Heat Shock Proteins in Farmed Gilthead Seabream (*Sparus aurata*): The Key to assessing their Welfare?

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Introduction

Aquaculture is an important economic activity worldwide. Steady growth in the industry has seen growing international concern over the welfare of farmed fish. Aquaculture practices, such as repeated handling, confinement and over-crowding, have been widely reported as chronic stressors affecting fish physiology and welfare status (Alves *et al*., 2010). Consequences of compromised physiology and welfare in turn lead to decreased growth rates and thus reduced profitability (Ibarz *et al*., 2010). Current research into welfare indicators in fish has highlighted the use of proteomics, in particular heat shock proteins (hsp), to identify molecular indicators of chronic stress in several fish species. One such species of particular importance in aquaculture in the Mediterranean Sea (Kyprianou *et al*., 2010; Feidantsis *et al*., 2009), and the focus of the following studies, is the gilthead seabream (*Sparus aurata)*.

Discussion

Parameters of fish welfare have historically been difficult to determine and measure (Alves *et al*., 2010). A wide range of neural, hormonal and behavioural indicators have been used to assess fish welfare. While plasma cortisol concentration remains the most commonly used indicator of stress in fish (Bertotto *et al*., 2010), it is limited by the fact that it is "highly vulnerable to handling and sampling effects" (Roberts *et al*., 2010). The use of heat shock proteins on the other hand complements plasma cortisol concentration measurement by presenting a way of assessing fish welfare without the risk of sampling and handling error (Vijayan *et al*., 1997).

Heat shock proteins, or "stress proteins", are produced in all cellular organisms when they are exposed to stress (Roberts *et al*., 2010). In fish species, there is recent evidence that heat shock proteins play an important role in the response to physiological, environmental and nutritional stress (Cara *et al*., 2005). Further evidence suggests that heat shock proteins can be used to stimulate resistance in fish against bacterial infections and to reduce stress during transportation, thus reducing overall fish mortality (Roberts *et al*., 2010).

Alves *et al*. (2010) conducted a study on gilthead seabream (*Sparus aurata*) to determine metabolic biomarkers of chronic stress. Some farmed gilthead seabream (n=44) were allocated to three different test environments:

1. optimum rearing conditions (control),
2. repeated handling, and
3. crowding at a high stocking density.

Alves *et al*. (2010) conducted comparative proteomic analysis of the livers of all three groups – this is reported to be the first time such an approach has been implemented (Alves *et al*., 2010). The results were favourable in regard to linking heat shock proteins with chronic stress; compared to the control group stressed individuals recorded a down-regulation of heat shock cognate protein 70 (hsc70). Unlike heat shock protein 70 (hsp70), which is produced in response to stress, hsc70 is expressed in cells without stress. This characteristic therefore indicates that down-regulation of hsc70 can be used as an interpretive biomarker to evaluate chronic stress in fish species (Alves *et al*., 2010). To further substantiate these claims, it was suggested by Alves *et al*. (2010) that the proteomic analysis be extended to include analysis of other tissues such as "plasma, brain and gills" (Alves *et al*., 2010).

Environmental stress poses a significant threat to commercial fish harvesting worldwide. Both low and high ambient water temperatures can have negative impacts on the welfare of the gilthead seabream. One particular disease affecting this species in the Mediterranean Sea is the “winter syndrome”, a pathological condition associated with long-term exposure to low ambient temperatures during the winter months (Kyprianou *et al*., 2010). To investigate this disease, Kyprianou *et al*. (2010) conducted an experiment to determine the molecular response of *Sparus aurata* when exposed to low temperatures. Individuals (n=5) were assigned to one of three treatment groups according to temperature: 18°C (control), 14°C and 10°C.

With regard to the heat shock proteins, Kyprianou *et al*. (2010) expanded on the method used by Alves *et al*. (2010) by extending the proteomic analysis to include the heart and red and white muscle of *Sparus aurata*. Results varied for hsp70: heart and liver hsp70 levels increased in individuals exposed to 14°C and 10°C. In the white and red muscle, hsp70 levels increased in only those individuals exposed to 10°C, indicating that there is variation between the heat shock protein response of different tissues to low ambient temperature. These findings were further supported by Ibarz *et al*. (2010), who found multiple physiological alterations, particularly in the liver, of gilthead seabream exposed to the cold.

In contrast, high ambient water temperatures associated with global warming have also been shown to compromise the welfare of gilthead seabream via expression of hsp70. Feidantsis *et al*. (2009) examined the effect of thermal stress on gilthead seabream, specifically hsp70 expression, in fish exposed to different water temperatures. Individuals (n=55-60) were exposed to one of five different water temperatures: 18°C, 22°C, 24°C, 26°C and 30°C. Proteomic analysis of the heart and white muscle yielded similar results: hsp70 levels increased slightly after exposure to 22°C and 24°C, whereas hsp70 levels increased sharply after exposure to 26°C and 30°C. Red muscle on the other hand showed significantly increased levels of hsp70 in treatments higher than 22°C.

These results, in conjunction with the results of Kyprianou *et al*. (2010), provide evidence that heat shock proteins, in particular hsp70, can be used to identify thermal stress in gilthead seabream (*Sparus aurata*), leading to better welfare management in terms of the thermal threshold that the species is able to tolerate in aquaculture environments.

Conclusion

As demonstrated by this research, the use of proteomics, in particular heat shock proteins, to assess chronic stress in the gilthead seabream (*Sparus aurata*), is a reliable tool that can be applied to all facets of species welfare management, namely stress management, nutrition, animal husbandry and transport.

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