine methanesulfonate, MS222 ($0.1 g l^{-1}$).
Careful netting and handling were implemented to minimize stress. Blood samples were withdrawn separately from the caudal vein of each individual and then divided into different test tubes depending on the parameter to be analyzed. Part of the blood collected in heparinized tubes was used for the immediate determination of haematocrit value. The remaining fraction was centrifuged and the obtained plasma was stored at -80°C. In order to extract

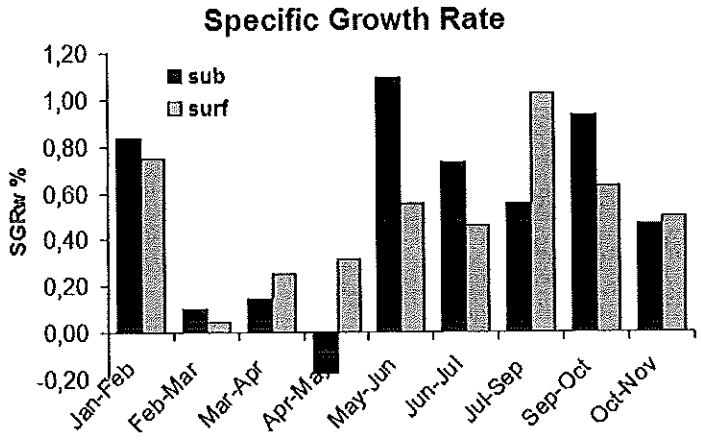


Figure 2: Specific daily growth rates in wet weight of *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages.

serum, blood samples not treated with heparin, were allowed to clot at 4°C, centrifuged at 1500g for 20 minutes and stored at -80°C until analysis.

## 2.2 Analytical assays

Haematological and biochemical parameters: all assays were carried out using commercial diagnostic kits.

Serum cortisol concentration was determined by a enzyme-linked (ELISA) immunoassay kit (*Alpha Diagnostic International*, USA).

Serum glucose and total protein levels were determined by kits respectively based on the colorimetric Glucose Oxidase-Peroxidase (GOD-POD) and chemical biuret-tartrate methods (*Sclavo Diagnostics*, Italy).

Plasma lactate was assayed by a kit that utilize the enzymatic colorimetric method of Lactate Oxidase-Peroxidase (LOD-POD) (*Roche Diagnostics*, Italy).

Haematocrit value (% red blood cell) was determined in heparinized capillary tubes after centrifugation in a standard micro-haematocrit centrifuge at 12000 g for 10 minutes and comparison of the capillary tubes with a reference scale.

## 2.3 Immunological parameters

Haemolytic activity against sheep erythrocytes were determined in serum samples, according to Caruso et al. [23].

Non specific haemolytic activity (SH50) was assayed using a 2.5% suspension of sheep red blood cells (SRBC) in phosphate buffered saline (PBS) containing 10 mM $Mg^{2+}$ and 2 mM $Ca^{2+}$ added to serially diluted serum in PBS-Ca-Mg buffer. After incubation at 22°C for 1 h, unlysed erythrocytes were removed by centrifugation and the content of haemoglobin in the supernatants measured at 540 nm [24]. The SH50 units were obtained from the concentration of serum giving 50% haemolysis of

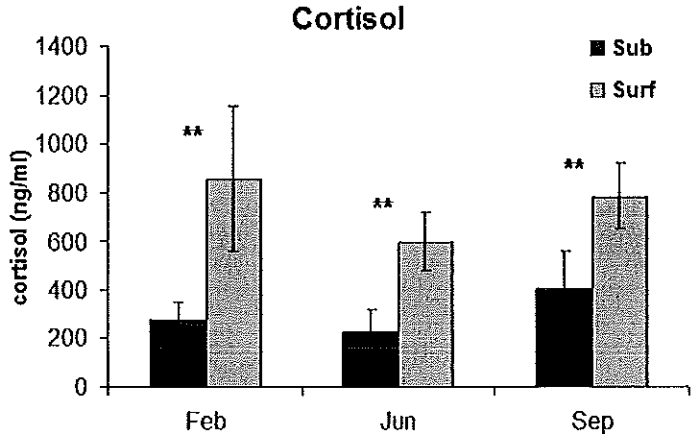


Figure 3: Cortisol concentration in *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

SRBC, where 100% haemolysis was calculated by incubation with distilled water of the SRBC suspension.

Lysozyme content was measured on samples of mucus, serum and kidney, according to the plate diffusion method [25, 26]. Lysozyme content was evaluated by measuring the diameter of lysis produced into 1% agar plates added with 0.05% *Micrococcus lysodeikticus* cells, incubated at 33°C for 22°C.

# 3 Results and Discussion

## 3.1 Growth parameters

Figure 1 shows the monthly trend of total wet weight of the two groups of *D. labrax* during the experimentation period. Although there were no significant differences in wet weight, fish cultured in submerged cage reached the largest size.

No significant differences were shown in specific growth rate between the two groups, even though group sub showed a peak in May-Jun and Sept-Oct. The two populations showed a positive trend, except for the month of Apr to May (Figure 2).

The overlapping of the growth curves for the fish cultured in surface cage and those cultured in submerged cage was also confirmed by the relative condition factor K. The relative condition factor showed that increases in weight and length did not lead to a corresponding increase in this factor.

Under the environmental conditions present during the trial, submerged and control European sea bass, experienced broadly similar light and temperature regimes, as control fish preferred to swim at depths in the cage which were similar to those at which submerged fish swam.

The results from this trial contrast with all previous commercial scale sea-cage

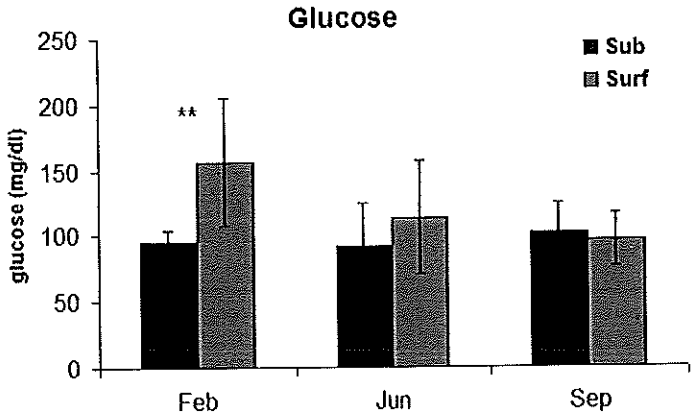


Figure 4: Glucose concentration in *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

submergence experiments which have invariably documented negative effects of submergence upon growth or condition [27, 16].

## 3.2 Haematological and biochemical parameters

As in other vertebrates, fish experiencing stress show several physiological changes that are expressed through a number of particular indicators [6].
Principal results of our study underline a clear relation between observed changes in physiological parameters and the farming type (submerged or surface cage). All biochemical and haematological parameters examined were significantly higher in sea bass cultured in surface indicating a lower state of welfare than fish in submerged cages.
Fish reared in surface cages showed a significantly higher cortisol levels ($p \leq 0.01$) than those of submerged cages (Figure 3). Blood cortisol levels are widely used as an indicator of stress condition in fish [6] because the extreme sensitivity of the HPI axis that is activated in response to most forms of stress in teleost fish.
An increase in cortisol levels means that fish are experiencing a stress and they are reacting with a typical "stress response", of adaptive value characterized by an immediate release of catecholamines and cortisol into the blood stream.
As shown in Figure 4, overall lower values of glucose are observed in submerged cage but the difference is significant ($p<0.01$) only in the February sampling.
Blood glucose levels have been also described to be affected by chronic and acute stress [28]. Indeed stress is typically associated with increased metabolic rate that is positively correlated to plasma glucose levels [29]. Therefore increased glucose concentrations are commonly explained by

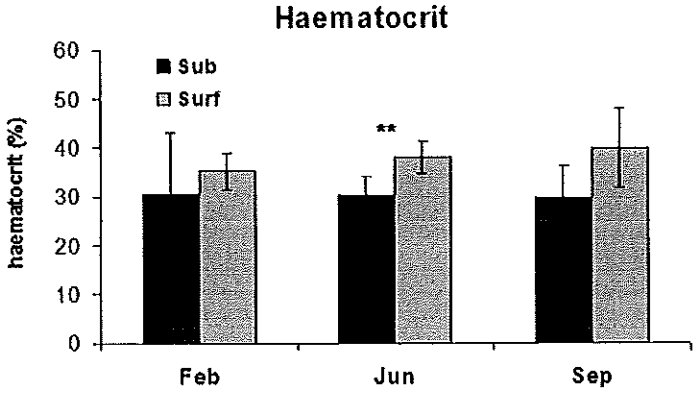


Figure 5: Haematocrit values in *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

the increased requirement of energy occurring during stress conditions.
Haematocrit value is also consistent with other parameters, showing significant higher value ($p \leq 0.01$) in surface cages in June (Figure 5).
In general haematocrit value rises in fish exposed to stress as a direct consequence of the increase in hearth-beat and the need of higher oxygen uptake [12].
The increase of lactate is used as a stress index and is related to glycolysis anaerobic that occurs when muscular activity is intense [30, 31], but statistical differences were not observed between cages.
It is also important underline that in *D. labrax* reared in submerged cages all considered parameters are maintained in the range of basal values reported in literature on the contrary fish of surface cages often exceed this range.

## 3.3 Immunological parameters

The non-specific or innate immune system is the most important resistance mechanism in fish [32, 33] and some of its components (e.g., lysozme, haemolytic and haemagglutinating activity) are used as indicators of immunocompetence in fish exposed to stress [34, 35].
As shown in Figure 6, fish of submerged cages showed significant higher values ($p \leq 0.01$) in non specific haemolytic activity (SH50), in June and September.
Lysozyme content was measured in different tissues or organs (mucus, plasma and kidney) but higher and significant difference ($p \leq 0.05$) was found in the mucus of seabass cultured in submerged cage only in September (Figure 7).
Mucus lysozyme represents the first defence mechanism against foreign agents [32, 26].
The detection of different immunological defence ability lead us to suppose that subemergence could have a positive effect

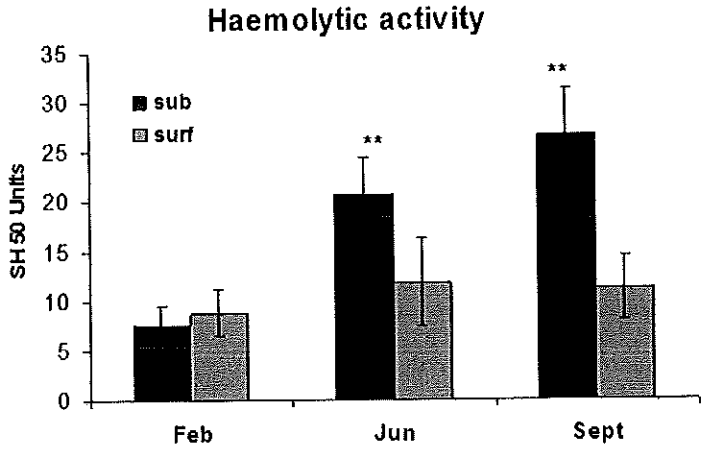


Figure 6: Haemolytic activity in *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

on some components of innate immune system of cultured fish.
Moreover results of immunological parameters are in agreement with those haematological and biochemical and seem to further confirm the hypotesis that submergence promotes sea bass welfare.

# 4 Conclusions and future perspectives

The research provides for the first time data on stress assessment in submerged cages, a particular rearing condition. In off-shore cages, where handling or other stressors currently acting in intensive practices do not affect the quality of the final products, the main negative factor that may influence the specimens reared is mainly referable to chronic stress condition with a continuous disturbance due to the growth itself and consequent increase of the specimen size.
Submergence seems to be a favourable condition for sea bass culturing, as evidenced by growth, haematological, biochemical and immunological parameters.
One reason for this could be linked to stress response from predation. In fact, due to predation activity (i.e. birds attacks), surface-based fish farming can become an environment favouring high mortalities, injury and stress; in a research on dynamics of predation, Dieperink [36] refers that a flock of cormorants are able to empty the pound net in about 30 minutes and consume 110 fish weighing a total of approximately 50 kg.
This hypothesis is, also, supported by studies on neuroendocrine perception of stress in fish where is demonstrated that prey/predator encounter is a stressful situation and induce a stress response with energy cost for escape [37].

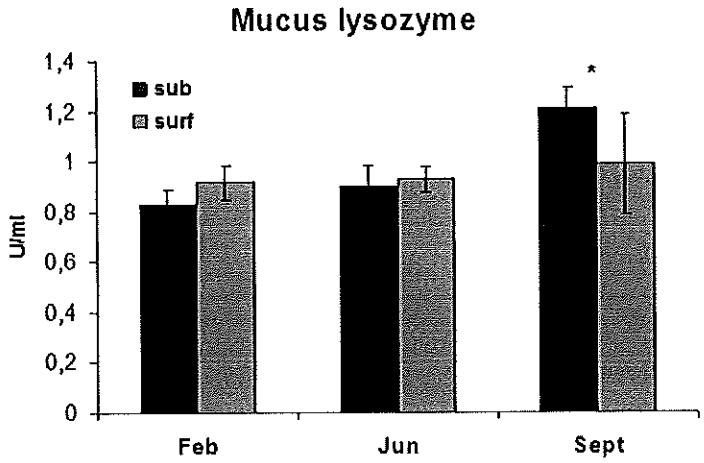


Figure 7: Lysozyme content in the mucus of *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

Rehnberg and Schreck [38] report in coho salmon (*Oncorhynchus kisutch*) an increase in plasma cortisol following chemosensory detection of a predator Woodley and Peterson [39] refer in long-nose killifish (*Fundulus majalis*) elevated plasma cortisol when exposed to visual stimulus of a predator similarly, Jarvi [40] reports that Atlantic salmon (*Salmo salar*) displayed a physiological response following predator exposure in the form of elevated blood glucose, lactate concentrations and chloride concentration.

Moreover literature reports a relationship between high plasma cortisol levels and immunosuppressive effect producing alterations in some components of immune system e.g., phagocytic activity [41], lysozyme activity [42], or serum haemolytic and agglutinating activity [34, 43, 44].

Among the parameters used in our experiment to monitor the response to stress induced by the different culture conditions, cortisol and glucose proved to be the most sensitive and therefore their determination is suggested in the early warning of metabolic alterations following sea-cage farming.

All these results lead us to conclude that the conditions in which fish are cultured do influence their welfare. Haematological and biochemical parameters suggest that sea bass reared in submersible cages show the best welfare condition compared to fish kept in surface cages. The high immunological reactivity displayed by fish reared in submersible cage also supports this conclusion.

This study lead us to suggest mariculture in submergible cages as a rearing practise that allows to minimize stress and therefore that favours animal welfare.

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