Chapter 6 - Conclusions

The rapid increase in the production and use of ENPs in coming years is a real fact, resulting in exposure and environmental impact (Gottschalk and Nowack, 2011). Therefore, understanding environmental exposure and the toxicity of ENPs provides the basis for assessing the environmental risks posed by these compounds. Knowledge on the hazard side is increasing exponentially (Klaine et al., 2008, Scown et al., 2010).

However, there are currently almost no specific trace analytical methods available to quantify nanomaterials in environmental samples, e.g. water, wastewater or biosolids (Kim et al., 2012, von der Kammer et al., 2012, Kühnel and Nickel, 2014). The fact that the physicochemical properties of the ENPs per se, as well as their interactions with immediate surrounding environments, determine their potential hazards, acute or chronic, direct or indirect, is extremely important (Nel et al., 2009, Bernhardt et al., 2010, Albanese et al., 2012, Keller et al., 2012). Lack of knowledge about the transport, fate, and behavior of ENPs in the aquatic environment largely constrains our ability to assess their potential risks in a quantitative and predictable way. In seawater aquatic ecosystems, for example, the presence of particles may increase the rate of sedimentation, making it difficult to determine the real risk (Keller et al., 2010).

Currently the only way to obtain information on existing levels of ENPS in the environment, and to study what might be the risks involved, is to model predicted environmental concentrations (PEC) and perform ecotoxicological/ecological studies starting from these. (Sun et al., 2014).

Right inside this context is inserted this research work, which tried to respond to the major issues related to the study of the toxic effects associated with the release of silver nanoparticles in the marine environment.

The work was not easy, as we found several issues related to the behavior of particles in seawater. This work has demonstrated that an interaction between silver nanoparticles and seawater, leading to the formation of aggregates that influence the silver amount available in the water column especially during the first few hours of exposure. To this aim, in this work, we used a mathematical model based on the logistic function to predict the actual silver dose in seawater column. This strategy proved very effective to describe at quantitative level the toxicity observed for the diffent types and levels of silver tested (Chp2). We could demonstrate, in fact, that silver toxiciy is primarily driven by the actual Ag dose and no-size effect could be proven. This feature was observed from ecotoxicological data (Chp2), bioenergetics (Chp3) and predicted high order level effects (Chp5).

In Chp4 we provided a mechanistic approach to explain toxicity of silver nanoparticle. First, we found that AgENP are preferentially accumulated to mussel digestive gland and in gills. However, ionic silver is avoided in the digestive tissue, as exposures to silver nitrate rendered only 6% metal accumulation in such tissue. Most of ionic silver, in fact, appeared to accumulate in gills. Moreover, about 40-50% of silver provided originally in colloidal form was found as soluble after 24 h, indicating that Ag found in gills, from NP exposure, is due to silver speciation (dissolution/oxidation) in seawater. We hypothized that sublethal biological effects due to AgENP exposre are therefore partiotioned between the two tissues. We find a confirmation of such hypothesis from the differential expression pattern of metallothionein and the carbolylated protein fingerprints observed in the two tissues. Furthermore, we demonstrated that AgENP are able to elicit oxidative damages, as resulted

from accumulation of membrane lipid peroxidation byproducts (lipofiscin granules) in the digestive gland and irreversible oxidation of proteins in both tissues.

Finally, it is relevant to note that this work considered 6 different levels of biological organization: the molecule level (silver bioaccumulated into mussel tissues); molecular level (gene expression study); biochemical level (oxidative stress related enzymes and protein carbonylation); physiological level (bioenergetics); organismal level (ecotoxicology); population level (DEB prediction of fecundity). This multilevel approach is one of the first comprehensive approach to link early warning effects with high order level ones. As known the first are highly predicive and analytical whilst the second are necessary for the realistic assessment of the ecosystem status. One powerful modeling framework for predicting the population-level impacts of contaminants from results of individual toxicity tests has proven to be the Dynamic Energy Budget (DEB) modelling (Muller et al., 2010, Jager et al., 2005, Jager et al., 2010). According to our research, we showed that NOEC and LOEC levels for high order level population effects are similar between ionic and nano silver, either ranging between 100 and 1000 ng/L. Most recent estimations within the NanoFATE project (Svendsen C., personal communication) are confirming the earlier predictions by Gottschalk et al.(2010) of nanoAg PEC around 1-10 ng/L in superficial waters. A risk assessment based on gross PEC/PNEC ratio will render a low risk for nanosilver (0.001/0.01). However, to date, there are no PEC predictions on marine environments, such as coastal and estuarine areas in which nanoparticles should undergo sudden aggregation and precipitation due to drop of their surface potential.

Our future perspective is to carry out silver chemical/physical speciation studies in mussel tissues, particularly the digestive gland where we know AgENPs (or AgENP derived silver) are accumulated, by means of synchrotron radiation source x-ray spectroscopy. Moreover, we

would like to further dissect the effects of ionic silver from those of AgENPs using high throughput molecular techniques such as high density microarrays and RNA massive sequencing (quantitative transcriptomics). To this aim, a high resolution array encompassing 15K Mytilus spp RNA probes was prepared. Finally, the proteomic work, that in this case was used a proxy of oxidative damage, will be completed with the identification of carbolynated features.