# The role of Proteomics in the study of the influence of Climate Change on seafood products

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**Abstract:**

The climate change influence over the oceans has been the subject of numerous studies, informs and strategies from different scientific perspectives, focused mainly in the ecological impact. The majority of the related studies have been focused in measuring or predicting the physical, chemical, geographical, sociological and economical consequences of this reality, which seems to be unstoppable, and only a few of them are devoted to detect the effects of the climate change over the quality of seafood products, wild or cultivated.

The stress produced in marine organisms by the consequences of climate change is reflected at the cell molecular level, being affected the metabolite concentration, the expression of proteins and their modifications. The study of the climate change may take advantage of these molecular changes, which may be used as a source of possible Biomarkers of its evolution.

After the genomic age, proteomics appears as a young but robust discipline for a global study of the protein content in cells, including their identification, possible modifications, quantification of differential expression and tissue localization, being the most adequate set of methodologies to evidence protein changes in marine organisms affected by climate variations. In the last decade proteomic technologies have experienced an exponential development, but the research has been mainly applied to biomedical and human health research, being scarcely applied to the study of the marine

environment. The application of the proteomics methods to study the effects of climate change over seafood, mainly from the safety point of view, is reviewed.

**Key words:** proteomics, seafood, climate change, food safety.

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**Summary:**

# 1. Introduction

Aquatic ecosystems, inland, coastal and marine, provide humans with resources for recreation, food and livelihood. Besides, they also perform many other important environmental functions, e.g. in relation to meteorological events, pollution etc., contributing to general human well-being (FAO 2009). The vital role in the regulation of the climate by the oceans, due to their inherent capacity to store and transport heat around the globe, has been recognized for long. Furthermore, the synergy of oceans currents and atmospheric winds produce the climate.

## 1.1. Oceans and climate change

According to several recently published FAO reports (FAO 2009), *Climate Change* is a natural process that takes place simultaneously on various timescales, in relation to the variation over time of earth's global climate or local climates, which may be the result of both, natural forces and of course, human activities. In this regard, it is important to distinguish between climate and weather, being the last the daily to weekly fluctuations in temperature, winds, precipitation, etc., while climate stands for what is expected on a season basis and relates quite closely to sun's variation (Fleming, Broad, Clement, Dewailly, Elmir, Knap, Pomponi, Smith, Solo, Gabriele & Walsh, 2006).

There is a general consensus about today's anthropogenic impact in the oceans which is the cause of the depletion of a varied range of living organisms. It is widely agreed, as well, that situation seems to be further exacerbated by risks derived from the global warming. From the environmental perspective, it is important to consider also that $CO_2$, emitted by human activities, is not only accumulated in the atmosphere; it permeates

into the ocean surface layers resulting in a big reduction of the water oxygen levels (Pörtner, Langenbuch & Michaelidis, 2005). Things are complicated further by the fact that a significant percentage of the world's population is living within 75 miles of the coast, and that number is rapidly increasing (Fleming, Broad, Clement, Dewailly, Elmir, Knap, Pomponi, Smith, Solo, Gabriele, & Walsh, 2006): microbial contamination by bacteria, viruses and protists direct or indirectly related to human activity is increasing and affecting the safety of seafood supply. Anthropogenic contamination has lead to an increasing threat: the contamination due to the high levels of heavy metals, polyaromatic hydrocarbons (PAHs), endocrine disrupting chemicals, pesticides, toxins derived from harmful algal blooms (HABs), swelling and other persistent environmentally substances, entering the marine food chain (Brunner, Jones, Friel & Bartley, 2009). On the other hand and as a consequence of the climate change, oceans, especially those at mid-latitudes and in the tropics, are warming and becoming more saline in certain zones. Conversely, in the subarctic Atlantic, the Southern Ocean and some areas of the Pacific Ocean, water is becoming fresher. Oceans vital role in climate regulation, due their capacity to store and transport heat around the globe, must not been forgotten. Climate change affects the intensity and frequency of sea currents, which flush and clean continental shelf areas in 75 percent of the world's major fishing grounds (FAO, 2008a). The oceans in the tropics and mid-latitudes will be less productive but, by contrast, cold water oceans will see greater productivity.

As a consequence of those cited physical phenomena and bearing in mind that fish and shellfish cannot tolerate swift rises in the water temperature, changes in their metabolic routes are produced, marine species distribution patterns will change, being more noticeable for the fish stocks at the edges of their species' range. Species, particularly those with shorter life spans, will change their lifetime cycle. In a parallel way, some

plankton species will bloom earlier, resulting in mismatches between the early life stages of fish and their prey, with the consequent decline in fish abundance. The combination of increasing temperatures and sea level rise will result in changes to coastal circulation patterns, thereby affecting nutrient supply, lagoon flushing, coastal erosion, and possibly ocean acidity and coral bleaching. These will affect both the reef-building capacity of corals as well as the spawning cycles of reef fishes and invertebrates (FAO, 2007). In summary, marine organisms will need to make continuous efforts in order to achieve their functional adaptation to those stressful situations in the oceans as well as under culture conditions (Schreck, Contreras-Sanchez & Fitzpatrick, 2001). As it is used in this context, stress must be understood as the physiological cascade of events that occurs when an organism is attempting to resist death or re-establish homeostatic conditions in face of an "insult". The stress response can be polymorphic with regard to species of fish, stage of maturity and type and severity of stressor. In this sense, biochemical indicators of stress and metabolism and the definition of new biosensors for marine control have been widely reviewed (Kröger, Piletsky, & Turner, 2002; Dahlhoff, 2004; Brun, Bricelj, MacRae, & Ross. 2008).

However, at the same time, the demand for sustainable sources of seafood has never been greater (Pitcher, 2008). It is noticeable that the expectation for the year 2030 is a production of near 133 million tones of seafood, combining that provided by the oceans and from aquaculture productions (FAO, 2008b). But, with many traditional fisheries depleted, due to a previous overexploitation, it appears that global capacity for seafood production has been reached or even exceeded, therefore in response to this decline and to the increasing demand for seafood products, aquaculture has become the fastest growing animal food-producing sector (Pelletier, Ayer, Tyedmers, Kruse, Flysjo, Robillard, Ziegler, Scholz & Sonesson, 2007). Aquaculture production is rising rapidly,

and by 2030 it is estimated that will be close to capture landings. Unlike terrestrial farming systems, in which the bulk of global production is based on a limited number of animal and plant species, in aquaculture more than 220 different farmed aquatic animal and plants species were reported in 2002 (Brander, 2007; Halweil, 2008). About a quarter of the marine fish capture is not utilized for direct human consume, but for the production of fishmeal as a source of feed for agriculture and aquaculture, so unless aquaculture and marine fishing can be decoupled, particularly through development of alternative sources of feed, aquaculture is unlikely to provide a solution to the problem of maintaining a sustainable supply of fish. Environmental degradation from aquaculture practices has been reported also (Cole, Cole, Gaydos, Gray, Hyland, Jacques et al, 2009). Their negative effects such as: organic pollution and eutrophication, a buildup of excess nutrients and wastes in some cases spilled to sea without any depuration process, cause at the same time algal bloom, depletion of oxygen, reduction in water quality and habitat destruction. Consequently, aquaculture farms can contribute, as well, to climate change.

**1.2. Climate change, global warming and seafood**

According to the aspects mentioned above and many referenced publications, some of the most profound and direct impact of climate change over the next few decades will be on agricultural and food systems and consequently on global food security (Gregory, Ingram, & Brklacich, 2005; Brown, & Funk 2008; Miraglia, Marvin, Kleter, Battilani, Brera, Coni et al, 2009) and obviously, on seafood products.

Food safety is defined by FAO (FAO, 2009) as “situation that exists when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious

food that meets their dietary needs and food preferences for an active and healthy life". This definition comprises four key dimensions of food supplies related to climate change, which in the specific case of seafood products are: seafood availability, seafood accessibility, seafood utilization and seafood stability.

**Availability of aquatic foods** will vary, positively and negatively, through changes in habitats, stocks and species distribution. These changes will occur inland, and in coastal and marine systems and will impact on aquaculture.

**Stability of aquatic food supply** will be impacted by changes in seasonality, increased variance of ecosystem productivity, supply risks and other issues that may also have large impacts on supply chain costs.

**Access to aquatic foods** will be affected by changes in livelihoods and catching or culture opportunities combined with transferred impacts from other sectors, competition for supply, etc.

**Utilization of aquatic products** and the nutritional benefits produced will be impacted by changes in range and quality of supply; market chain disruptions; greater food safety issues; and reduced opportunities to consume preferred products. This is particularly critical for countries with high per capita fish consumption.

Those four keys are reinforced in the specific case of seafood and according to "FAO expert workshop on climate change implications for fisheries and aquaculture report" (FAO Report 2008, Brunner, Jones, Friel & Bartley, 2009) because "fisheries and aquaculture play a crucial role for food supply, food security and income generation".

Fish is highly nutritious, rich in micronutrients, minerals, essential fatty acids and proteins. It represents a valuable supplement to diets that otherwise lack essential vitamins and minerals, providing nutrients that have particular importance in natal and child health and development. In rich countries much attention is being given to the

related health benefits, and official recommendations for European and North Americans are to eat more fish. Furthermore, fish products provide to more than 2.8 billion people (2.6 billion from developing countries) with about 20 percent of their intake of animal protein per capita, and in some cases, like small island and several developing countries, fish contributes to, or exceeds, 50 percent of the **total animal protein** intake.

Food safety policies and research must be focused in detecting and rigorously quantifying risks for consumers as soon as possible by the use of precise and secure methodologies. Concerning to seafood quality, it is important to emphasise the nutritional maintenance of seafood properties but also the contaminant tradeoffs (Yaktine, Nesheim, & James, 2008). But controlling seafood safety and quality management is more difficult than the assessment of terrestrial food, due to the obvious great number of consumable marine species, their accessibility and biodiversity, the difficulty for oceans toxicological control and many other causes.

It is unquestionable that climate change is one of several emerging and unresolved issues that affect the marine environment and requires more attention by the administration and by the scientific community (Aricò, 2008). Great effort is being made to develop strategies to predict and quantify, from the ecological and productive marine perspective, the effects the climate change (Brander, 2007; Brunner, Jones, Friel, & Bartley, 2009; Pelletier, Ayer, Tyedmers, Kruse, Flysjo, Robillard, Ziegler, & Sonesson, 2007; Pitcher, 2008). Proteomics, like other “omics” approaches (Davies, 2009), has a potential for the study of food products, and specifically for seafood (Halweil, 2008).

## 2. Proteomics

Proteins play crucial role in almost every biological process. Besides providing structural support, they are responsible of an ample variety of physiological functions (catalysis, transport and sensing) in all living systems. Whereas the genome of an organism (entire set of genetic material) is more or less constant and specific, the proteome (entire complement of proteins expressed by a genome) is highly dynamic. Expression and quantity of proteins varies between different types of cells in the same organism and in the same type of cells, in response to a very diverse stimuli and environmental factors.

### 2.1. Definition and methodologies

The proteome reflects the biological context of one particular biological system at a particular time. As a discipline, proteomics not only includes the structural and functional knowledge of proteins, but also the study of their modifications, the interactions between them, the study of their intracellular localization and the quantification of their expression. In this sense, Proteomics is the large-scale study of one proteome under different perspectives:

a. Expression proteomics
b. Differential expression proteomics
c. Cellular map and interaction proteomics.
d. Posttranslational modification studies or Modificomics.

a. - Expression proteomics is focused on large-scale characterization and quantification of all components of a particular proteome, under precise and determinate conditions.

Under the proteome term can be included organisms, tissues, cells or sub-cellular components.

b. - Differential expression proteomics compares dynamic changes in the proteome of two or more different samples. This involves the differential identification and quantification of the components in a determinate proteome in dependence with the changes derived from the different states. These changes can be due to external or internal features like cellular differentiation, pathologic alterations, chemical, physical and biological agents or conditions, etc.

c. - Interaction proteomics or cellular map proteomics studies the relationships among proteins in one determined biological system (Blackstock & Weir, 1999). This approach responds to the systems biology concept in which proteins are considered as part of dynamic molecular complexes and not as individual and isolated components.

d. - Proteins can suffer posttranslational modifications (PTMs) in response to a wide range of extra- and intra-cellular signals. This is the subject of fourth type of proteomic approach (Hunter, 1995). More than 300 different PTMs have been described (Aebersold & Goodlett, 2001; Jensen, 2004); among them, phosphorylations and glycosylations are the most abundant in the literature although protein lipidations, nitrosylations, sulfations or oxidative modifications are also responsible for many essential functions. Posttranslational modifications regulate many different purposes in various cellular processes such as enzyme activation/inactivation, signal transduction, gene expression, cellular protein localization, protein/protein interactions and protein stability. Genomic data can partly be used for the prediction of PTMs, and specific software and databases are rapidly evolving (Xue, Li, Wang, Feng & Yao, 2006; Reinders & Sickmann, 2007; Abu-Farha, Elisma, Zhou, Tian, Zhou & Asmer, 2009). Protein modifications are site-specific and polymorphic, often transient, time and

location specific. The study of PTMs, called "modificomics", is very difficult and requires the use of a versatile battery of methodologies. PTMs constitutes an area of interest frequently studied in proteomics research and especially for our specific issue, "modificomics" will yield many important insights.

From the analytical perspective, in the last ten years there has been an exponential development concerning to proteome analysis techniques (Chen & Pramanik, 2008; Gehlenborg, Yan, Lee, Yoo, Nieselt, Hwang, Aebersold & Hood, 2009; Wepf, Glatter, Schmidt, Aebersold & Gstaiger, 2009) and sample preparation (Cañas, Piñeiro, Calvo, López-Ferrer & Gallardo 2007), thanks mainly to mass spectrometry (MS) detection methodologies improvement and the information obtained has been in a new conceptualization: Systems Biology, which has constituted an inflexion point for the study and understanding of this and many other scientific disciplines.

Mass spectrometry is the most comprehensive and versatile tool in large-scale proteomics and their approaches, advances and applications have experimented rapid growth in last decades. MS analysis is based on the ionization of chemical compounds to generate charged molecules in the gas phase under high vacuum, which masses are measured after a separation on the basis of their mass-to-charge (m/z) ratio. Further, molecule fragments may be produced by diverse mechanisms as Collision Induced Dissociation (CID) and the "so-called" fragment-ions separated and measured as well by their m/z ratio. Spectra can be produced in a fraction of time, giving information about the molecular weight or the structure of a compound. Basic to the operation of MS instruments, which operate under high vacuum, are the *ion source,* which produce gas phase ions (or, in the case of electrospray ionization, move ions that exist in solution into the gas phase); the *mass analyzer*, which sorts the ions by their masses by applying electric or magnetic fields; and the *detector*, which measures the abundances of ions.

There are different ion sources and mass analyzers combining up in different type of mass spectrometers. The ionization techniques traditionally used for volatile organic compounds were not applicable to peptides and proteins molecules. It can be intuitively realized that it is not easy to ionize and put into gas phase big and polar molecules without any fragmentation or decomposition. The development, in the late 80s, of two new ionization techniques, electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), allowed for first time the acquisition of mass spectra with peptide or protein amounts lower than 1 pmol, opening the door for rapid and sensitive protein characterization (Yates, Ruse & Nakorchevsky, 2009). Today MS is not just an establish tool for structural proteomic research, it is more than ever at the forefront of functional and expression proteomics, being used for protein quantification thanks to the stable isotope labelling procedure improvements, like: "isotope-coded affinity tags" (ICAT), "isotope tagging reagents for relative and absolute quantitative" (iTRAQ), "stable isotope labelling with amino acids in cell culture" (SILAC) or $^{18}O$ post-digestion labelling (Bettmer, Montes Bayón, Ruiz Encinar, Fernández Sánchez, Fernández de la Campa & Sanz Medel, 2009). An in-depth analysis of state of the art of proteomic instrumentation is out of the scope of this article and readers are referd to some of the recent reviews concerning mass spectrometric protein analysis (Batoy, Akhmetova, Miladinovic, Smeal & Wilkins 2008; El-Aneed, Cohen & Banoub, 2009; Ahmed, 2008; König 2008; Yates, Ruse & Nakorchevsky, 2009; Lee, Soper & Murray 2009; Pan, Aebersold, Chen, Rush, Goodlett, McIntosh et al, 2009).

Bearing in mind the variety of available technical options, two main different proteomic work-flow approaches are considered, as it is outlined in figure 1:

**Bottom-up proteomics**:

Proteins are first purified by different methodologies, typically bidimensional gel electrophoresis (2-DE), and selected protein spots are digested to peptides, using typically trypsin as the proteolytic enzyme. Later, peptides masses and either, fragment ions produced by CID, are measured in a mass spectrometer. Using specialized software for searching protein databases, called search engines, a comparison between experimental MS data and the theoretically predicted from the information contained in data-bases is performed. In this way, spectra are assigned to putative proteins by, either peptide mass fingerprinting (PMF), or by Fragment Mass Fingerprinting (FMF), using in this case the peptide fragmentation spectra. Putative peptide sequences, which are used for the identification of the corresponding proteins, are assigned. This is the workflow known as the "classical" Bottom-Up proteomic approach.

Alternatively, the crude protein extract can be digested directly, without any previous separation, and the produced peptides separated by multi dimensional separation by liquid chromatography coupled to a mass spectrometer. Peptides are identified from the fragmentation spectra, as described earlier, making use of search engines. This Bottom-Up approach is known as multidimensional protein identification, MudPit.

**Top-Down proteomics**

In Top-Down proteomics, intact proteins, without any previous enzymatic digestion, are identified and characterized by deducing a partial amino acid

sequence after fragmentation in a tandem mass spectrometry experiment. Proteins are previously purified by various chromatographic procedures and analyzed by MS/MS using high resolution mass spectrometers such as Fourier Transform ion cyclotron resonance (FTICR) or Orbitrap. Protein exact masses are obtained in these high resolution mass spectrometers and, consequently, modifications may be deduced from the difference between theoretical and experimental masses. Fragmentation studies may provide the exact localization of modifications. Nevertheless, spectra produced are very complicated and software for top-down is not so developed as that for bottom-up proteomics.

**2.2. - PTMs in marine organisms**

Methodological approaches able to detect subtle changes in the expression of individual proteins and amino acid sequence modifications, constitute an excellent tool in order to reflect biological responses to stimuli, facilitating the search for Biomarkers (Smith, Salaberria, Cash & Pärt, 2007; Nesatyy & Suter 2008; Ahmed, 2009), which can be explanatory of a biological situation or may function as "flags" to discriminate groups of organisms revealing sets of specific peptides for a given condition (Kussmann, Affolter & Fay, 2005). Specific structural modifications are reflected by changes in the relative molecular mass ($M_r$) of the corresponding amino acid/s, resulting in a change in the $M_r$ of the original protein, so the determination of the stochiometry of these specific modifications opens a possibility for the evaluation of the effect of the climate change in marine organisms.

Until now, the majority of specific protein modifications described in marine organisms, originated by climate change effects, are due to the oxidative stress caused by

environmental pollution, thermal stress and exposition to ultraviolet radiation. As an example, high levels of these modifications have been showed by 2-DE and immunoblot in the digestive glands of the marine bivalves (Chora, McDonagh, Sheehan, Starita-Geribaldi, Roméo, & Bebianno, 2008). Metals in aquatic environments can generate the formation of reactive oxygen species (ROS), producing effects as carbonylation and ubiquitination of proteins (McDonagh, Tyther, & Sheehan, 2006). Specific staining methods to detect carbonyl groups in proteins separated by 2-DE have been developed (Talent, 1998). These methods involve the derivatization of the carbonyl group with dinitrophenylhydrazine (DNPH) prior to the electrophoresic separation, detecting it, on the blot membrane after transfer, using immunostaining with an anti-DNP antibody. The spots of interest can be later analyzed and identified by MS/MS.

The methods used to detect ubiquitination are based in the separation of the proteins by 2-DE and the specific staining using anti-ubiquitin antibodies after blotting the proteins to a membrane (McDonagh, Tyther & Sheehan, 2006 a, b). Mass spectrometric methods may be used to study this type of modification using characteristic mass shifts and fragment ions. After tryptic digestion, the ubiquitinylated proteins produces diglycine branched peptides that can be used as markers in MS/MS analysis (Wang, Li, Dangott & Wu, 2006).

Other common modification that may be produced due to oxidative stress is the glutathionylation of proteins which is the reaction between free cysteine groups and the reduced form of glutathione, catalyzed by glutaredoxin. This modification was found in proteins from the digestive glands in the blue mussel *Mytilus edulis* by 2-DE and western blot using anti-GSH antibodies (McDonagh, Tyther & Sheenan, 2005).

Also, in relation with oxidative stress, metallothioneins, a family of Cys-rich proteins with the capacity of bind metal atoms, both physiologically important (Zn, Cu, Se), as those xenobiotic (Cd, Hg, Ag), have been studied due their role in the protection of marine organisms against metal toxicity and oxidative stress. Proteomics may offer new sensitive and specific tools for their determination as new potential biomarkers for oxidative stress conditions (McDonagh & Sheenan, 2008; Kjersem, Aa, Meier, Goksøyr & Grøsvik, 2008).

The described modifications may be considered as the top of the iceberg of a, yet undiscovered, higher number of potential protein chemical transformations related to climate change. Table 1 describes an overview of the strategic workflow to evidence the main PTMs. Specific details about different modifications, like shifts in amino acid molecular masses and procedures to carry out specific sample enrichment, are shown. Methodologies used include 2-DE, blotting, and all those sample preparation techniques useful for the purification of peptides and proteins with an specific modification, incluiding the use of commercial kits (Cañas, Piñeiro, Calvo, López-Ferrer & Gallardo, 2007).

## 3. Proteomics applications to evaluate climate change effects over marine products

In marine biology, proteomics allows a better understanding of the mechanisms underlying changes in fish and invertebrates protein metabolism induced by alterations due to biotic or abiotic variables (Viant, 2007; Fraser & Rogers, 2007), although at present, the majority of the studies are related to species held under laboratory conditions. Next, applications which can be considered as examples of potential role of

proteomics in the assessment of climate change effects on marine organisms are described.

### 3.1. Environmental applications

The applications of proteomics to environmental toxicology have been developed from two perspectives: (i) the identification of new molecular targets for toxic substances with the aim to know their mechanism of action, and (ii) the utilization of the information produced by changes in the patterns of protein expression for a predictive screening of toxic compounds action (Nesatyy & Suter, 2008). A recent review about the application of proteomics to the evaluation of cellular response in marine animals exposed to a toxin mixture is explanatory (Sala, Ronzitti, Sasaki, Fuwa, Yasumoto, Bigiani et al, 2009).

As it was mentioned above, although the application of proteomics to marine science has been not so extensive as the applications in Biomedicine (Nunn & Timperman, 2007), proteomics methodologies have been applied in ecological studies in the marine context, mainly to evaluate and to define different and specific environmental protein biomarkers and stress responses (Viarengo, 2006; Johnson & Browman, 2007; Nesatyy, Ammann, Rutishauser, Suter, 2006; Nesatyy & Suter, 2008 Sheehan, Tyther, Dowling & McDonagh, 2007; Amelina, Apraiz, Sun & Cristobal, 2007; Prunet, Cairns, Winberg & Pottinger, 2008). Therefore, proteomics has been used to evaluate the modifications of proteins like, metallothionein, cholinesterase, cytochrome p450 monooxigenase system and other related to ecotoxicology issues in a variety of "sentinels" marine species defined as excellent indicators for environmental stress and potential health threats for humans (Stewart, Gast, Fujioka, Solo-Gabriele, Meschke, Amaral-Zettler,

Polz, Collier, Strom, Sinigalliano, Moeller & Holland, 2008). In that sense, recently, a new discipline dedicated to this topic has been described: Ecotoxicproteomics (Gomiero, Pampanin, Bjørnstad, Larsen, Provan, Lyng & Andersen 2006).

Most important "sentinel species" from the seafood point of view are bivalves (Saavedra Carlos & Bachére 2006), mainly mussels (Zorita, Apraiz, Ortiz-Zarragoitia, Orbea, Cancio, Soto, Marigómez & Cajaraville, 2007) and clams (Dowling, Hoarau, Romeo, O'Halloran, Van Pelt, O'Brien & Sheehan, 2006).Their abundance, wide geographical spread, taxonomic variety, and close contact with aqueous sources of environmental toxicology, as they have high capacity for adaptation to environmental pollution, makes them the indicators of choice for marine pollution. There are, as well, some reports on the evaluation of the environmental impact over some vertebrate aquatic species like rainbow trout (*Onchorynchus mikiss*) (Smith, Salaberria, Cash & Pärt, 2007), Atlantic salmon (*Salmo salar*) (Provan, Bjørnstad, Pampanin, Lyng, Fontanillas, Andersen, Koppe & Bamber 2006; Salbu, Denbeigh, Smith, Heier, Teien, Rosseland, Oughton, Seymour & Mothersill, 2008) cod (*Gadus morhua*) (Smith, Tunsjo, 2007), flatfish (*Limanda limanda*) (Ward, Wei, Cheng, Billingham, Martin, Johnson, Lyons, Feist & Stentiford, 2006), zebra fish and some other (Kültz, Fiol, Valkova, Gomez-Jimenez, Chan & Lee, 2007), which may be considered as potential sentinel species. In all the cited examples, proteomics tools have been used (Bainy, 2008).

A compilation of the use of proteomics as a tool for evaluating the climate change consequences over the marine environment is showed in Table 2. It must be pointed out that, in most cases, it is going to be possible to derive conclusions about seafood safety from the environmental research studies.

### 3.2. Seafood applications

Proteomics is built upon the foundations of genomics, in such way that a lack of genomic information on a particular species can substantially limit the success in protein identification (Graham, Elliott & Van Eyk, 2005). Sequence data from marine fish and shellfish species is scarce in databases, limiting the easiness with which proteomics information can be obtained (Piñeiro, Barros-Velázquez, Vázquez, Figueras & Gallardo, 2003). Japanese pufferfish (*Takifugu rubripes*), zebra fish (*Danio rerio*) and the estuarine fish (*Fundulus heteroclitus*) are among the few marine organisms for which the complete genome sequences are available, facilitating the application of proteomic research to the marine environment (Parrington, & Coward, 2002; Burnett, Bain, Baldwin, Callard, Cohen, Di Giulio, Evans, Gómez-Chiarri, Hahn, Hoover, Karchner, Katoh, MacLatchy, Marshall, Meyer, Nacci, Oleksiak, Rees, Singer, Stegeman, Towle, Van Veld, Vogelbein, Whitehead, Winn & Crawford, 2007; Wang, MacKenzie, De Souza, Zhong, Goss & Li 2007; Lemeer, Pinkse, Mohammed, Van Breukelen, Hertog, J. Slijper & Heck 2008).

Proteomics has already been applied to specific aspects of seafood research and technology, but there are no reviews or compiled information focused on the study of the influence of the climate change over seafood using proteomics methodologies. According to the available scientific information, the published proteomics applications related with seafood production and safety have been classified in three main items: (i) food safety studies, (ii) authentication and taxonomic applications and (iii) nutritional aspects. References on the applications to wild and cultivated species have been included for these aspects.

### 3.2.1. Seafood safety applications: microbiological, toxicological, allergenic, parasitological and pathological issues.

The main effects of climate change and global warming over seafood are related to their safety (Miraglia, Marvin, Kleter, Battilani, Brera, Coni et al, 2009). As it was described above, fish and other marine organisms must make a high physiological effort and develop adaptation mechanisms to survive the stress caused by those fluctuating conditions and contaminants. Those strategies can be flawed by energetic losses and vulnerability to pathogen infections, which are more noticeable in cultivated species. Besides, human activities have also contributed to increase the microbial contamination degree in the oceans.

Although the application of "OMICS" technologies to food safety research is increasing (Davies, 2009), specially with the purpose of understanding the complexities of pathogen behaviour at the molecular level (Fratamico, 2008), specific research in seafood pathogens has been scarce (Martinez & James, 2004). However, it is possible to find many general references about proteomic tools applied to fast bacterial classification from early years (Lay 2001; Emerson, Dworzanski & Snyder, 2005; Dworzanski, Deshpande, Chen, R., Jabbour, Snyder, Wick & Li, 2006; Agulto, Liu & Liu, 2008) and applications to the study of common pathogenic seafood bacteria such as *Listeria* and *Staphylococcus* genus (Hain, T., Chatterjee, S. S., Ghai, R., Kuenne, C. T., Billion, Steinweg, C., Domann, Kärst, Jänsch, Wehland, Eisenreich, Bacher, Joseph, Schär, Kreft, Klumpp, Loessner, Dorscht, Neuhaus, Fuchs, Scherer, Doumith, Jacquet, Martin, Cossart, Rusniock, Glaser, Buchrieser, Goebel & Chakraborty, 2007; Guilbaud, Chafsey, Pilet, Leroi, Prévost, Hébraud, & Dousset 2008; Ho & Reddy, 2008; Scott & Cordwell, 2009), mentioning nutrient limitations and starvation (Schweder,

Markert & Hecker, 2008) or salt concentration response (Wu, Lin, Wang, Ye, Xiao, Wang & Peng 2006) as climate change consequences.

According to environmental studies and as it was mentioned above, the increase of bloom-forming cyanobacteria and other toxins producers is another consequence of the climate change related to seafood safety. Contamination of marine products by algal toxins is a widespread phenomenon, posing significant threats to human and animal health. Proteomics methodologies have been applied to cyanobacteria research more extensively than to other marine microorganisms (Ow & Wright, 2009). Biomarkers in toxin-producers species have been characterized (Chan, Sit, Lam, Hsieh, Hodgkiss, Wan, Ho, Choi, Wang & Dudgeon 2006) but lately, the research is focused to the study of algal toxins response in fish and shellfish by means of protein markers defined by proteomics (Mezhoud, 2008; Ronzitti, Milandri, Scortichini, Poletti & Rossini, 2008 and Sala, Ronzitti, Sasaki, Fuwa, Yasumoto, Bigiani et al, 2009; Castielli, De la Cerda, Navarro, Hervás & De la Rosa, M. A. 2009). In parallel, cyclic peptides which are marine biotoxins, especially microcystins, have been characterized using different mass spectrometry techniques (Liu, Ng, Meluzzi, Bandeira, Gutierrez, Simmons et al, 2009; Ferranti, Fabbrocino, Nasi, Caira, Bruno, Serpe et al, 2009; Draper, Xu & Perera, 2009). Allergenic proteins, pivotal from the point of view of seafood safety have studied as well using proteomics techniques. As it is already known, fish and shellfish contain different and well known allergens, like tropomyosins and parvalbumins (Taylor, 2008). During the last years, proteomics has been applied to food allergies studies (Carbonaro 2004 Chardin & Peltre, 2005). Isoforms of allergen proteins have been characterized in fishing products (Permyakov, Karnoup, Bakunts & Permyakov, 2009), as it has been done with specific peptide sequences indicative of parvalbumin presence in hake manufactured products (Carrera, Cañas, Piñeiro, Vázquez & Gallardo, 2006). Shellfish

allergy has been studied (Motoyama, Suma, Ishizaki, Nagashima & Shiomi 2007) and some allergens have been included in the data base using proteomic strategies: arginine kinase from *Penaeus monodon* (Yu, Lin, Chiang & Chow, 2003, ), the black tiger shrimp, and myosin light chain from the white leg Pacific shrimp (*Litopenaeus vannamei*) (Ayuso, Grishina, Bardina, Carrillo, Blanco, Ibáñez, Sampson, Beyer, 2008) and several commercial specific ELISA tests have been developed using those prawn proteins.

Parasite detection in clinical and animal samples has been other food safety issue in which proteomics has been applied (Barrett, Jefferies & Brophy, 2000), using immunologic and molecular procedures. The work of Lefevre and co-workers makes reference to proteomics focused on host-parasite interactions mentioning an aquatic host (Lefèvre, Adamo, Biron, Missé, Hughes & Thomas, 2009).

Related to cultivated marine species safety, proteomic strategies has been used to protein monitoring in aquaculture pathology (Booy, Haddow, Ohlund, Hardie & Olafson, 2005), characterization of the virus which causes the white spot syndrome in cultivated shrimp (Huang, Zhang, Lin, Xu, Hu & Hew, 2002; Li, Lin, Chen, Wu, Lim, Loh, Tang & Hew, 2007) and the effects over fish of endocrine disruption chemicals (EDCs) (Martyniuk & Denslow, 2009).

There are safety risks associated with the use of chemical compounds in animal feeds, including aquafeeds, which may include: veterinary drugs residues, persistent organic pollutants, chemical and solvents residues used in agriculture, heavy metals and mineral salts. Biological contamination in animal feeds is possible, as well: *Salmonella* and transmissible spongiform encephalopaties, which are detected using proteomics methodologies (Tacon & Metian, 2008), among others.

### 3.2.2. Taxonomic and seafood authentication applications

Fish stock distribution and sustainability has suffered seriously as a consequence of the climate change. Variations in ecosystems composition and population and the decrease of autochthonous species have been produced, along with an increase in the presence of foreigner species in traditional fishing grounds, because these species belonging to same genera and also with similar morphological features may be producers of toxic compounds originating a risk for consumers' safety. Robust molecular tools based in nucleic acid and protein analysis are today available for the differentiation of very similar species.

Tools for taxonomic authentication for gadoid species have been developed using proteomics methodologies (Piñeiro, Vázquez, Marina, Barros-Velázquez & Gallardo, 2001; Martinez & Fritzs, 2004; Carrera, Cañas, Piñeiro, Vázquez & Gallardo, 2007) and a specific fast analytical procedure has been patented in Spain (patent Spanish number 200603287). The presence or absence of specific peptides, which may be detected by their masses using mass spectrometry in fresh, frozen, or processed fish, can evidence species authenticity in a short analysis time.

The use of proteomics for taxonomic purposes has been extended to other marine species like, Pleuronectiformes (flat fishes) and Perciformes, as mullets, seabasses, seabreams, etc (Mazzeo, De Giulio, Guerriero, Ciarcia, Malorni, Russo & Siciliano 2009) and more recently to prawns and shrimp (Ortea, Canas, Calo-Mata, Barros-Velázquez & Gallardo 2009).

### 3.2.3. Nutritional and quality seafood applications

A key scientific objective in nutrition research is to determine the role of the diet in metabolic regulation to improve human health. Depending on individual genotypes, nutrients may modify the metabolic pathways through regulating the expression of genes and the translation of proteins, improving health status (Kussmann & Affolter, 2006; Zhang, Yap, Wei, Chen & Chen, 2008). Nutritional proteomics or Nutriproteomics (Barnes & Kim, 2004) means the application of the proteomics methodologies to nutrition-related research and, among other topics, focus on the interaction of bioactive food ingredients with proteins (Schweigert, 2007), which may be investigated following two different procedures: (i) searching for the effect of bioactive food ingredients on protein synthesis via gene expression and, (ii) examining the interaction of these ingredients with proteins looking for post-translational modifications or for small molecule/protein interactions.

As it has been done with other omics-based nutrition approaches (nutritranscriptomics, and nutrimetabolomics), most of nutriproteomics investigations have been performed using animal models (Wang, Li, Dangott & Wu, 2006). There are only some applications of nutriproteomics to seafood as source of bioactive ingredients. One of them, is the study of the molecular mechanisms regulated by the consumption of PUFA (Mutch, Wahli & Williamson, 2005; De Roos, Geelen, Ross, Rucklidge, Reid, M., Duncan, Caslake, Horgan, & Brouwer 2008). Proteomics has been used to identify proteins which serum levels were altered by the administration of long chain ω-3 polyunsaturated fatty acids (LC-PUFA) from fish oil. A comparison was made with the alterations produced when sunflower oil, rich in oleic acid, was dispensed. A down-regulation of the serum proteins related to coronary heart disease in the patients whose

diets were supplemented with fish oils, was evident.Proteomics applications to the study of nutritionally relevant proteins have been reported (Alomirah, Alli & Konishi, 2000; Kvasnicka, 2003), also focusing on their influence in human health (Kussmann, Affolter & Fay, 2005). In that context, fish and other seafood products contain nutritional and relevant proteins, related to organoleptic fish products properties and consequently to their quality degree, with a high content of essential amino acid, which are abundant in the edible parts. The structure of these proteins can be affected during the process of manufacture and storage (Herrero, 2008).

Proteomics methodologies are the most adequate to evaluate the changes occurred in proteins, as those which occurred during cod and tuna frozen storage (Kjærsgård, Nørrelykke & Jessen 2006a; Kjærsgård, Nørrelykke, Baron & Jessen, 2006b; Schiavone, Zilli, Storelli & Vilella, 2008; Kinoshita, Sato, Naitou, Ohashi & Kumazawa, 2007) and pre-slaughter activity (Morzel, Chambon, Lefèvre, Paboeuf & Laville, 2006).

Monitoring nutritional differences between wild and farmed species is other of the applications of nutriproteomics (Monti, De Napoli, Mainolfi, Barone, Guida, Marino & Amoresano, 2005; Eriksson, 2005; Reddish, St-Pierre, Nichols, Green-Church & Wick, 2008). Cultivated species adaptation to global climate change has received special attention by researchers. Proteomics strategies have been used in preliminary physiological and biochemical investigations (Martin, Cash, Blaney & Houlihan; Overturf & Gaylord, 2009). The relationship between cold acclimation and sarcoplasmic proteins expression in carp muscle, have been studied (McLean, Young, Doherty, Robertson, Cossins, Gracey, Beynon & Whitfield, 2007). When fishes were cooled to 10ºC, there was an accumulation for the fragments of the enzyme creatine-

kinase (CK), consistent with the observed higher muscle CK proteolysis associated with the decrease of water temperature.

## 4. **Challenges**

Understanding the adaptation strategies of marine organisms to the global climate change must be based in multidisciplinary studies from an "ecosystem approach", defined as a comprehensive and holistic vision for the understanding and anticipation of the ecological changes, assessing the full range of consequences, and developing appropriate management responses. Consequently, ongoing study of the climate change phenomenon and its impact on the fisheries ecosystem and therefore on seafood products, is crucial. At this point, proteomics, developed to study complex systems, demanding sophisticated, robust and fast analytical methodologies, capable of identifying in parallel hundreds of seafood proteins, with a variety of PTMs at different expression levels, by means for instance of microarray technologies (Zhang, Yap, Wei, Chen & Chen 2008), can play a strategic role. However, the preliminary steps of that work are impeded for the scarce number of sequenced marine proteins. So a first basic stage of sequencing research is indispensable.

Proteins can be related to pollution initiation or progression, safety, quality and nutrition topics, constituting in this sense important biomarkers for the detection of pollution, food safety, and nutritional value. But, to define, quantify and understand the significance of those protein biomarkers, even in early stages, in order to develop prevention strategies, a multidisciplinary collaboration is needed. The successful research must include disciplines as diverse as: Physiology, Genetics, Ecology, Cell Biology, Analytical Chemistry, Oceanography, Bioinformatics, Zoology and Botany

among others and, of course, to be planned from the Systems Biology approach. At this point, the first and easier step which must be focused is, obviously, to the study of cultivated species, because aquaculture is a valuable food supply and economic support for many countries (Cole, Cole, Gaydos, Gray, Hyland, Jacques et al, 2009) and it can be controlled from the food safety and ecological points of view.

## 5. Conclusions

From the literature discussed in this review, it is possible to consider proteomics as an indispensable scientific set of methodologies with an enormous potential in order to evaluate climate change effects on the production of food, and more specifically on the production of seafood, wild and cultivated. Nevertheless, until now only few research groups are implicated in the application of proteomics to seafood research, being most of the attention focused on seafood safety.

The lack of genome sequencing for most of the marine organisms, together with the difficulty in the *de novo* interpretation of peptide fragmentation spectra, has precluded a more extensive use of proteomics mass spectrometry techniques in the field of seafood production. Nevertheless, marine organisms DNA sequencing projects are underway and software for protein identification from mass spectrometry data is improving, facilitating the interpretation of spectra and the comparison. So, perhaps it is not too presumptuous to think that in few years proteomics will be widely used in seafood production studies.

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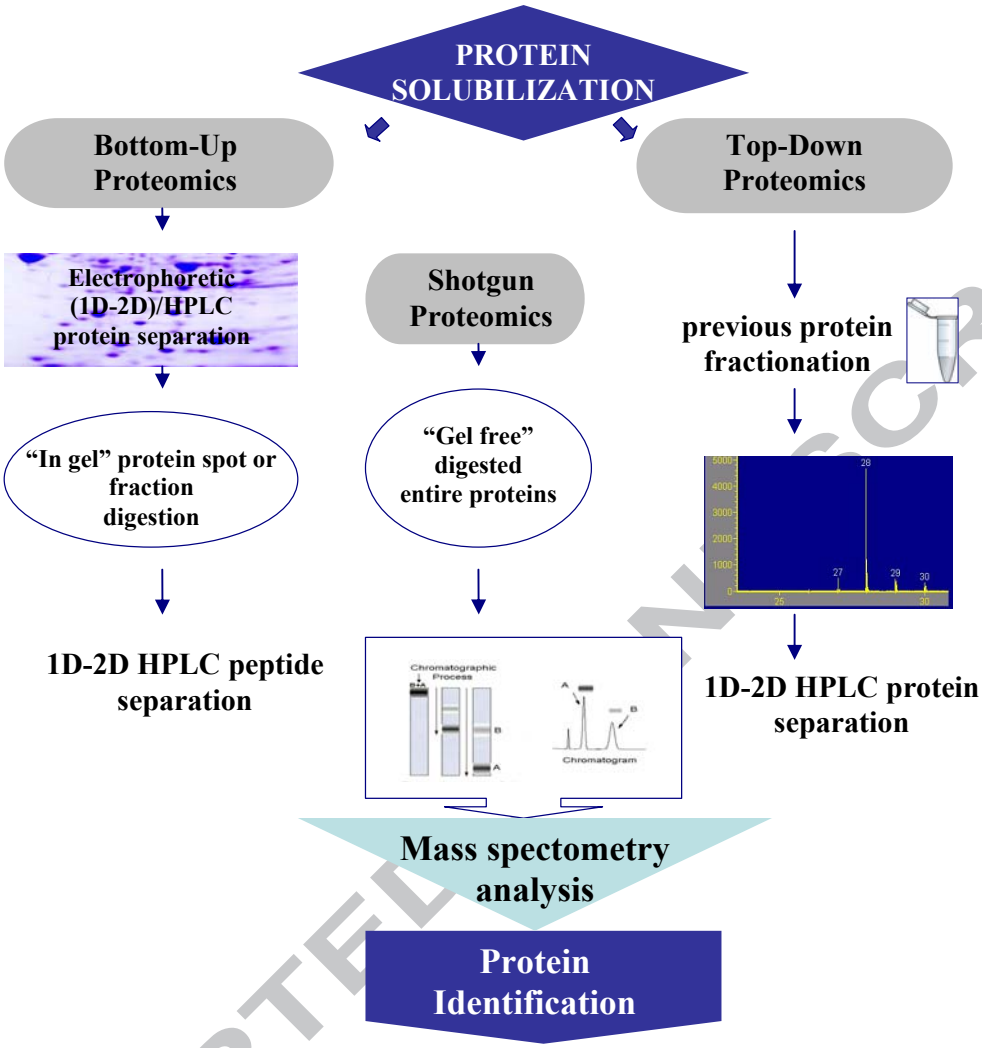


Figure 1: Proteomics strategies workflow

1

| MODIFICATION | AMASS (DALTONS) (AA) | SAMPLE ENRICHMENT METHODOLOGY OR COMMERCIAL REAGENT | DETECTION PROCEDURE BY 2-DE/WESTERN BLOT | MASS SPECTROMETRY (BOTTOM-UP APPROACH) |
|---|---|---|---|---|
| **PHOSPHORYLATION** | +80 (S, T, Y) | IMAC, $TiO_2$, SIMAC, $ZrO_2$, PAC, anti-pY, -pS, pT<br>Ion exchange chromatography | - Horizontal trail of spots (multiple p*I* variants)<br>- Specific staining: ProQ Diamond™<br>- Labeling phosphopeptides with $^{32}P$ or $^{33}P$ and autoradiography<br>- Anti-pS, -pT, -pY | - look a characteristic mass shifts in parent and fragment ion of + 80Da<br>-look for MS/MS signature ion of 216 Da pY and -79 or -63 Da for pT, pS or pY.<br>-monitor neutral loss of $H_3PO_4$ and $MS^3$ or MSA of the neutral loss (98, 49, 32.6 Da) |
| **GLYCOSYLATION**<br>**N-LINKED**<br>**O-LINKED** | >800 (N)<br>203, 800 (S,T) | - Affinity chromatography with lectins<br>- HILIC | - Multiple p*I* variants spots<br>- Specific staining: ProQ Emerald™ | -detecting the respective oxonium ion *m/z* 163 (hexose), 204 (HexNAc), 292 (sialic acid), 366 (hexose-HexNAc) |
| **OXIDATION** | +16 (C) | Reduction, then alkylation or S-S reactivity "biotin switch". Sulfinic acid reactivity with dimedone | Derivatization of carbonyl groups with DNPH prior to electrophoresis or into the membrane and immunobloting with anti-DNP | -look a characteristic mass shifts in parent and fragment ion of + 16, 32, 48 Da<br>- $MS^3$ neutral loss $CH_3SOH$ (64, 32, 21.3 Da) |
| **CARBONYLATION** | +30 (P, R, K, T) | Biotin hydrazide labeling and avidin affinity chromatography | Derivatization of carbonyl groups with DNPH prior to electrophoresis or into the membrane and immunobloting with anti-DNP | -$MS^3$ neutral loss CO (30, 15, 10 Da) |
| **HYDROXYLATION** | +16 (K, T, P, D) | | | -look a characteristic mass shifts in parent and fragment ion of + 16 Da |
| **ACETYLATION** | +42 (N-term, K, S) | Anti-acetyllysine | | - look a characteristic mass shifts in parent and fragment ion of + 42Da |
| **UBIQUITINATION** | >1000 | Pierce Ubiquitin Enrichment Kit™ | 2-DE separation and western blot with anti-ubiquitin | -look for a characteristic mass shifts in parent and fragment ion of +114 Da (Gly-Gly) |
| **METHYLATION** | +14 (A, M, K, S, T, N) | | | -look a characteristic mass shifts in parent and fragment ion of + 14 Da |
| **NITROSYLATION** | +34 (C) | Reduction, then alkylation or S-S reactivity "biotin switch" | Western with anti-SNO | - look a characteristic mass shifts in parent and fragment ion of + 30Da<br>- monitor neutral loss NO (30, 15, 10) |
| **ACYLATION:**<br>**FARNESYL**<br>**MYRISTOYL** | +204<br>+210 | Fatty acyl-biotinyl-exchange labeling<br>Immunoprocipitation | | - look a characteristic mass shifts in parent and fragment ion of + 204, 210, 238 Da<br>- monitor neutral loss |

| | | | | |
|---|---|---|---|---|
| **PALMITOYL** | +238 (C) | | | |
| **SULFATION** | +80 (Y) | | | -$MS^3$ neutral loss $SO_3$ (80, 40, 26.6 Da) |
| **XENOBIOTIC MODIFICATIONS** | Cd, Cu, Ni, Zn, Se | | | look a characteristic mass shifts in parent and fragment ion according to the metal ion |

**Table 1.** Overview of the workflow and of the classical proteomic strategies to evidence the main PTMs.

List of abbreviations: IMAC (Immobilized Metal Ion Affinity Chromatography), HILIC (Hydrophilic interaction liquid chromatography), PAC (phosphoramidate chemistry), DNPH (2,4-dinitrophenylhydrazine), DNP (2,4-dinitrophenol), SIMAC (sequential elution from IMAC), Anti pS (anti-phosphoserine), Anti pT (anti-phosphothreonine), Anti Py (anti-phosphotyrosine), Anti SNO (Anti-S-Nitroso-Cysteine).

1

| SUBJECT | ENVIRONMENTAL SEAFOOD PROTEOMICS APPLICATIONS TO EVALUATE CLIMATE CHANGE EFFECTS | REFERENCES |
|---|---|---|
| **PATHOLOGY** | Adaptive response of winter ulcer causative agent in farmed Atlantic salmon and cod. | *Tunsjø, Paulsen, Mikkelsen, L'Abée-Lund, Skjerve & Sørum, 2007* |
| | Environmental stressors effects over fish immune system | *Ripley, Iwanowicz, Blazer, & Foran, 2008* |
| | Mussel immune response changes | *Bussell, Gidman, Causton, Gwynn-Jones, Malham, K., Jones, Reynold,& Seed 2008* |
| | Dab liver tumor detection | *Ward, Wei, Cheng, , Billingham, Martin, A., Johnson, Lyons,., Stentiford, 2006* |
| **THERMAL AND OXYGEN FLUCTUATIONS** | Hemocyanin binding changes in crayfish (only IEF) | *Powell, & Watts, 2006* |
| | Metabolic responses in juvenile cod | *Pérez-Casanova, Afonso, L., Johnson, Currie, & Gamperl, 2008* |
| | Baseline expression of HSPs in Mediterranean species | *Lejeusne, Pérez, Sarrazin, & Chevaldonné 2006* |
| | HSP functions and role : review | *Evgen'ev, Garbuz, & Zatsepina, 2005* |
| | Effects in growth performance and fecundity of cod and eelpout (HSP). Not proteomics | *Pörtner, Langenbuch, & Michaelidis, 2005* |
| | Myoglobin Expression levels influenced by hypoxia in carp. (microarrays) | *Fraser, De Mello, Ward, Rees, Williams, Fang, Gracey, ,& Cossins, , 2006* |
| | Oxidative proteomic stress and HSP in clams | *Dowling, Hoarau, Romeo, O'Halloran, Van Pelt, O'Brien, & Sheehan, , 2006* |
| | Oxidative stress in mussels | *McDonagh, & Sheehan, 2007* |
| | Redox proteomics in mussel | *McDonagh, & Sheehan 2006 a y b* |
| | Ubiquitination and carbonylation as oxidative stress indicators in clams | *Chora, McDonagh, Sheehan, Starita-Geribaldi, Roméo, & Bebianno 2008* |
| | Proteomic of mud crab gill under low temperature adaptation | *Wang, Kong, Wang, & Li, 2007* |
| **ENVIRONMENTAL TOXICOLOGY** | Zebra fish model in assessment | *Scholz, Fischer, Guñdel, Kuster, Luckenbach, & Voelker, 2008.* |
| | Mussel as sentinel in pollution | *Zorita, Apraiz, Ortiz-Zarragoitia, Orbea, Cancio, Soto, Marigómez, & Cajaraville, 2007* |

| | | |
|---|---|---|
| | Heavy metals and stress effects in Atlantic salmon | *Salbu, Denbeigh, Smith, Heier, Teien, Rosseland, Oughton, Seymour, & Mothersill, 2008* |
| | Goldfish liver adaptation to environmental stress | *Wang, Wei, Wang, Chan, & Dai, 2008* |
| | Rainbow trout proteomic endocrine disruptors characterization as biomarkers | *Smith, Salaberria, Cash, & Parït, 2007* |
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1 Table 2: Marine environmental proteomics applications
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