Ph.D. SCHOLARSHIPS ON THE THEMATIC AREAS OF INNOVATION AND GREEN

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Ph.d. in RESEARCH METHODS IN SCIENCE AND TECHNOLOGY - Ciclo XXXVII

Bound Topic of Research

"Mitigation strategies of pollution impacts in coastal ecosystems"

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Climate change and new farming contamination scenarios by marine biotoxins: risk reduction with innovative forecast approaches of toxic events for a sustainable and safe production management of marine aquaculture

THEME: ☐ INNOVAZIONE ☒ GREEN

RESEARCH PROPOSAL:
summary description of the research proposed by the candidate structured as follows:
- *introduction of the problem in the international scientific context,*
- *significance of the matter,*
- *method through which the problem will be addressed*
- *objective and expected findings,*
- *bibliography.*

Introduction of the problem in the international scientific context

Phytoplankton delivers key ecosystem services at the core of marine food webs by providing food and oxygen and sequestering CO_2. Some naturally occurring species can however, produce a variety of biotoxins capable of accumulating in shellfish tissues, because of their filter feeding strategy, and are the so-called HA (Harmful Algae).
Events called HABs (Harmful Algal Blooms) can represent an intoxication risk, in case of contaminated shellfish consumption.
Harvesting has to be suspended in case toxins concentrations exceed the regulatory limits imposed by the EU835/2004 regulation.
These suspensions of course represent significant economic losses to the aquaculture sectors.
Even though these suspensions cannot be avoided, EWS (Early Warning Systems) aim to forecast bloom occurrence in order to mitigate the socio-economic impact.
HABs interest coastal areas all over the world: from the Mediterranean to the North-East Atlantic Ocean and South-East Asia. They are

	characterized by different initiating factors (e.g., nutrient availability; stratified, mixed and/or frontal water masses, calm seawaters etc.). Coastal environments, which are fundamental in the generation of ecosystem services, such as fishery and aquaculture, are subjected to increased anthropogenic pressures, because of increasing productivity of human populations and effects of climate change combined with eutrophication processes. **Significance of the matter** Shellfish productions represent a strategic activity in the Adriatic Sea, for both Italian and Eastern coasts. Biotoxins contamination is known since 1989 in the Adriatic Sea, and it occurs seasonally, compromising harvesting and causing substantial economic losses. The most concerning algal species for the Adriatic Sea are: • *Alexandrium minutum* (PSP), • *Alexandrium pacificum* (PSP), • *Gonyaulax polyedra* (YTX) • *Lingulodinium polyedrum* (YTX), • *Dinophysis* spp. (DSP), which represent the most relevant group in this area. DSP (Diarrhetic Shellfish Poisoning) toxins represent the most frequently reported cases of seafood contamination in the Mediterranean Sea, and specifically in the Adriatic Sea too, where toxicity events occurred on both western and eastern sides, causing recurrent closures of shellfish farms. DSP is caused by the consumption of sufficient amounts of okadaic acid (OA) group toxins, which includes OA and dinophysistoxins (DTX-1, DTX-2, and DTX-3) and it is characterized by digestive symptoms, such as nausea, vomiting, diarrhoea, chills and abdominal pain. The triggers for toxin production are unclear, but environmental factors, as climate change, increase of sea water temperatures, reduction in pH and increase in nutrient availability are hypothesized to have a role in the formation of algal blooms that could therefore, increase. **Objective and expected findings:** This project aims to develop innovative forecasting systems for HABs in the Adriatic Sea, by developing and implementing physical, chemical, biological and mathematical models and remote sensing and monitoring systems. To do so it will be crucial to define physical, chemical and biological drivers so that they can be used, by continual monitoring and

	models, in a period of risk forecasting and analysis, in order to ensure a more efficient management and mitigation of the potential negative impacts. The final product will be a computerised early warning system for biotoxin contamination of Adriatic mussel farms, which will be shared with the involved stakeholders, so that it can be used in the management of coastal services. **Method through which the problem will be addressed:** The project will start with a one-year sampling phase of mussel farms of the Emilia Romagna and Marche coasts. Seawater samples will be collected on the surface with polyethylene bottles, to analyse phytoplankton assemblages, the environmental parameters, and by a phytoplankton net (mesh size: 10-20 µm), target harmful microalgal species, since (e.g.) *Dinophysis* species occurrs in the thermocline. This will give us an integrated sample of the water column. The methodology bottle sampling and net hauls is described in the European Standard EN 15972:2011. Common environmental parameters used in the model training phase are: • day • distance from coastline (m) • wind maximum speed (km/h) • wind direction • atmospheric pressure • cloud cover (okta) • water transparency (m) • sea surface temperature (°C) • salinity • dissolved oxygen (mg/L) • oxygen saturation (% sat.) • chlorophyll *a* (µg/L) • pH • N-NO_3 (µM) • N-NO_2 (µM) • N-NH_3 (µM) • P-PO_4 (µM) • total P (µM) • Si-SiO_2 (µM) Phytoplankton will be quantified by the use of the Utermöhl (1958) method, and target harmful phytoplankton species by molecular qPCR methods. Presence/absence data will be associated with oceanographic and predictive variables (e.g., day of the year, distance from coastline and meteorological variables) to prepare the model. Further, mussel samples will be collected together with water samples in mussel farms in order to carry out chemical and molecular

	analyses by HPLC and qPCR, respectively, for the determination of the presence of target toxins and harmful algal species. **Bibliography:** • Fernandes-Salvador J. A. et al., Current Status of Forecasting Toxic Harmful Algae for the North-East Atlantic Shellfish Aquaculture Industry, Frontiers in Marine Science, Volume 8, 2021, DOI=10.3389/fmars.2021.666583 • Zingone A. et al., Toxic marine microalgae and noxious blooms in the Mediterranean Sea: A contribution to the Global HAB Status Report, Harmful Algae, Volume 102, 2021, 101843, ISSN 1568-9883, https://doi.org/10.1016/j.hal.2020.101843. • Valbi, E. et al. A model predicting the PSP toxic dinoflagellate Alexandrium minutum occurrence in the coastal waters of the NW Adriatic Sea. Sci Rep 9, 4166 (2019). https://doi.org/10.1038/s41598-019-40664-w • Penna A. et al., Potentially harmful microalgal distribution in an area of the NW Adriatic coastline: Sampling procedure and correlations with environmental factors, Estuarine, Coastal and Shelf Science, Volume 70, Issues 1–2, 2006, Pages 307-316, ISSN 0272-7714, https://doi.org/10.1016/j.ecss.2006.06.023. • Taylor M. et al., Outbreak of Diarrhetic Shellfish Poisoning Associated with Mussels, British Columbia, Canada. Marine Drugs. 2013; 11(5):1669-1676. https://doi.org/10.3390/md11051669
Briefly highlight the coherence characters between the project, the SNSI, and the PNR with reference also to the capacity to foster innovation and interchange between the world of research and the productive world in the field innovation, digital and enabling technologies, as well as the potential scientific, economical and social repercussion.	The project is consistent with the thematic area **5.4.2 Industria Intelligente e Sostenibile, Energia e Ambiente dell'SNSI 2014-20** for the technological path of development with national priority: Sistemi e tecnologie per il water e il waste treatment. The final product of the PhD project will provide an early warning system for biotoxin contamination of Adriatic mussel farms. Actions will be coordinated with the stakeholders' network (local producers and cooperative societies, sanitary control agencies, ARPAM etc.), that will have to contribute to the local coastal services management. Clear and coherent strategies are expected and will be focalised on conservation of the

	coastal marine ecosystem and on sustainable development through actions to prevent marine biotoxins risk contamination. An integrated and innovative approach about toxic algal phenomena in some areas of the Adriatic Sea is intended with this proposal, to allow an efficient coastal ecosystem service management. Moreover, the project is coherent with the **PNR 2021-2027** references, **chapter 5.6 Prodotti Alimentari, Bioeconomia, Risorse Naturali, Agricoltura, Ambiente**: • 5.6.1 Green technologies: Articolazione 3. Prevenzione della contaminazione del suolo e delle acque • 5.6.5 Conoscenza, innovazione tecnologica e gestione sostenibile degli ecosistemi marini, articolazione 1: Conoscenza degli ecosistemi marini e della fascia costiera The overall objective of this PhD proposal is to develop innovative systems to forecast toxic algal events in the Adriatic Sea, by developing and implementing mathematical models, remote sensing and monitoring systems: physical, chemical and biological drivers, available through physical models and continual monitoring, will be used to do a risk assessment and forecast. This will allow a more efficient management and mitigation of potential negative impacts of biotoxin contamination. This project therefore aims to protect and improve coastal ecosystem services and local productivity and economies via an integrated approach. When knowledge about marine ecosystem, especially coastal ones, is present, solutions to try to mitigate impacts can in fact be put into place (displacements of the farms in a non-impacted area; anticipated collection of mussels, if possible). Realizing a new and integrated approach requires an analysis of existing measures: present coastal activity managing practices are not ready yet to the effects and consequences of climate change. Human activities can facilitate the introduction of exotic (and potentially toxic) species that can become invasive, thus generating a variation of the local phytoplankton community. Invasiveness of these species is likely, since the Mediterranean Sea is considered to be in a “tropicalization” phase, because of climate change effects.

	Expected results will be quantifiable by offering a control system for good management practices and to guarantee an optimal protection of mussel farms. This new knowledge will allow a reduction of the impacts both on the environment and on human health with sustainable solutions: this project aims to reduce waste of feedstock, by using circularity and reuse. Meetings with involved parts for training and follow up will be online, when possible, in order to cut back on unnecessary travel and therefore reduce CO_2 emissions. There will also be initiatives to rise awareness about the issue and environmental sustainability among the layman, such as conferences and articles.