Parco Scientifico e Tecnologico della Sardegna

PortoConte Ricerche

THE IMPACT OF TEMPERATURE CHANGES ON THE LIVER METABOLISM OF GILTHEAD SEA BREAM (SPARUS AURATA)

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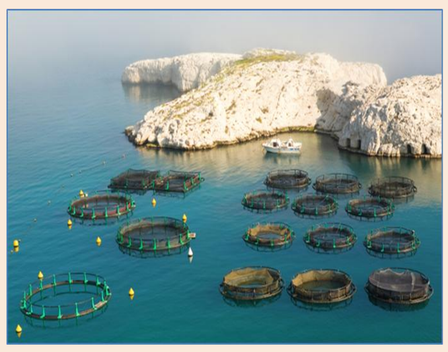
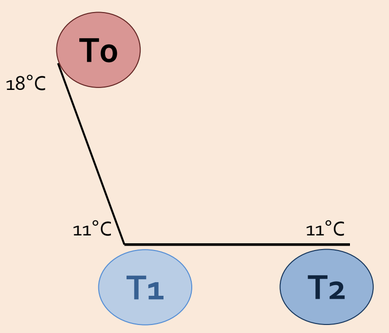
Background



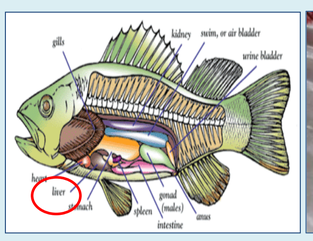
The sea bream (Sparus aurata) is very sensitive to low temperatures. Cold temperatures are considered a source of stress, and cause fasting and a reduced growth performance. Therefore, aquaculture feed producers are strongly interested in the optimization and production of specific feeds for the winter period, and on the impact of cold temperatures on the farmed fish metabolism.
The impact of temperature reduction on the liver proteome of sea bream is not still well investigated. For this reason, a feeding trial with three temperature steps of four week each (**18°C constant (To)**; **a decreasing temperature ramp down to 11°C (T1)**; and **11°C constant (T2)**) was carried out using a standard feed composed by a vegetable source integrated with fish flour. A proteomic approach was applied to assess the impact of different temperature variations on the metabolism of gilthead sea breams (Sparus aurata). Specifically, liver protein expression profiles were evaluated by shotgun proteomics using FASP (filter-aided sample preparation), MS/MS, and label-free differential analysis.

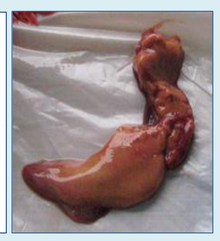
Scheme of the feeding trial adopted

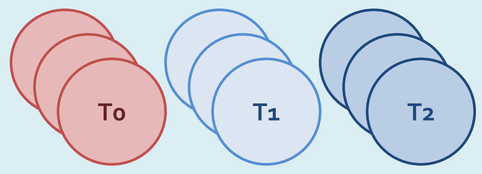


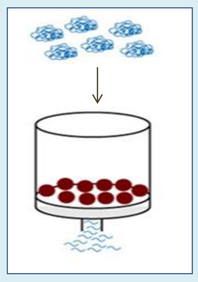
Shotgun proteomics of sea bream liver exposed to low temperatures

In order to evaluate the different protein profiles of liver during temperature changes, three biological samples per temperature variation were analyzed by shotgun proteomics. Peptide mixtures obtained by filter digestion (FASP) were subjected to LC-MS/MS analysis on a Q-TOF hybrid mass spectrometer, and protein identification was performed using Proteome Discoverer.









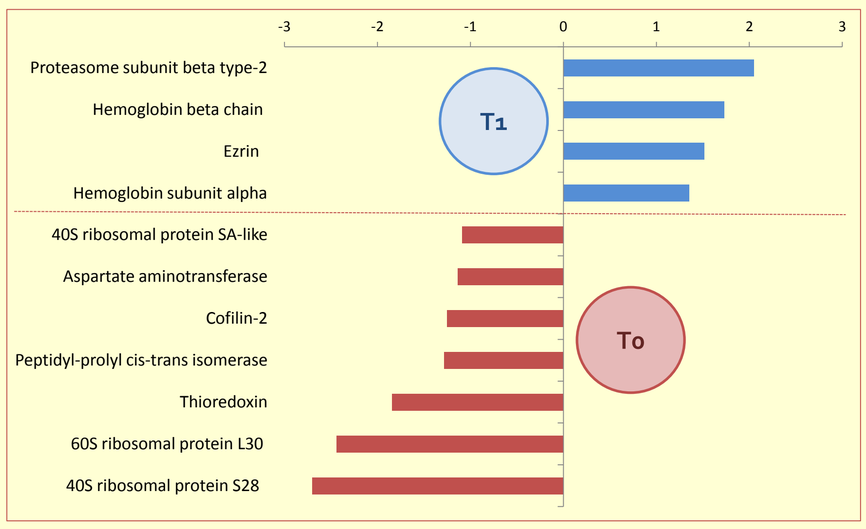
Identifications	To	T1	T2
# Proteins	585	620	654
# Peptides	1340	1328	1448
# PSMs	5098	4274	4837

Differential comparison of protein abundance changes in To and T1 temperature steps

Proteomic analysis of the three biological samples collected at To and T1 produced 773 total protein identifications. A total of 43 proteins were differentially abundant by considering a P value <0.05 and a Log Ratio NSAF (RNSAF)>0.5 or <–0.5 as indicated in the table reported below.

Log Ratio NSAF >0.5 <-0.5	T1/To	To>	T1>
# Differential Proteins	43	24	19

Differential proteins T1/To Log Ratio NSAF >1 and <1



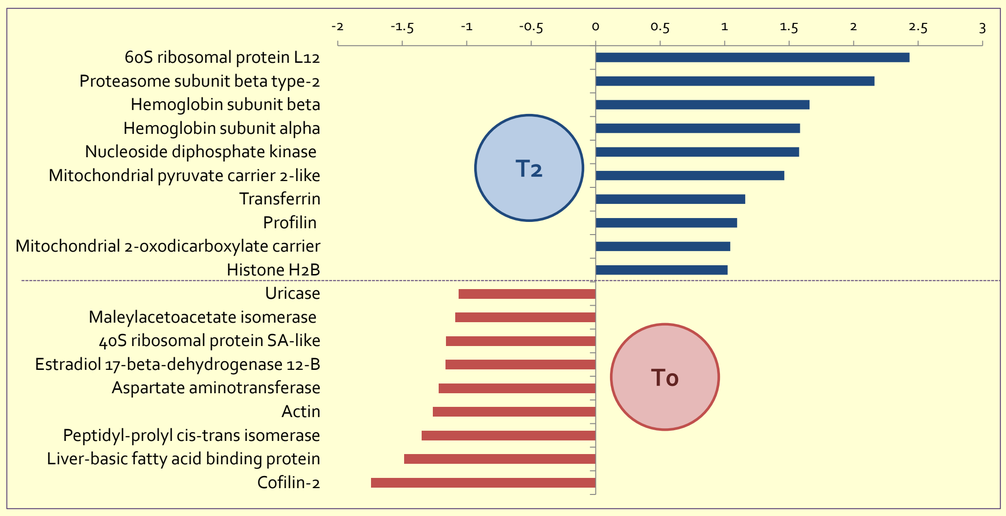
The bar graph reports the main differential proteins (#11) with a Log Ratio >1 or <1 considering a P value <0.05.
Four of these differential proteins were highly expressed in T1, and were mainly implicated in proteolysis (**proteasome subunit beta type-2**). The other 7 proteins, augmented in To are involved in protein synthesis (ribosomal proteins), actin-binding proteins (Cofilin-2), amino-acid metabolism (**aspartate aminotransferase**), or in the protection from oxidative damage (**Thioredoxin**).

Differential comparison of protein abundance changes in To and T2

In the comparison of To versus T2, 814 total proteins were identified. Among these, 65 were differentially abundant when considering a P value <0.05 and a Log Ratio NSAF (RNSAF)>0.5 or <–0.5 as indicated in the table reported below.

Log Ratio NSAF >0.5 <-0.5	T2/T0	T0>	T2>
# Differential Proteins	65	33	32

Differential proteins T2/To Log Ratio NSAF >1 and <1



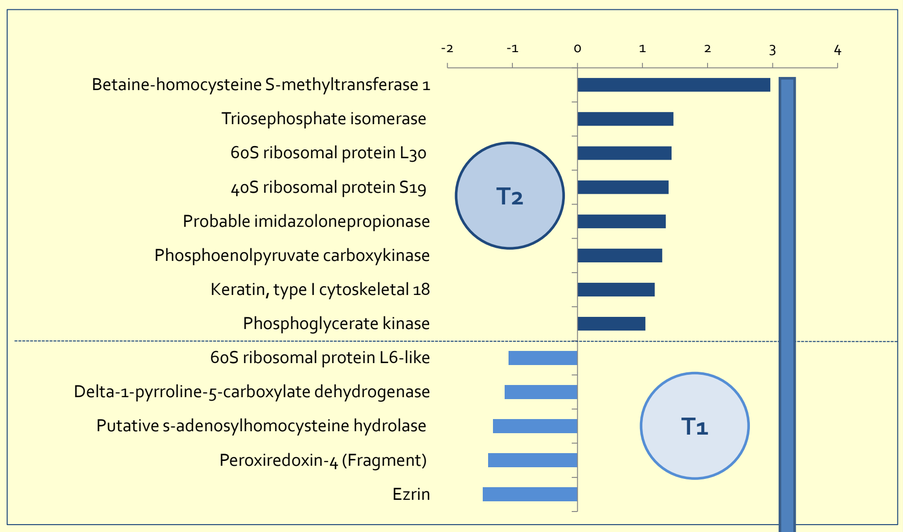
The bar graph reports the main differential proteins (#19) with a Log Ratio >1 or <1 considering a P value <0.05.
Ten of these were highly expressed in T2, and were mainly implicated in proteolysis (**proteasome subunit beta type-2**), in the conversion of energy (**nucleoside diphosphate kinase**), **transferrin** and mitochondrial protein carriers such as **2-oxodicarboxilate carrier.** In the opposite way, 9 proteins augmented in To are involved in the metabolism of fatty acids (**Fatty acid binding protein**) and metabolism of amino-acids such as **aspartate aminotransferase** and in the protection from oxidative damage (**Uricase**).

Differential comparison of protein abundance changes in T1 and T2 temperature steps

A total of 812 protein identifications were considered for comparison of of protein abundance changes in T1 versus T2. A total of 25 differential proteins were observed, with a P value <0.05 and Log Ratio NSAF (RNSAF)>0.5 or <–0.5 , as indicated in Table shown below.

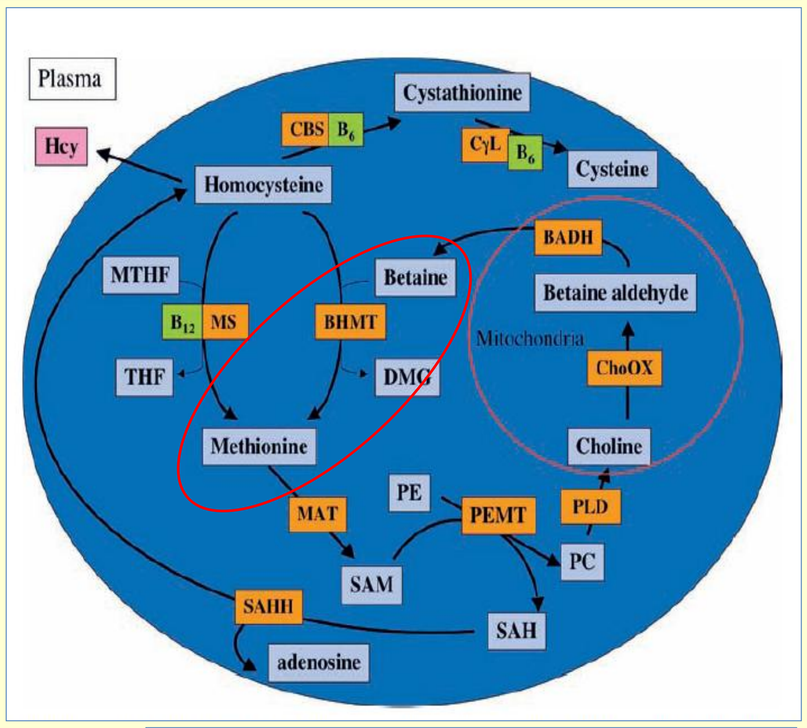
Log Ratio NSAF >0.5 <-0.5	T2/T1	T1>	T2>
# Differential Proteins	25	8	17

Differential proteins T2/T1 Log Ratio NSAF >1 and <1



The bar graph reports the major represented differential proteins (#13) with a Log Ratio >1 or <1 considering a P value <0.05.
8 of these differential proteins were highly expressed in T2, one of them is **BHMT** (**betainehomocysteine S-methyltransferase** (Log Ratio NSAF 2.96) already seen before in a precedent feeding trial with a commercial feed) is implicated in the methionine metabolism. Others are implicated in glycolysis such as **triosephosphate isomerase, phosphoenolpyruvate carboxykinase** and **phosphoglycerate kinase**. In T1 are augmented scavengers proteins (**Peroxiredoxin**),**s-adenosylhomocystein hydrolase** implicated in adenosine and homocysteine synthesis from s-adenosylhomocysteine and **delta-1-pyrroline-5-carboxylate dehydrogenase** involved in amino-acid metabolism.

Metabolic pathway of Betaine homocysteine methyltransferase (BHMT)



M. A. Pajares and D. Pérez-Sala, Cellular and Molecular Life Sciences, 2006, 63 (23), 2792-2803)

BHMT is involved in the superpathway of methionine degradation, as well as in the methionine salvage pathway.
Specifically, BHMT catalyzes the conversion of betaine and homocysteine to dimethylglycine and methionine.
Here, BHMT overexpression is probably related to its involvement in the oxidative stress balance. In fact, BHMT regulates the levels of S-adenosyl-methionine (SAM), which is a biosynthetic precursor of glutathione. The changes in BHMT levels can therefore reflect the oxidative stress caused on hepatocytes by cold. Similar alterations in liver metabolism were observed by Ibarz and coworkers before when studying the response of the liver proteome in response to cold stress. Their studies highlighted alterations in protein expression similar to this study, that were linked to a reduced ability to respond to oxidative stresses upon exposure to cold.

Conclusions

The proteomic analysis of fish liver exposed to low temperature can contribute significantly to understand protein changes induced by this stressful environmental condition, and assist in the formulation of feeds with high performances also at lower farming temperatures.

Future perspectives

The knowledge acquired by low temperatures effects on the farmed fish metabolism is being currently used to evaluate the performance of feeds optimized for colder water temperatures (**Winter Feed**). These trials are involving changes in feeds and the water temperature. The feeds are specifically designed for use in the different seasonal atmospheric conditions.