Parco Scientifico e Tecnologico della Sardegna

PortoConte Ricerche

IMC International Marine Centre

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PROTEOMIC CHARACTERIZATION OF THE SEA URCHIN ROE FOR UNDERSTANDING AND MONITORING QUALITY

Stefania Ghisaura[1], Grazia Biosa[1], Daniela Pagnozzi[1], Roberto Anedda[1], Maura Baroli[2], Barbara Loi[2], Tonina Roggio[1], Sergio Uzzau[1], Maria Filippa Addis[1]

1 Porto Conte Ricerche Srl, Loc. Tramariglio, Alghero (Sassari), Italy; 2International Marine Center, Loc. Sa Mardini, 09170 Torregrande, Oristano, Italy

**Corresponding author: ghisaura@portocontericerche.it*

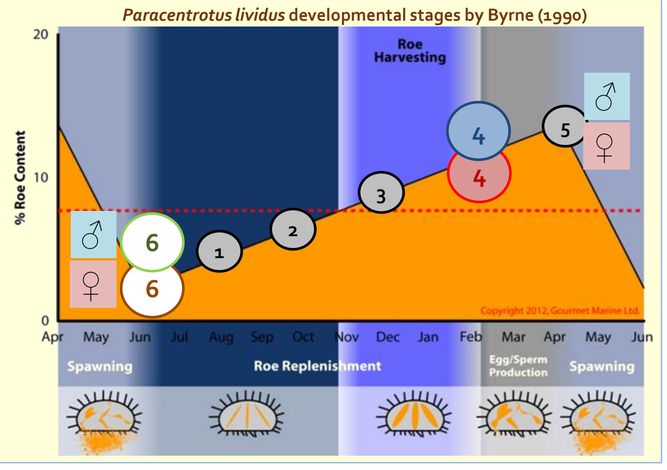
Background

The sea urchin ***Paracentrotus lividus*** (Lamarck, 1816) is the most abundant echinoid found throughout the Mediterranean Sea and in the eastern Atlantic Ocean. *P. lividus* gonads are considered a delicacy in many parts of the world for their flavor and taste and, in recent years, their increasing demand has resulted in commercial overexploitation of wild population. This species is becoming an important candidate for the emerging aquaculture industry in Europe. *P. lividus* gonad quality is strongly influenced by the season/reproductive period, geographic location, gender, and diet. During the reproductive cycle, *P. lividus* gonads undergo important structural changes classified in different developmental stages. One of the most exhaustive classification system of *P. lividus* is described by Byrne (1990) and divides the annual cycle in 6 developmental stages:

1: recovery; 2: growing; 3: pre-mature; 4: mature; 5: spawning; 6: spent.

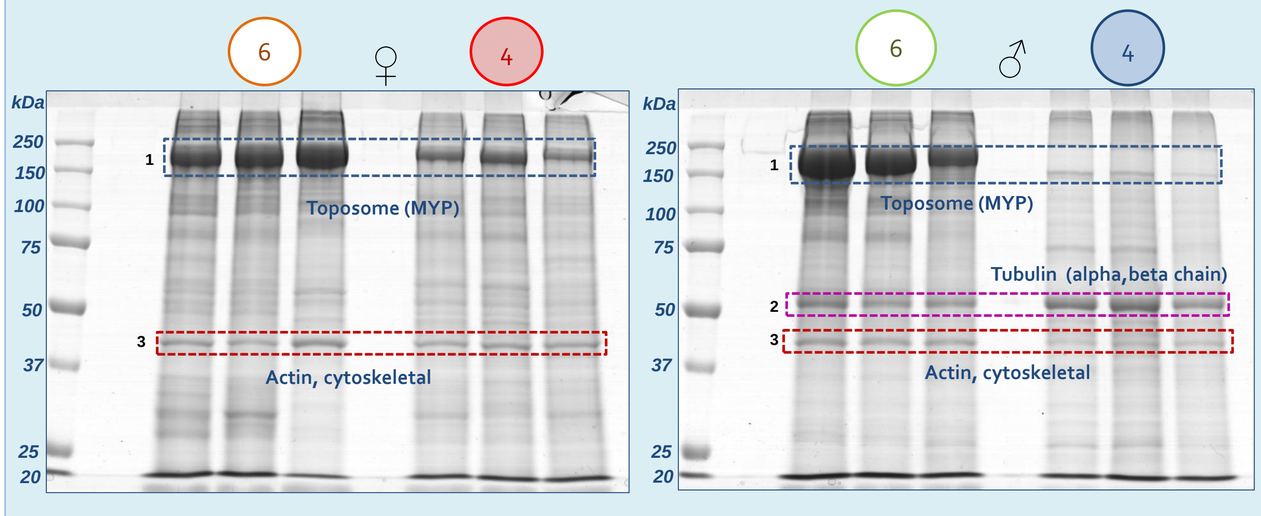
In order to characterize quality, proteins of wild sea urchin gonads from *P. lividus* of the Sardinian sea in different zones were analyzed. In this work, female and male gonads of two opposite seasonal reproductive stages - 4: mature; 6: spent - were characterized by : 1) **Gel-based proteomics**, represented by a densitometric analysis of monodimensional protein profiles and characterization of stages and sexes ; 2) **Shotgun proteomics**, with FASP (filter-aided sample preparation) , MS/MS, and label-free differential analysis.

Paracentrotus lividus developmental stages by Byrne (1990)

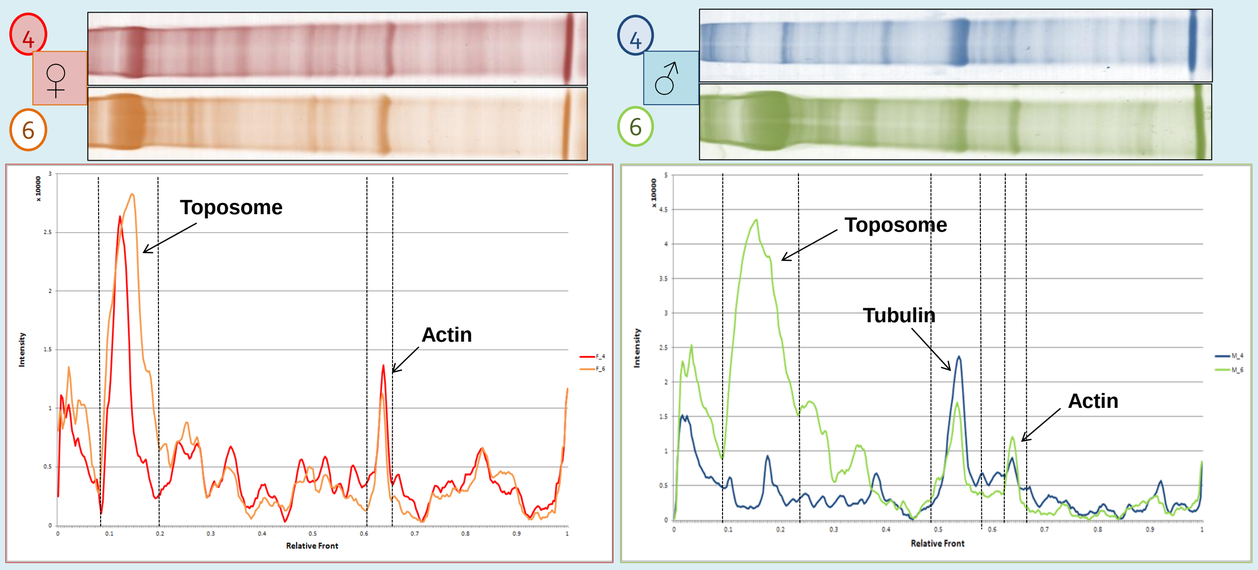


Gel-based proteomics

In order to evaluate the different profiles of stage 4 (mature) and 6 (spent) for both sexes, 8 samples for every category were analyzed by 1D-SDS PAGE. Patterns were scanned and compared using QuantityOne software (Bio-Rad). The densitometric analysis showed interesting differences among categories and similarities within the groups. Three samples per stage and sex with the same intensities pattern were chosen in order to evaluate differences among stages and sexes. The most representative and differential bands for females and males in stage 4 and 6 were analyzed by LC-MS/MS.



1D-SDS PAGE of three biological samples per stage and sex: stage 6-female, stage 4-female, stage 6-male, and stage 4-male selected for the proteomic analysis. Two differential protein bands for females (as indicated by numbers 1, 2) and three for males (as indicated by numbers 1-3), corresponding to different MWs and signal intensities were cut and trypsin digested for LC-MS/MS identification. **Band number 1** is mainly constituted by Toposome (MYP) for the two sexes. **Band number 2** is mainly Tubulin (alpha and beta chain) and **band number 3** is mainly actin (cytoskeletal).

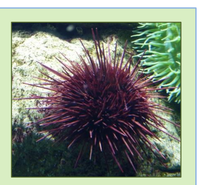


Ratio values	♀6/♀4	♀4	♀6
Toposome (MYP) ratio	1.7	-	-
Toposome (MYP) /Actin ratio	-	3.3	7

Ratio values	♂6/♂4	♂4	♂6
Toposome (MYP) ratio	8.17	-	-
Toposome (MYP) /Actin ratio	-	1.7	15.6
Toposome (MYP) /Tubulin ratio	-	0.6	6.1
Tubulin/Actin ratio	-	3.8	2.5

Densitometric analysis of the three biological samples per stage and sex: The first and second panel report the densitometric analysis of stage 6-female and stage 4-female, and of stage 6-male and stage 4-male, respectively, with the main representative peaks selected for Area calculation: two peaks for females:Toposome and Actin; three peaks for males: Toposome, Tubulin and Actin. On the top the monodimensional profile of the two stages (4 and 6) is reported.
Tables below report the ratio values of the area of main representative peaks: Toposome (MYP) ratio, Toposome (MYP)/ Actin ratio , Toposome (MYP) /Tubulin ratio and Tubulin /Actin ratio.

Conclusions

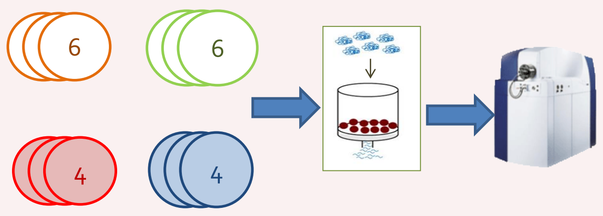


This study represents a step forward in understanding the reproductive cycle of the sea urchin and in providing new protein markers of sex and maturation stage, enabling to speed up current procedures, usually based on histochemical analysis.

- **Specific differences in the proteome of female and male gonads and in gonads at different stages of maturation were identified and quantified, that can be now measured with a simple electrophoretic and densitometric assay.**
- **A profound characterization of the proteome changes occurring in sea urchin gonads according to sex and along maturation was obtained.**

Shotgun proteomics

The same three biological samples per stage and sex used for 1D-SDS-PAGE were analyzed by shotgun proteomics in order to obtain a wider picture of the proteome of stages 4 and 6 in females and males. 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.



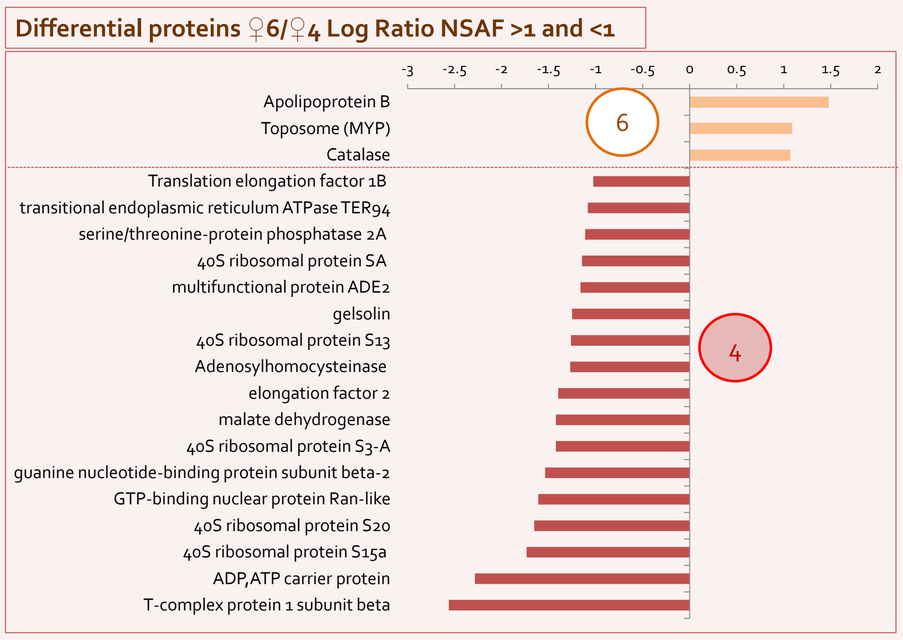
Identifications	♀4	♀6	♂4	♂6
# Proteins	202	135	262	142
# Peptides	514	398	727	464
# PSMs	2251	1805	3200	2711

Differential comparison of protein abundance changes in stage 4 and 6 females

Differential analysis of three biological samples per stage 4 and 6 females identified 242 total proteins. From these, 33 differential proteins showed statistically significant differences considering a P value <0.05 and a Log Ratio NSAF (RNSAF)>0.5 or <–0.5 as indicated in the table.

Log Ratio NSAF >0.5 <-0.5	♀6/♀4	♀4 >	♀6 >
# Differential Proteins	33	28	5

Differential proteins ♀6/♀4 Log Ratio NSAF >1 and <1



The bar graph reports the major differential proteins (#20) with a Log Ratio >1 or <1 considering a P value <0.05.
17 of these differential proteins were highly expressed in stage 4 females, mainly implicated in protein synthesis (**ribosomal proteins, elongation factor 2-like** etc). The other 3 proteins, augmented in stage 6 females, are involved in fat transport (**Apolipoprotein B-100 like**) or in the protection from oxidative damage (**catalase**). **Toposome** is mainly involved in accumulation of protein reserves in nutritive phagocytes, and plays a role in embryo development.

Differential comparison of protein abundance changes in stage 4 and 6 males

304 total proteins were identified in stage 4 males versus stage 6 males in the comparison of protein abundance changes. 59 differential proteins showed statistically significant differences with a P value <0.05 and Log Ratio NSAF (RNSAF)>0.5 or <–0.5 as indicated in the table.

Log Ratio NSAF >0.5 <-0.5	♂6/♂4	♂4 >	♂6 >
# Differential Proteins	59	49	10

Differential proteins ♂6/♂4 Log Ratio NSAF >1 and <1

The bar graph reports the major differential proteins (#24) with a Log Ratio >1 or <1 considering a P value <0.05.
20 of these differential proteins were highly expressed in stage 4 males, being for the most part mitochondrial proteins (**cytochrome c oxidase, ATP synthase** etc) or cytoskeletal proteins (**dynein arm light chain, Tektin-B1, ropporin-1-like protein-like, radial spoke head protein 9 homolog**).The other 4 proteins were augmented in stage 6 males and are **thymosin beta, Toposome (MYP) ,actin, apolipoprotein B-100,** and **catalase.**