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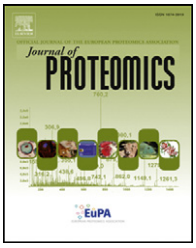

Proteomic-based comparison between populations of the Great Scallop, *Pecten maximus*☆

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ABSTRACT

Comparing populations residing in contrasting environments is an efficient way to decipher how organisms modulate their physiology. Here we present the proteomic signatures of two populations in a non-model marine species, the great scallop *Pecten maximus*, living in the northern (Hordaland, Norway) and in the center (Brest, France) of this species' latitudinal distribution range. The results showed 38 protein spots significantly differentially accumulated in mantle tissues between the two populations. We could unambiguously identify 11 of the protein spots by Maldi TOF–TOF mass spectrometry. Eight proteins corresponded to different isoforms of actin, two were identified as filamin, another protein related to the cytoskeleton structure, and one was the protease elastase. Our results suggest that scallops from the two populations assayed may modulate their cytoskeleton structures through regulation of intracellular pools of actin and filamin isoforms to better adapt to their environment.

Biological significance

Marine mollusks are non-model organisms that have been poorly studied at the proteomic level, and this article is the first studying the great scallop (*P. maximus*) at this level. Furthermore, it addresses population proteomics, a new promising field, especially in environmental sciences.

This article is part of a Special Issue entitled: Proteomics of non-model organisms.

1. Introduction

Measurement of natural variation, within and between populations, is of major concern for population biologists and ecologists. Indeed, populations of organisms can reflect the overall history of adaptations and the evolutionary forces that have shaped them, and is often the starting point for functional ecological studies [1]. Application of proteomics to natural population comparison, i.e. "population proteomics", was originally developed to find cancer-specific biomarkers in humans [2,3]. However, this new research field quickly gathered interest in the environmental sciences. For example, it was successfully applied to differentiate eight ecotypes of *Arabidopsis thaliana* on the basis of their two dimensional gel

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electrophoresis (2-DE) proteomic signatures [4]. Several 2-DE based studies were also carried out on natural populations of marine organisms, i.e. mollusks [5–7] and fishes [8–10]. Notably, population proteomics was successfully used to discriminate natural populations of the European Hake (*Merluccius merluccius*) [11]. Here we describe a 2-DE based proteomic study comparing two populations of the great scallop, *Pecten maximus*.

P. maximus is a suspension-feeding bivalve mollusk living along the north-east Atlantic coasts from Norway to Morocco, and extending into the Mediterranean Sea in the western part of the Alboran Sea. Like many other marine bivalves, *P. maximus* adults are sessile and live on the sea floor, whereas larvae have a long planktonic phase lasting up to 6 weeks [12,13]. Given its large potential to spread, *P. maximus* populations are expected to show low genetic differentiation among populations [14]. It is noteworthy that population genetic studies of *P. maximus* do not show consistent structure. Two studies using polymorphic allozyme loci were carried out on *P. maximus* populations from the United Kingdom and France, and neither of them revealed well-defined genetic structure among the populations studied [15,16]. By contrast, studies based on PCR-RFLP (Restriction Fragment Length Polymorphism) and RAPD (Random Amplified Polymorphic DNA) [17,18] showed a genetic separation in one enclosed area, the population of Mulroy Bay, Ireland. Studies of Norwegian populations based on mitochondrial haplotypes showed negligible genetic differentiation [19]. Nevertheless, pooled haplotype frequencies of the population from Norway show significant differences with the pooled haplotypes of all the populations studied, including samples from UK, France and Ireland [20].

The low level of genetic differentiation observed between populations contrasts with the differences in life-history traits (e.g. reproduction, shell growth) highlighted in these populations [21–25]. Proteomic signatures of natural populations are known to depend on both environmental and genetic factors. Therefore, one could expect that population proteomics should be a better way than genetic studies to discriminate populations living in contrasting environments. The aim of this study was therefore to identify potential biomarkers, at the protein level, that could be used to discriminate populations. In this context, we compared the mantle tissue proteomes of *P. maximus* from two different populations living in the northern part (Norwegian population) and in the center (Bay of Brest population) of the species' latitudinal distribution range. These two locations are highly contrasted, especially in terms of temperature, photoperiod, and food availability. Since proteins are the effectors of cells, the identification of such biomarkers could also provide valuable insights into the physiological and phenotypic differences between the populations of Norway and France.

2. Materials and methods

In order to identify meaningful biomarkers of geographic differentiation, efforts were made to avoid physiological biases. Animals of similar sizes were collected and sampling periods were chosen to match a similar physiological state for the scallops. In order to partially control for temporal variability, animals were sampled at two different time points. Abundances of protein spots were then compared between populations at each sampling date. Only spots differentially expressed between populations, whatever the sampling date, were considered as valuable candidates.

2.1. Sampling

Individuals of *P. maximus* were collected from the Bay of Brest (Brittany, France) by SCUBA diving in Spring 2011, at a depth of 10 m (Fig. 1). Five animals were collected before the first phytoplankton bloom (21/03/2011; average shell height, standard deviation: 93.2 mm, 5.0 mm) and five more during the first bloom (04/04/2011; 104.3 mm, 6.9 mm). Similarly, specimens were sampled from Bjørnafjorden (Hordaland, Norway; Fig. 1) in Spring 2012 before (04/04/2012; 103.0 mm, 6.0 mm) and during the first phytoplankton bloom (17/04/2012; 108.4 mm, 2.7 mm). Animals were dissected within 2 h after sampling and the mantle tissue was snap-frozen in liquid nitrogen and kept at −80 °C until protein extraction.

In the Bay of Brest, environmental parameters were monitored using a CTD profiler (SBE-911, Sea-Bird Electronics Inc., Bellevue, WA, USA) from January to December 2011 at the site of sampling, as described in Chatterjee et al. [26]. For the sampling site in Bjørnafjorden, a CTD profiler (model 204, SAIV AS, Bergen, Norway) logged data from December 2011 to April 2012. The data were downloaded weekly (Table 1).

2.2. Protein extraction

Mantle tissue was crushed with a mixer mill (MM400, RETSCH, Haan, Germany) and kept frozen during the crushing using liquid nitrogen. One hundred milligrams of tissue powder was homogenized in 100 mM Tris–HCl (pH 6.8) containing 1% of protease inhibitor mix (GE Healthcare, Saclay, France). After centrifugation (20 000 g, 5 min, 4 °C), pellets were discarded and supernatants were pipetted to new tubes. Nucleic acids were then enzymatically removed following manufacturer's instructions (nuclease mix, GE Healthcare). Samples were precipitated at 4 °C using 20% trichloroacetic acid (1/1:v/v, overnight). After centrifugation (20 000 g, 30 min, 4 °C), pellets were washed with 70% acetone and re-suspended in urea/thiourea buffer (2 M thiourea, 7 M urea, 4% CHAPS, 1% DTT) containing 1% IPG (pH 4–7, GE Healthcare). Protein concentrations were determined using a modified Bradford assay [27], and all samples were adjusted to 400 μg of proteins in 250 μl urea/thiourea buffer.

2.3. Two-dimensional electrophoresis

Prior to isoelectric focusing, IPG strips (pH 4–7, 13 cm, GE Healthcare) were passively rehydrated with 250 μl of protein solution in wells for 14 h. Isoelectric focusing was conducted using the following protocol: 250 V for 15 min, 500 V for 2 h, gradient voltage increase to 1000 V for 1 h, gradient voltage increase to 8000 V for 2 h 30, 8000 V for 3 h, and reduced to 500 V (Ettan IPGphor 3, GE Healthcare). To prepare for the second dimension SDS-PAGE, strips were incubated in equilibration buffer (50 mM Tris–HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS and 0.002% Bromophenol Blue) for two 15 min-periods, first

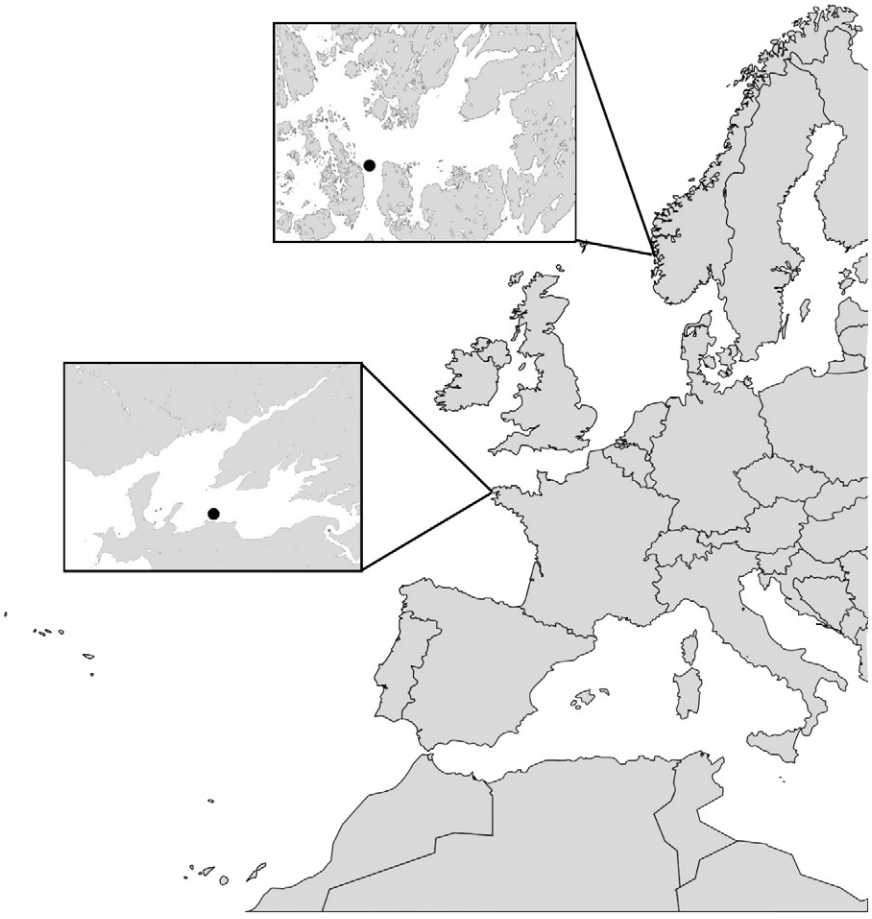

Fig. 1 – Sampling sites of *Pecten maximus* populations from Bjørnafjorden (Hordaland, Norway) and the Bay of Brest (Brittany, France). Black points indicate precise sampling sites.

with 1 $g{\cdot}l^{-1}$ dithiothreitol and then with 48 $g{\cdot}l^{-1}$ iodoacetamide. IPG strips were then placed on top of 12% polyacrylamide gels, which were run in 10 °C thermo-regulated device (SE 600 Ruby, Amersham Biosciences) at 10 mA per gel for 1 h and then 30 mA per gel until complete migration. Gels were subsequently stained with Coomassie Blue (PhastGel, GE Healthcare) and unspecific coloration was destained with a solution containing 30% methanol and 7% acetic acid. The resulting gels were scanned with a transparency scanner (Epson Perfection V700) in gray scale with 16-bit depth and a resolution of 400 dpi.

2.4. *Gel image analysis and statistical analysis of protein abundances*

Images were aligned and spots were detected and quantified using the Progenesis SameSpots software (version 3.3, Nonlinear Dynamics, Durham, NC, USA) applying the automated algorithm. All detected spots were manually checked and artifact spots were removed. Data were exported as volume raw values and statistical analyses were conducted in R [28] using the packages prot2D [29] and Limma [30] from the Bioconductor suite [31]. Data were normalized (quantile normalization) and the samples from each sampling date from a location were compared to the samples from the other location (with 5 bio-replicates per site per date). For comparisons, we used a moderated *t*-test, which is a modified *t*-test, for which the standard errors have been moderated across spots, increasing the reliability of the test [29,30]. Once the values of moderated *t*-test were calculated, a global correction by false discovery rate (fdr) [30] was applied, in order to take into account multiple comparison issues and paired-comparison correction. Spots with an fdr threshold lower than 0.1 and an absolute fold change superior to 2 were considered as differentially expressed.

Table 1 – Sampling site and parameters at the time of sampling.

Site	Coordinates	Date	Temperature	Chl-a (μg/l)	Salinity
Bay of Brest	48° 17′ 43.32″ N	21/03/11	9.8 °C	0.89	34.10
	4° 27′ 12.78″ W	04/04/11	11.3 °C	1.69	34.10
Bjørnafjorden	60° 5′ 1.14″ N	04/04/12	7.1 °C	0.46	30.36
	5° 16′ 26.04″ E	17/04/12	7.3 °C	1.53	31.89

2.5. Mass spectrometry

Proteins for which abundance changed between populations were excised from gels and prepared for analysis by mass spectrometry (MS). Gel pieces were first washed in 50 mM ammonium bicarbonate (BICAM), and then dehydrated in 100% acetonitrile (ACN). Gel pieces were vacuum-dried, and rehydrated with BICAM containing 0.5 μg sequencing grade porcine trypsin (Promega, Madison, WI, USA), and incubated overnight at 37 °C. Peptides were extracted from the gels by alternative washing with 50 mM BICAM and ACN, and with 5% formic acid and ACN as described in Galland et al. [32]. Between each step, the supernatants were pooled, and finally concentrated by evaporation using a centrifugal evaporator (Concentrator 5301, Eppendorf, Hamburg, Germany). Samples were then resuspended in trifluoroacetic acid (TFA; 0.1% in water). Peptide solutions were mixed with the α-cyano-4-hydroxycinnamic acid (HCCA, 10 mg·ml^{-1} of a ACN/TFA/water (60/4/36:v/v/v) solution), and spotted on a polished steel target using the dried droplet method. Peptides were then analyzed by Matrix-Assisted Laser Desorption Ionization Time-of-Flight tandem mass spectrometry (MALDI TOF–TOF mass spectrometry) in positive ion reflector mode with an Autoflex III (Bruker Daltonics, Billerica, MA, USA) mass spectrometer. The FlexControl software (v3.0, Bruker Daltonics) was set up to acquire successively full spectra and MS/MS from the dominant peaks. Mass spectra were analyzed with FlexAnalysis (v 3.0; Bruker Daltonics) by applying the following conditions: TopHat algorithm for baseline subtraction, Savitzky–Golay analysis for smoothing (0.2 m/z; number of cycles: 1) and SNAP algorithm for peak detection (signal-to-noise ratio: 6 for MS and 1.5 for MS/MS). The charge state of the peptides was assumed to be +1. Fragments of porcine trypsin were used for internal mass calibration.

Proteins were identified with the PEAKS software (v 5.3, Bioinformatics Solutions, Waterloo, ON, Canada), using MS/MS-based identification and de novo peptide sequencing. A custom-made EST database (see below) was used with the following search parameters: carbamidomethylation of cysteine was set as a fixed modification; oxidation of methionine and phosphorylation of serine, threonine or tyrosine were set as variable modifications; one missing cleavage during trypsin digestion was allowed. Protein identification was considered as unambiguous when a minimum of two peptides matched with a minimum score of 20. False discovery rates were also estimated using a reverse database as decoy.

The EST database was constructed by combining *P. maximus* sequences issued from Illumina RNAseq sequenced from mantle tissues (available at http://ramadda.nerc-bas.ac.uk/repository; see [33]) and from hemocyte cells [34]. Overall, the database included a total of 252 888 *P. maximus* expressed sequence tags (ESTs). EST sequences were annotated by homology searches against a non-redundant protein database using the Blast algorithm from NCBI with an e-value cut-off of 1·e^{-10} [35].

3. Results

A total of 648 spots were detected across all the replicates from both locations (Fig. 2). Paired comparisons between scallops from France and Norway for each week of sampling showed that most of the differences between populations were common across sampling dates, for a total of 38 protein spots significantly differentially expressed (Table 2). Overall, 23 proteins were more abundant in mantle tissues from French scallops (spots Fr-1 to 23, Fig. 2) and 15 were significantly more abundant in Norwegian samples (spots No-1 to 15, Fig. 2). Direct comparisons between samples from the same location at different sampling dates showed no difference for the French scallops, and 8 differentially accumulated proteins for the Norwegian samples (different from the 38 protein spots differentially accumulated between location). Based on the differential accumulation of 38 spots, Norwegian and French populations formed distinct clusters (Fig. 3).

Eleven out of 38 differentially accumulated proteins could be unambiguously identified by Maldi TOF–TOF mass spectrometry. Eight of these proteins were identified as actin isoforms (Table 3). Given these results, spots were re-analyzed by excising other gels and care was taken to avoid contamination, but we obtained the same results (data not shown). Although the 8 proteins were all identified as actin, they matched to 4 different EST sequences, for which in silico translation gave 3 different protein sequences (as protein sequences from contig_6520 and rep_c_100421 displayed 100% identity). These sequences were aligned with sequences of actin from *Pecten* sp. (GenBank NCBI Accession no. 1101351B) and closely related species, Pacific oyster *Crassostrea gigas* (EKC38058.1) and Farrer's scallop *Azumapecten farreri* (AAP88387.1). Only one EST sequence was long enough to be considered as complete (contig_6250). This sequence showed 91% similarity with actin from *Pecten* sp. and *C. gigas* and 94% with the sequence from *A. farreri*. Among the most expressed proteins in Norwegians samples, two were identified as Filamin C and one was identified as elastase, a metalloprotease.

4. Discussion

Differentiation of natural populations is generally studied by classical population genetic approaches. In the case of the great scallop, previous studies did not show clear genetic structure between biogeographically distinct populations [15–21]. Nevertheless, these populations exhibit very different life history traits. For instance one of the major differences between French and Norwegian populations is their rate and scope of growth [22,36]. Indeed, French scallops grow faster but their asymptotic sizes are smaller than those of Norwegian scallops [22,36]. In this study, the use of 2-DE based proteomics allowed us to successfully discriminate two populations of *P. maximus* living in the northern limit (Norway) and in the center (France) of its latitudinal range. Using a sampling strategy aiming at reducing the effects of physiological variations (two samplings at two week interval), classical 2-DE separation of proteins and accurate statistical tools we recently developed [29], we showed that 38 protein spots were significantly differentially accumulated between the two populations.

Comparative proteomics is a powerful tool classically used to identify proteins differentially regulated in response to changing environments, providing information on the mechanisms of adaptation of organisms [37]. Such studies

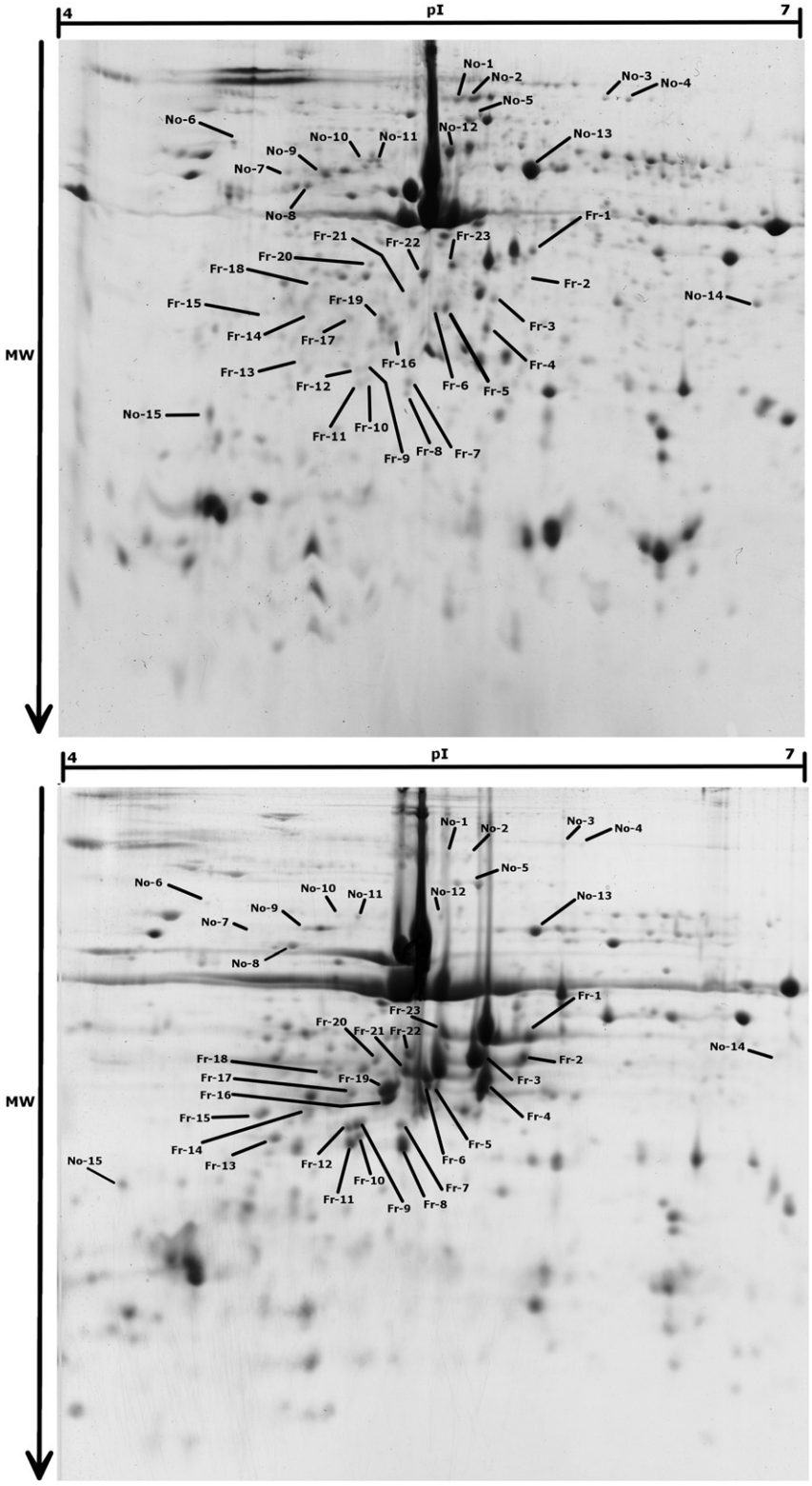

Fig. 2 – Representative bi-dimensional gels (pH 4–7, SDS-PAGE 12%) for *Pecten maximus* mantle proteins from Norway (upper gel) and France (lower gel). Spots whose abundance change between the two populations are arrowed.

are often conducted by subjecting organisms to contrasted controlled conditions, taking care to minimize the effects of both the genetic component and the multiple confounding factors that may impact the response of organism. However, there is a need to study the organisms in their natural context. For this reason, more and more proteomic studies dealing with natural populations in their natural environments have been published in recent years. Most of these studies aimed

Table 2 – Significantly * differentially accumulated protein spots between the two tested populations, at the different sampling dates. Number at the margin corresponds to protein spots significantly differentially accumulated shared by the comparisons of the same row/column.

	Bjørnafjorden 04/04/12	Bjørnafjorden 17/04/12	Shared spots
Bay of Brest 21/03/11	63	104	48
Bay of Brest 04/04/11	93	94	59
Shared spots	49	66	38

* Moderate *t*-test paired-comparison, fdr <0.1, absolute fold change >2, n = 5.

at determining the molecular adaptation of organisms to particular environments and rely on the comparisons of populations from contrasted environments. For example, in an ecotoxicological context, the comparisons of the liver proteomic signatures from three populations of the flatfish *Platichthys flesus* living in contrastingly polluted estuaries, recently allowed our group to propose new proteins as biomarkers of xenobiotic exposure [32].

Population proteomics has already proved efficient to discriminate populations of marine organisms, e.g. snails [5], mussels [6,7], and fishes [8,10,11]. Furthermore, as the proteomic signature of a given organism is in fact the result of multiple parameters, including both its genetic background and the effects of environmental parameters on its physiology, identification of the differentially expressed proteins

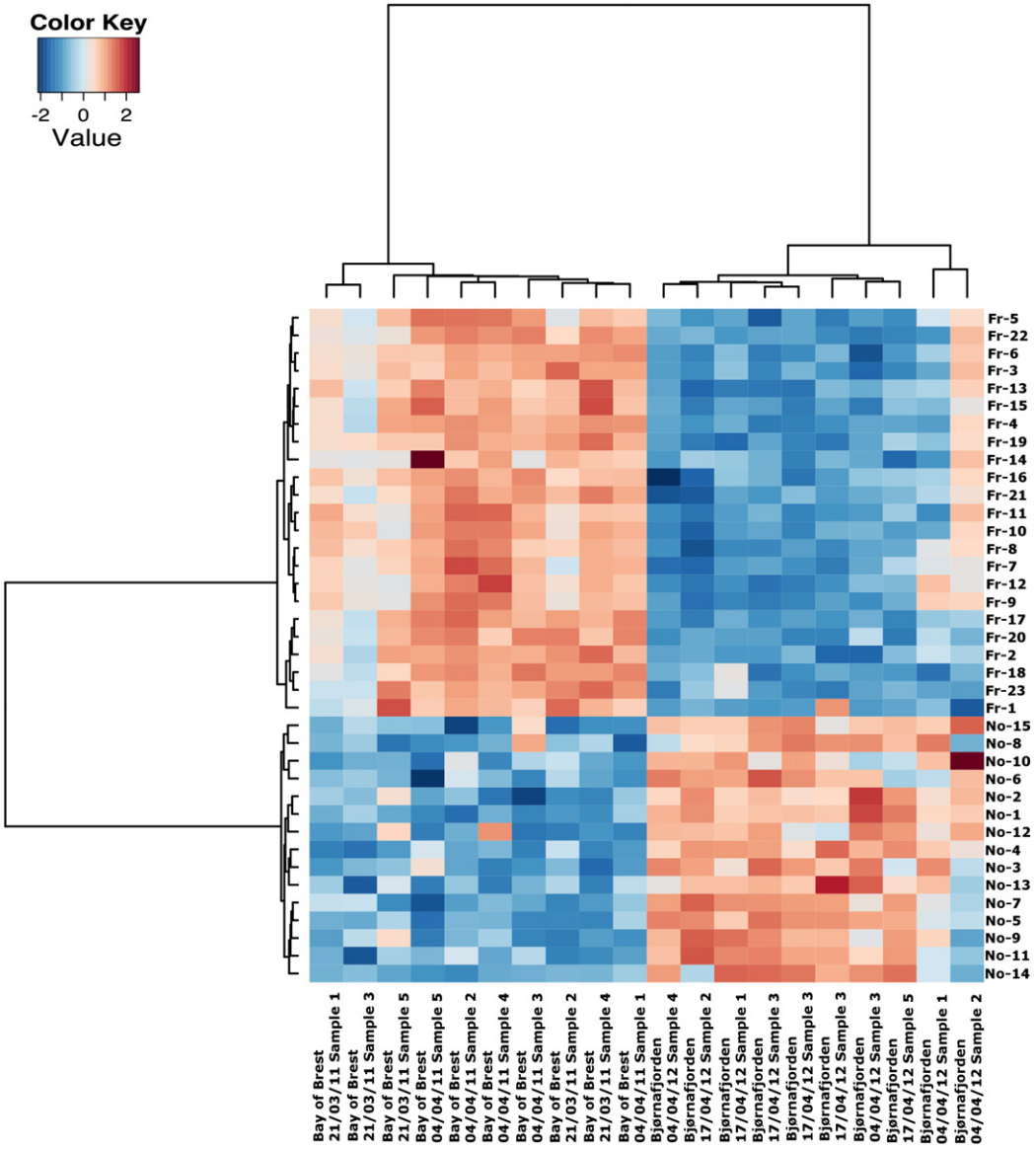

Fig. 3 – Two-way hierarchical clustering analysis with Euclidean distance and Ward's aggregation method of proteins from *Pecten maximus* mantle whose abundance change between French and Norwegian populations. Columns represent gel replicates: samples 1 to 5 from first and second sampling dates in France (Bay of Brest, 21/03/11 and 04/04/11, respectively) and samples 1 to 5 from first and second sampling dates in Norway (Bjørnafjorden, 04/04/12 and 17/04/12, respectively). Rows represent the 38 proteins whose abundance change between the populations (with labels from Table 3).

Table 3 – List of *Pecten maximus* mantle tissue proteins identified by MS/MS whose abundance change between samples from France and Norway. [a]

Spot	−10logP	% cov.	Peptide sequences	Assignation	NCBI	EST	MW	AA
Fr-4	666.54	18	SYELPDGQVSTRGNER.C GYSFTTTAER.G QEYDESGPSIVHR.A	Actin [*Pecten* sp.]	1101351B	scallop_rep_c_68146	42 kDa	374
Fr-23	521.94	13	SYELPDGQVSTRGNER.C QEYDESGPSIVHR.A					
Fr-2	1452.26	14	QEYDESGPSKVHR.C SYEEPDGQVATDGNER.A VAPEEHPVYATEAPTNPK.C GYSFTTTAER.T	Actin-2 [*Crassostrea gigas*]	EKC38058.1	contig_35831	42 kDa	376
Fr-6	1341.09	12	GYSFTTTAER.G SYEEPDGQVATDGNER.A VAPEEHPVLLTEAPLNPK.G					
Fr-9	594.56	12	SYEHPDGQVRTXGNER.K GYSFTTTAER.G	beta-Actin, partial [*Cipangopaludina cathayensis*]	ACT22633.1	scallop_rep_c100421	21 kDa	184
Fr-10	492.53	12	SYEHPDGQVRTXGNER.K GYSFTTTAER.G					
Fr-21	583.29	17	SYEHPDGQVRTXGNER.K GYSFTTTAER.G					
Fr-5	1361.49	6	GYSFTTTAER.G QEYDESGPSIVHR.A SYEXPDGQVKTRGNER.C NHC(+57.02) PSSTNNENQDHRS(+79.97)TR.G	Actin [*Azumapecten farreri*]	AAP88387.1	contig_6250	42 kDa	376
No-3	799.91	4	GEINQPC(+57.02)EFNIYTR.G VTESDIGSLMATIR.A FNDEHIPQSPYR.G VYVTPSIGDAR.A	Filamin-C [*Crassostrea gigas*]	EKC28512.1	contig_13763	324 kDa	3016
No-12	981.70	3	FNDEHXPQSPYR.T GEENQPC(+57.02)EFNXYTR.A VYVTPSIGDAR.A					
No-10	214.01	3	SAYDQGQNPTPYYR.G IGTWENDEEGR.A	Elastase [*Lottia gigantea*]	ESO85431.1	contig_1	75 kDa	645

[a] Changes are considered as statistically significant for an fdr <0.1 (using moderate *t*-test paired-comparison) and absolute fold change >2. Mass weight (MW) and number of amino acids (AA) are computed from NCBI sequences. −10logP is the score given by PEAKS software (v5.3).

should give insights into the adaptation mechanisms of animals to their specific environment.

In this study, we could identify 11 out of the 38 differentially accumulated proteins. This ratio of identification, while relatively low (29%), appears quite similar to those commonly obtained for non-model species [38,39]. As a consequence, we can only see a part of the differences observed between populations. Ten out of eleven proteins identified were closely related to the cytoskeleton structure. Indeed, 8 distinct protein spots were identified as actin, suggesting that this protein is expressed in *P. maximus* as several isoforms, which would be differentially regulated between the two assayed populations.

Isoforms of actin are widespread in eukaryotes [40]. Mammals and birds are known to possess 6 different genes encoding actin isoforms. Four of these isoforms are related to muscular functions ($\alpha_{skeletal}$-actin, $\alpha_{cardiac}$-actin, α_{smooth}-actin, γ_{smooth}-actin), and two are related to the cytoskeleton (β_{cyto}-actin and γ_{cyto}-actin) [41]. In marine invertebrates, isoforms of actin are less known and no classification has yet been reported. However, some studies have described the existence of different forms of actin in some mollusks, like cephalopods [42], gastropods from the Planorbidae family [43] and abalone [44]. In bivalve mollusks, DNA–DNA (southern) hybridization in *Placopecten magellanicus* revealed the existence of 12 to 15 genes encoding actin [45] and the presence of several actin isoforms within the cytoplasm of *Chlamys farreri* has also been inferred [46]. However, the most complete information can be gathered from the Pacific oyster *C. gigas*, as its full genome was recently completely sequenced [47]. In this species, thirteen different genes were annotated as actin.

In all, the mass spectrometry data obtained from eight protein spots matched with four different contigs encoding at least 3 different actin isoforms. Several protein spots identified as actin isoforms matched with the same contig. It should be noted that protein isoforms are not necessarily encoded by different genes, and could rather be the result of alternative splicing or post translational modifications (PTMs). It is noteworthy that actin is known to be a major target of PTMs such as acetylation, ADP-ribosylation, arginylation, methylation, and phosphorylation [48].

Actin is one of the most essential and abundant intracellular proteins. Actin microfilaments are involved in numerous major cellular functions such as maintaining cell shape, cell division, endocytosis, exocytosis, secretion, signal transduction, and

regulation of enzyme activities [49,50]. Interestingly, isoforms of actin have both overlapping and unique cellular functions [41]. Thus, biochemical evidence suggests that properties, and therefore functions, of actin microfilaments may vary according to the mix of actin isoforms in the filament [51]. Considering our results, we postulate that scallops from the different locations may modulate their intracellular pools of actin isoforms to adapt to their particular environment.

Filamin is another protein related to the cytoskeleton that was identified in our study. Two different spots were identified as filamin, which could be different isoforms of this protein. Like actin, different isoforms of filamin have already been observed in other organisms, including mammals (i.e., Filamins A, B and C [52]), and three isoforms have already been characterized in bivalves [53]. Filamins belong to a family of proteins initially considered as cytoskeleton organizers, as they cross-link microfilaments of actin through their actin binding domain [52]. However, recent studies in mammals have shown that filamins can also interact with several cell proteins other than actin, including membrane receptors, ion channels, enzymes, signaling pathway proteins and transcription factors [54,55], and was shown to regulate the transforming growth factor-β (TGF-β) signaling, thus mediating cell proliferation [56].

The differences observed in the cytoskeleton organization and regulation could play a key role in growth efficiency. The cytoskeleton may have a major role in adjusting the biological rhythms through the sensing of environmental parameters [57], and could thus be related to the very different growth rates already described for French and Norwegian populations of scallops [22,36].

In conclusion, our results show that proteomic analysis is very efficient in discriminating two populations of *P. maximus* living in contrasted habitats. Identification of the differentially accumulated proteins in the two assayed populations suggests a crucial difference in cytoskeleton structure and functioning. However, as 27 proteins differentially regulated between populations could not be identified, other functions may be implied. Future genetic and physiological studies might focus on actin and filamin and their different isoforms. An extensive analysis of these particular proteins would provide useful information on the genetic local adaptation versus phenotypic plasticity debate.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jprot.2014.03.026.

REFERENCES

[1] Karr TL. Application of proteomics to ecology and population biology. Heredity 2007;100:200–6.
[2] Nedelkov D. Population proteomics: addressing protein diversity in humans. Expert Rev Proteomics 2005;2:315–24.
[3] Nedelkov D, Kiernan UA, Niederkofler EE, Tubbs KA, Nelson RW. Population proteomics: the concept, attributes, and potential for cancer biomarker research. Mol Cell Proteomics 2006;5:1811–8.
[4] Chevalier F, Martin O, Rofidal V, Devauchelle A-D, Barteau S, Sommerer N, et al. Proteomic investigation of natural variation between *Arabidopsis* ecotypes. Proteomics 2004;4:1372–81.
[5] Martínez-Fernández M, Rodríguez-Piñeiro AM, Oliveira E, Páez de la Cadena M, Rolán-Alvarez E. Proteomic comparison between two marine snail ecotypes reveals details about the biochemistry of adaptation. J Proteome Res 2008;7:4926–34.
[6] López JL, Mosquera E, Fuentes J, Marina A, Vázquez J, Álvarez G. Two-dimensional gel electrophoresis of *Mytilus galloprovincialis* differences in protein expression between intertidal and cultured mussels. Mar Ecol Prog Ser 2001;224:149–56.
[7] López JL, Marina A, Vázquez J, Álvarez G. A proteomic approach to the study of the marine mussels *Mytilus edulis* and *M. galloprovincialis*. Mar Biol 2002;141:217–23.
[8] Carrera M, Cañas B, Piñeiro C, Vázquez J, Gallardo JM. Identification of commercial hake and grenadier species by proteomic analysis of the parvalbumin fraction. Proteomics 2006;6:5278–87.
[9] Carrera M, Cañas B, Piñeiro C, Vázquez J, Gallardo JM. De novo mass spectrometry sequencing and characterization of species-specific peptides from nucleoside diphosphate kinase B for the classification of commercial fish species belonging to the family Merlucciidae. J Proteome Res 2007;6:3070–80.
[10] Ortea I, Cañas B, Gallardo JM. Mass spectrometry characterization of species-specific peptides from arginine kinase for the identification of commercially relevant shrimp species. J Proteome Res 2009;8:5356–62.
[11] Gonzalez EG, Krey G, Espiñeira M, Diez A, Puyet A, Bautista JM. Population proteomics of the European hake (*Merluccius merluccius*). J Proteome Res 2010;9:6392–404.
[12] Beaumont AR, Barnes DA. Aspects of veliger larval growth and byssus drifting of the spat of *Pecten maximus* and *Aequipecten* (*Chlamys*) *opercularis*. ICES J Mar Sci 1992;49:417–23.
[13] Gosling E. Reproduction, settlement and recruitment. In: Gosling E, editor. Bivalve molluscs: biology, ecology and culture. Blackwell Publishing Ltd.; 2003. p. 131–68.

[14] Saavedra C, Peña JB. Nucleotide diversity and Pleistocene population expansion in Atlantic and Mediterranean scallops (*Pecten maximus* and *P. jacobaeus*) as revealed by the mitochondrial 16S ribosomal RNA gene. J Exp Mar Biol Ecol 2005;323:138–50.
[15] Beaumont AR, Morvan C, Huelvan S, Lucas A, Ansell AD. Genetics of indigenous and transplanted populations of *Pecten maximus*: no evidence for the existence of separate stocks. J Exp Mar Biol Ecol 1993;169:77–88.
[16] Wilding CS, Latchford JW, Beaumont AR. An investigation of possible stock structure in *Pecten maximus* (L.) using multivariate morphometrics, allozyme electrophoresis and mitochondrial DNA polymerase chain reaction-restriction fragment length polymorphism. J Shellfish Res 1998;17:131–40.
[17] Wilding CS, Beaumont AR, Latchford JW. Mitochondrial DNA variation in the scallop *Pecten maximus* (L.) assessed by a PCR-RFLP method. Heredity 1997;79:178–89.
[18] Heipel DA, Bishop JDD, Brand AR, Thorpe JP. Population genetic differentiation of the great scallop *Pecten maximus* in western Britain investigated by randomly amplified polymorphic DNA. Mar Ecol Prog Ser 1998;162:163–71.
[19] Ridgway GMI, Dahle G, Høisœter T. Population genetics of *Pecten maximus* of the Northeast Atlantic coast. Sarsia 2000;85:167–72.
[20] Beaumont A. Chapter 10 Genetics. In: Shumway SE, Parsons GJ, editors. Scallops: biology, ecology and aquaculture, vol. 35. Elsevier; 2006. p. 543–94.
[21] Mackie LA, Ansell AD. Differences in reproductive ecology in natural and transplanted populations of *Pecten maximus*: evidence for the existence of separate stocks. J Exp Mar Biol Ecol 1993;169:57–75.
[22] Chauvaud L, Patry Y, Jolivet A, Cam E, Le Goff C, Strand Ø, et al. Variation in size and growth of the great scallop *Pecten maximus* along a latitudinal gradient. PLoS One 2012;7:e37717.
[23] Paulet YM, Lucas A, Gerard A. Reproduction and larval development in two *Pecten maximus* (L.) populations from Brittany. J Exp Mar Biol Ecol 1988;119:145–56.
[24] Strand Ø, Nylund A. The reproductive cycle of the scallop *Pecten maximus* (Linnaeus, 1758) from two populations in western Norway, 60N and 64N. An International Compendium of Scallop Biology and Culture World Aquaculture Society; 1991. p. 95–105.
[25] Strohmeier T, Duinker A, Lie O. Seasonal variations in chemical composition of the female gonad and storage organs in *Pecten maximus* (L.) suggesting that somatic and reproductive growth are separated in time. J Shellfish Res 2000;19:741–8.
[26] Chatterjee A, Klein C, Naegelen A, Claquin P, Masson A, Legoff M, et al. Comparative dynamics of pelagic and benthic micro-algae in a coastal ecosystem. Estuar Coast Shelf Sci 2013;133:67–77.
[27] Ramagli LS. Quantifying protein in 2-D PAGE solubilization buffers. In: Link AJ, editor. 2-D Proteome Anal Protoc, vol. 112., Methods in molecular biology; 1999. p. 99–103 [Springer, Totowa, NJ]. http://dx.doi.org/10.1385/1-59259-584-7:99.
[28] R Core Team R version 3. 0. R: a language and environment for statistical computing. Vienna Austria: R Found Stat Comput; 2013 [www.R-project.org].
[29] Artigaud S, Gauthier O, Pichereau V. Identifying differentially expressed proteins in two-dimensional electrophoresis experiments: inputs from transcriptomics statistical tools. Bioinformatics 2013;29:2729–34.
[30] Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004;3 [Article 3].
[31] Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004;5:R80.
[32] Galland C, Dupuy C, Capitaine C, Auffret M, Quiniou L, Laroche J, et al. Comparisons of liver proteomes in the European flounder *Platichthys flesus* from three contrasted estuaries. J Sea Res 2013;75:135–41.
[33] Artigaud S, Thorne MAS, Richard J, Lavaud R, Jean F, Flye-Sainte-Marie J, et al. Deep sequencing of the mantle transcriptome of the great scallop Pecten maximus. Mar genomics 2014. http://dx.doi.org/10.1016/j.margen.2014.03.006 (in press).
[34] Pauletto M, Milan M, Moreira R, Novoa B, Figueras A, Babbucci M, et al. Deep transcriptome sequencing of *Pecten maximus* hemocytes: a genomic resource for bivalve immunology. Fish Shellfish Immunol 2014;37:154–65.
[35] Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–402.
[36] Strand Ø, Parsons GJ. Chapter 21 Scandinavia. In: Shumway SE, Parsons GJ, editors. Scallops: biology, ecology and aquaculture, vol. 35. Elsevier; 2006. p. 1067–91.
[37] Tomanek L. Environmental proteomics: changes in the proteome of marine organisms in response to environmental stress, pollutants, infection, symbiosis, and development. Ann Rev Mar Sci 2011;3:373–99.
[38] Forné I, Abián J, Cerdà J. Fish proteome analysis: model organisms and non-sequenced species. Proteomics 2010;10:858–72.
[39] Dowd WW. Challenges for biological interpretation of environmental proteomics data in non-model organisms. Integr Comp Biol 2012;52:705–20.
[40] Khaitlina SY. Functional specificity of actin isoforms. Int Rev Cytol 2001;202:35–98.
[41] Perrin BJ, Ervasti JM. The actin gene family: function follows isoform. Cytoskeleton 2010;67:630–4.
[42] Carlini DB, Reece KS, Graves JE. Actin gene family evolution and the phylogeny of coleoid cephalopods (Mollusca: Cephalopoda). Mol Biol Evol 2000;17:1353–70.
[43] Adema CM. Comparative study of cytoplasmic actin DNA sequences from six species of Planorbidae (Gastropoda: Basommatophora). J Molluscan Stud 2002;68:17–23.
[44] Bryant M, Flint H, Sin FT. Isolation, characterization, and expression analysis of three actin genes in the New Zealand black-footed abalone, *Haliotis iris*. Mar Biotechnol 2006;8:110–9.
[45] Patwary MU, Reith M, Kenchington EL. Isolation and characterization of a cDNA encoding an actin gene from sea scallop (*Placopecten magellanicus*). J Shellfish Res 1996;15:265–70.
[46] Ma H, Mai K, Liufu Z, Xu W. Cloning and characterization of an actin gene of *Chlamys farreri* and the phylogenetic analysis of mollusk actins. Chinese J Oceanol Limnol 2007;25:304–9.
[47] Zhang G, Fang X, Guo X, Li L, Luo R, Xu F, et al. The oyster genome reveals stress adaptation and complexity of shell formation. Nature 2012;490:49–54.
[48] Terman JR, Kashina A. Post-translational modification and regulation of actin. Curr Opin Cell Biol 2013;25:30–8.
[49] Pollard TD, Cooper JA. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Annu Rev Biochem 1986;55:987–1035.
[50] Welch MD, Rosenblatt J, Skoble J, Portnoy DA, Mitchison TJ. Interaction of human Arp2/3 complex and the *Listeria monocytogenes* ActA protein in actin filament nucleation. Science 1998;281:105–8.
[51] Bergeron SE, Zhu M, Thiem SM, Friderici KH, Rubenstein PA. Ion-dependent polymerization differences between mammalian β- and γ-nonmuscle actin isoforms. J Biol Chem 2010;285:16087–95.

[52] Van der Flier A, Sonnenberg A. Structural and functional aspects of filamins. Biochim Biophys Acta 2001;1538:99–117.
[53] Méndez-López L, Hellman U, Ibarguren I, Villamarín JA. Filamin isoforms in molluscan smooth muscle. Biochim Biophys Acta 1824;2012:1334–41.
[54] Popowicz GM, Schleicher M, Noegel AA, Holak TA. Filamins: promiscuous organizers of the cytoskeleton. Trends Biochem Sci 2006;31:411–9.
[55] Nakamura F, Stossel TP, Hartwig JH. The filamins: organizers of cell structure and function. Cell Adh Migr 2011;5:160–9.
[56] Zhou A-X, Hartwig JH, Akyürek LM. Filamins in cell signaling, transcription and organ development. Trends Cell Biol 2010;20:113–23.
[57] Shweiki D. The physical imperative in circadian rhythm: a cytoskeleton-related physically resettable clock mechanism hypothesis. Med Hypotheses 1999;53:413–20.