**Final publishable summary report** - The FP7-IRSES-PEOPLE BIOVADIA (2011-2015) is a joint exchange program aimed to study the biology and the biodiversity of a group of unique and original marine microalgae, the ‘blue diatoms’, and to investigate the possible applications of their specific blue pigments.

**Rationale** - Microalgae are at the base of most aquatic trophic chains, they are among the fastest growing photosynthetic organisms, they contribute to about half of the planet's primary production, many of them produce unique and valuable compounds (pigments, antioxidants, peptides, essential fatty acids, oils, bioactive molecules), and their biodiversity is high, yet they are a vastly underexploited bio-resource. Only a few strains of microalgae are actually exploited on an industrial scale (less than a dozen, including those used as feed in aquaculture), and microalgae-derived products are currently limited to a few niche markets. *Haslea ostrearia* and the ‘blue diatoms’ producing specific and rare blue pigments in nature are good candidates to join this club. However, their distribution and ecology around the world is not well known, limiting their potential exploitation.

**An old model… -** The marine microalga *Haslea ostrearia* (‘Blue Navicula’) is the first ever described blue diatom known for its ability to produce a water-soluble blue pigment, marennine (Fig. 1), which accumulates mainly at the apical regions of cells, before being excreted in the water. In oyster ponds of western France, where *H. ostrearia* sometimes becomes dominant, marennine is responsible for ‘greening’ of oyster gills, a phenomenon that increases palatability and therefore market value of the oysters (red label ‘fines de claires vertes’, produced in Marennes-Oléron Bay) (Fig. 2). *Haslea ostrearia* has long been considered the only microalga known, worldwide in distribution, to produce marennine, and for centuries, the greening of oysters was the only economic use of ‘Blue Navicula’.

**… but new prospects** - Recently, a new species of blue *Haslea*, which produces a blue pigment slightly different from marennine, has been discovered in the Black Sea, and described as *Haslea karadagensis*. The presence of a second blue *Haslea*, in a region where *H. ostrearia* had already been observed, questioned our knowledge of the biodiversity of these peculiar microalgae. A method for extraction and purification of marennine has been proposed, and pure marennine has been reported to have a wide range of biological properties in vitro (antioxidant, antibacterial, antifungal, antiviral, and antiproliferative activities), which could be interesting for aquaculture, cosmetics, food or health industry. To promote the blue *Haslea* to the point where they would be ready for industrial exploitation, different prerequisites should be met, such as improved understanding of the biology of the organisms and of the chemical structure and properties of the pigments, proof of concept of large-scale cultivation, and assessment of their exploitation potential. Some of these prerequisites were the objectives of the program BIOVADIA, which involved different partners in Europe, North-America and Australia: Université du Maine (PI), Université de Nantes, Université de La Rochelle and Université de Bretagne Sud in France; Cardiff University in UK; Karadag Natural Reserve in Ukraine; University of Crete in Greece; Université du Québec à Rimouski, Université Laval, and Mount Allison University in Canada; University of Rhode Island in the USA; University of Tasmania in Australia.

The program BIOVADIA consisted in 7 experimental work packages (WP1 to WP7), each to get insights into different issues regarding the ‘blue diatoms’ (*Haslea ostrearia*-like diatoms) and their marennine-like pigments, and a 8th WP, for the transfer of knowledge between partner institutions, and the dissemination of the results. The main goal of WP1 was to collect blue *Haslea* from different geographic areas, to characterize and identify them, and when appropriate, to describe new species. If several clones of a same strain/species could be derived from natural samplings, their life cycle and modalities of sexual reproduction would be studied (WP2). The description of new strains/species of blue diatoms using morphometrics and molecular markers was the main objective of WP3, whereas the study of their ecophysiology was the one of WP4. The objectives of WP5 were to improve the methods previously used to extract and purify the blue pigments and to increase our knowledge about the chemical nature of these peculiar biomolecules. In WP6, the production of these pigments should be improved and their potential of exploitation explored. The study of their biological activities would also be carried on, mainly in the context of aquaculture (objective of WP7).
The consortium achieved, and even went beyond most of the objectives of the program, although not all the visits initially programmed between partners took place. Many sampling campaigns were conducted in different countries and continents. After collection and isolation, strains of blue Haslea were characterized, by comparing the morphology of their silicified cell wall (frustule), the UV-visible spectrum of their pigments, and the sequences of some genetic markers from their plastid, mitochondrial and nuclear genomes. This information led to the discovery of four new species of blue Haslea, one in the Mediterranean Sea (Haslea provincialis), two in the Canary Islands (Haslea silbo sp. nov. and Haslea acoran sp. nov.), and one in the Java Sea (Haslea sp.). Our knowledge of factors controlling sexual reproduction and breeding experiments with geographically distant populations of H. ostrearia demonstrated that mitochondria transmission to the next generation is uniparental in diatoms.

A first phylogenetic analysis revealed that the blue Haslea species form a well defined cluster inside the genus Haslea. The geographic distribution of these species seems quite diversified, from global to endemism (Fig. 3). Furthermore, a genome sequencing approach was chosen to unravel the biodiversity of the blue Haslea. The assembly and annotation of the chloroplastic and mitochondrial genome of H. ostrearia are in progress, which will allow establishing a more complete molecular phylogeny. Additionally, the transcriptome sequencing of H. ostrearia coupled to a bioinformatic analysis allowed identifying 8 candidate genes for the biosynthesis of other high-value compounds (highly branched isoprenoids and sterols). The chemical structure of marennine has not been fully resolved yet, but relevant advances were made in the comprehension of the complex structure. Different experiments showed that the molecule behaves as polyanion; an important part of it has a glycosidic nature; aromatic rings, and different types of quinone groups are also present. Regarding anti-microbial activities, marennine has been shown to inhibit the growth of the pathogenic marine bacteria Vibrio splendidus, and to enhance larval survival of the blue mussel Mytilus edulis and the scallop Placopecten magellanicus exposed to this pathogen. If it was confirmed at the industrial scale that marennine or Haslea culture supernatant could be used as an alternative for antibiotics to control pathogens in hatcheries and bivalve production, this could be of great interest for sustainable aquaculture, by reducing the need for treatment by prophylactic antibiotics, an environmental issue especially pertinent to intensive aquaculture.

On the academic side, the BIOVADIA program has generated collaborations between partners within European countries, which will be at the basis of future research programs. Moreover, since the start of the project, 2 book chapters and 11 research articles have been published or are in press in scientific peer-reviewed journals, and as many are submitted or in preparation. The results were also presented in national and international conferences (oral communications and posters). More information about BIOVADIA consortium, achievements and outputs are available in the web site (biovadia.univ-lemans.fr).

Regarding socio-economic prospects, the BIOVADIA results will form the baseline for future research and development programs between both academic and non-academic partners from different countries. Indeed, algae producing companies and other end-user aquaculturists are interested by such potential developments. Although the chemical structure of marennine is not fully resolved yet, blue Haslea species are promising candidates for industrial-scale developments, not only in light of the economic importance of the oyster-producing industry (e.g. 10^5 t/yr with a value of 200 M€ in France), but also of the large spectrum of putative applications of marennine-like pigments, either as anti-microbial compounds, or natural blue pigments. For instance, out of the scope but a spin-off of the BIOVADIA program would be the possible utilization of Haslea biomass using a biorefinery approach, by exploring algal molecular biodiversity and developing processes for selective recovery of blue pigments and other molecules. Blue biotechnology, especially with blue Haslea, will allow identifying new high-value compounds, including natural blue colorants, which are attractive for the cosmetic and food industries.