

<b>Shellfish Pathology - Key Factor in Mussel Farming. A Black Sea Tailored Approach within the Shellfish Aquaculture Demonstrative Center (S-ADC)</b> <i>(Victor Niță, Magda-Ioana Nenciu, Simion Nicolaev, Houssam Hamza)</i>	<b>“Cercetări Marine”</b> <b>Issue no. 49</b>  <b>Pages 92 - 101</b>	<b>2019</b>
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## **SHELLFISH PATHOLOGY - KEY FACTOR IN MUSSEL FARMING. A BLACK SEA TAILORED APPROACH WITHIN THE SHELLFISH AQUACULTURE DEMONSTRATIVE CENTER (S-ADC)**

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### **ABSTRACT**

The Black Sea is acknowledged to be a unique aquatic ecosystem, characterized by complex aquaculture - environment interactions, whereby a tailored approach of this field is absolutely necessary. Black Sea aquaculture, although not as developed as in the Mediterranean, is exposed to the spread of aquatic diseases and pathologies that can cause heavy losses to farmers, as well as generate public health problems. The 10<sup>th</sup> session of the Scientific Advisory Committee on Aquaculture (CAQ) of the General Fisheries Commission for the Mediterranean (GFCM) (Turkey, March 2017) recognized that environmental protection is a major concern and stressed the importance of tackling all environmental aspects related to aquaculture at sea, including the use of risk analysis and data recording to assess aquaculture - environment interactions, as well as the vulnerability of ecosystems and the ecological services they provide. Furthermore, the GFCM adopted, at its 41<sup>st</sup> Session (Montenegro, October 2017), a strategy for the sustainable development of Mediterranean and Black Sea aquaculture (Resolution GFCM/41/2017/1). Target 2 of this strategy aims in particular at enhancing interactions between aquaculture and the environment, while ensuring animal health and welfare in order to minimize potential negative externalities, ensure adequate and responsible aquaculture management in the region and guarantee food safety and food quality. In this context, in the frame of NIMRD's Shellfish Aquaculture Demonstrative Center (S-ADC), a dedicated training course was organized during 6-10 May 2019, focusing on preventing and controlling mussel pathologies by acquiring knowledge of bacterial contamination, as well as early diagnosis. The course included both theoretical and practical modules (sampling, laboratory analyses, field visits). Also, complex issues were addressed, such as legislative and institutional gaps in the classification of mollusks from the veterinary point of view for consumption and Hazard Analysis and Critical Control Points (HACCP). The trainees represented institutions from Bulgaria, Georgia, Turkey, Russia, Ukraine and Romania, and the lecturers were both NIMRD and foreign experts in shellfish pathology.

**Key-Words:** pathology, mussels, training, microbial monitoring, HACCP, public health

## AIMS AND BACKGROUND

Currently, shellfish aquaculture is not developed to its full potential in the Black Sea region (except for Bulgaria) due to, on the one hand, environmental constraints, and, on the other hand, an unclear legislative framework (Niță et al., 2019). In this context, the Shellfish Aquaculture Demonstrative Center (S-ADC) in the Black Sea is the outcome of the FAO-GFCM Bucharest (2016) and Sofia (2018) declarations to enhance the regional cooperation in the sector, marked by a strong political commitment to increase collaboration with a view to promoting the rational exploitation of marine living resources, sustainable development of aquaculture and mitigation of threats marine environment and vulnerable species are facing. Mussel (*Mytilus galloprovincialis* Lamarck, 1819) farming development in the Black Sea was the first training effort of the Shellfish Aquaculture Development Center hosted by the National Institute for Marine Research & Development “Grigore Antipa” (NIMRD), Constanta, Romania. Focused on the holistic approach of the industry development recommendations, it aimed to follow the steps from the “farm up to the fork” and make understandable the gaps in the process (legal, scientific, administrative, production).

This effort was first operated in Romania (training, monitoring, mussel production, packaging) during 14-28 September 2018 (Niță et al., 2018). Subsequently, in 2019, the training programme developed during 6-10 May focused on shellfish pathologies and monitoring, by acquiring knowledge on bacterial contamination and early diagnosis. The course comprised both theoretical and practical modules (sample collection, laboratory analysis, field visits). Moreover, complex issues were addressed, such as settling the legislative gaps on zoo-sanitary classification of shellfish for consumption on the internal market and export (Nenciu *et al.*, 2019).

## EXPERIMENTAL

The main drawback for developing shellfish aquaculture in Romania is the lack of coordination between institutions (Sanitary Veterinary Directorate, Public Health Directorate, Romanian Waters Administration). The establishment of the Shellfish Aquaculture Demonstrative Center (S-ADC) in the frame of the NIMRD - General Fisheries Commission for the Mediterranean (GFCM) collaboration aims at the promotion of scientific, technical and technological bases for bivalve shellfish aquaculture in Romania. The methodology used is focused training for targeted beneficiaries: on the one hand, national and local management authorities involved in aquaculture planning, management, sanitary control, and, on the other hand, representatives of the private sector, especially the small-scale producers with limited investment capacity, as well as potential and existing investors. As such, the training programme developed during 6-10 May 2019

involved 10 participants from all Black Sea riparian countries: Ukraine (2), Romania (2), Bulgaria (1), Turkey (2), Georgia (2), Russian Federation (1), who deepened the knowledge on shellfish pathologies, as a key factor in mussel farming.

The European sanitary control of live bivalve mollusks is based on the Council Directive (EC) No. 492/91, which had previously set the hygiene rules for the production and marketing of live bivalve mollusks. Food safety monitoring of shellfish production areas in the European Community is currently regulated by the “Hygiene Package”, which entered into force on 1 January 2006, repealing the Directive (EC) No. 492/9. This legislative package includes Regulations (EC) No. 852/2004 and 853/2004, which target industry professionals, Regulation No. 854/2004, aimed at competent authorities, responsible of carrying out official sanitary controls, and Regulations (EC) No. 854/2004, 2073/2005 and 882/2004, which refer to end-product standards required for bivalve mollusks (European Community, 2004). The detailed implementation of classification and monitoring programmes following Regulation (EC) No. 854/2004 is the responsibility of competent authorities and may vary between Member States. Recently, European Regulation (EU) 2019/627 of 15 March 2019 replaced Regulation No. 854/2004, with no significant changes concerning shellfish microbial monitoring and control.

The risk of contamination of shellfish with bacterial and viral pathogens is evaluated by reference to the sources and types of fecal contamination (human and animal) in the vicinity of the shellfish production areas and the results obtained, based on the indicator bacteria *Escherichia coli*, from samples taken in these areas. Areas are classified following a full assessment of this risk and the classification given to an area determines whether shellfish harvested in that area require post-processing treatment and, where appropriate, the level of such treatment (Anon., 2017). The method used for determining the microbial contamination of mussel samples with *E. coli* is detection and Most Probable Number (MPN) technique specified in EN/ISO 16649-3.

## RESULTS AND DISCUSSION

In order to overcome the gaps and drawbacks in the Black Sea region, the training programme on shellfish pathologies was tailored to include 7 modules and their related theory and practical classes, involving:

**Module 1 - Basic Bivalves Biology:** the theoretical part of the first Module (Basic bivalves biology) of the training course consisted of presenting general data on the Black Sea benthic biocoenoses and their main characteristics. The main species of mollusks suitable for exploitation and

aquaculture at the Romanian Black Sea coast were presented. Moreover, the main habitats where mussels can be found in the Black Sea, their adaptations and physiological races were outlined. In the second part of the presentation, general data on mussel anatomy and physiology were presented. Emphasis was placed on their development cycle, breeding, and the factors that influence them. The practical hands-on part of the module consisted of presenting to the trainees the steps of performing a dissection and observations on the anatomy of the mussels (Fig. 1 left).

**Module 2 - Legislative framework:** the theoretical part of Module 2 covered the current European legislative package for the production and marketing of live bivalve mollusks (“Hygiene Package”, which entered into force on January 1<sup>st</sup>, 2006, repealing the Directive (EC) No. 492/91), focusing specifically on the sections which interest bivalve mollusk microbiological issues with regard to monitoring and classification of the production or relaying areas (Directive 91/492/EEC). Also, the following topics were approached: Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin; Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption; Directive no. 97/923 (2006/113/EC) “Shellfish Waters” implementation in Romania, adapted to the specific Black Sea conditions and related national legislation; accomplished steps for the implementation of the EU Directive in Romania; the parameters measured and the minimal/maximal values accepted, and which are included in the Annex of the Directive; sampling sites from designated marine areas for growth and economic exploitation of mollusks in Romania; recommendations and measures for complete fulfillment of the requirements of the “Shellfish Waters” Directive in Romania. The practical hands-on part of the module aimed at providing the trainees with the expertise for field work at sea, enabling the trainees to acquire the skills needed for the shellfish monitoring activities. The field course dealt with practical issues on monitoring the shellfish and surrounding seawater and included both seawater and mussel sample collection, preparation and storage before microbiological analysis (Fig. 1 right). Also, the practice covered: visit of the Chemistry and Biochemistry laboratories from NIMRD; short demonstration of laboratory methods used for analyzed parameters according to the “Shellfish Waters” Directive.



**Fig. 1.** Practical work: dissection and observations of mussel anatomy (left) and sample collection, preparation and storage before microbiological analysis (right) (*original photos*).

**Module 3 - Microbial monitoring and harmful algal blooms:** the theoretical part of Module 3 covered the following topics: Microbes - this course gave an introduction to principal pathogens and indicator bacteria in bivalves and their surrounding waters. The trainees were familiarized with the fundamental concept of fecal indicator bacteria (FIB) monitoring and health risk due to pathogenic microorganisms associated with marine shellfish. Harmful Algal Blooms (HABs)- introduction to the harmful algal species and their harmful effects as algal blooms to the shellfish harvests for human consumption. The present status in Black Sea was analyzed, with emphasis on the recommendation of the establishment of a registered National Laboratory for providing monitoring service to the administrative/veterinary management decisions for shellfish harvesting ban/free. The practical hands-on part of the module was focused on: *E. coli* estimation - in this practical course, the trainees learnt about the conventional method (the most probable number MPN technique) used for enumerating *E. coli* in shellfish and their growing seawater to determine their sanitary quality and to predict the risk of exposure to enteric pathogens (Fig. 2 right). Algal cells estimation - methodologies and equipment (planktonic nets, microscopes software) for phytoplankton sampling were demonstrated, in addition to sampling analysis and species detection through specific species identification keys for Black Sea region (Fig. 2 left).

**Module 4 - Mussel pathology and diseases (shellfish pathology with focus on virology, pathology of shellfish and impact on mussel quality).** Knowing the pathologies that can occur in mussels is an important aspect for the growth, development, marketing, and the evolution of natural populations. Early recognition of diseases and their further reporting is an important aspect in protecting aquatic biodiversity, as well as fisheries and aquaculture. As prevention is always better than the treatment, the module aimed to help interested factors in recognizing diseases in mussels and to understand their importance in ecosystems or aquaculture. The main illnesses that may occur, the causative pathogens, the disease syndrome, as well as

measures to prevent and control the illness were described. The main viral infections potentially affecting shellfish were introduced, focusing on: production and regulatory context, surveillance effort in the EU, main shellfish protozoan parasites, focus on the virus OsHV-1. The course was followed by practical applications in order to exemplify the work in detecting and recognizing pathogens.



**Fig. 2.** Practical work: algal cells estimation (left) and *E. coli* estimation by the MPN technique (*original photos*).

**Module 5 - Fisheries and aquaculture under Maritime Spatial Planning (MSP), Maritime Spatial Planning and Allocated Zones for Aquaculture (AZA):** this module aimed to integrate mussel aquaculture into the wider multi-use approach of the maritime space, in order to better understand co-location and co-existence perceptions with tourism and other marine uses. Around the Black Sea area, there is urgent need for active aquaculture spatial planning and area management that accounts for the range of uses of marine space, and human interactions in general, focused on a core requirement to increase space for aquaculture. In the Black Sea area there has been an expansion of aquaculture in recent years, but comprehensive regulation has been slower to develop. GFCM's Resolution 36/2012/1, providing guidelines on allocated zones for aquaculture (AZA), is not a mandatory regulation, but the resolution acts as a basic framework to guide GFCM contracting parties in the establishment of a spatial management of aquaculture, to avoid any potential contamination and/or conflicts. The training concerning Fisheries and Aquaculture under Maritime Spatial Planning (MSP) was focused on the main important aspects regarding these important fields by the spatial and temporal point of view taking into account the both development and relation with other maritime activities. The course included the dimension of marine fisheries and aquaculture (MFA) in the world, Black Sea and the Romanian Economic Exclusive Zone (EEZ). The boundaries establishment, mapping of human existing activities in the sea space, mapping future demands of marine space, useful for the elaboration, developing and evaluating of the maritime spatial and

management plan are the main steps of MSP, including MFA.

**Module 6 - Mussel safety and quality management (implementation of HACCP principles).** Module 6 covered the following topics: WHO Codex Alimentarius & EU legislation requests for mussel production collection and distribution for human consumption; design, construction and operation procedures for shellfish depuration and dispatch center; shellfish product value chain and the role of the Hazard Analysis and Critical Control Points (HACCP) plans as quality management systems.

**Module 7 - Country Reports:** all participants were provided with a template and shared information as requested. The **main conclusions** after each country representative presented their reports were the following: **Bulgaria** - there is a prevention and control programme in the Veterinary Medicine University. HACCP is desired to be implemented in fish/shellfish farms. There is a proposal for a regional regulatory system for zoo-sanitary control. **Georgia** - there is no HACCP implemented. **Romania** - there is HACCP implemented in all processing units (fish and shellfish). HACCP was implemented in the RAS (recirculating aquaculture system) turbot farm, in order to ensure product quality for the market (internal and exports). At national level, not many farms have HACCP implemented. **Russian Federation** - there is no HACCP implemented. **Turkey** - for the Eastern Black Sea region, there is a project on fish diseases. **Ukraine** - there is no HACCP system for fish implemented. HACCP would help the local producers to provide quality along the production chain, both for internal market and exports.

On the third day of the training course, the trainees travelled to Bulgaria, to observe the operation of a large-scale mussel farm (Fig. 3).



**Fig. 3.** Field visit at a large scale operational mussel farm (Bulgaria)  
(original photos).

The visit was planned by contacting in advance the farm manager, Mr. Nayden Stanev, who kindly agreed to receive onboard his vessel the participants and demonstrate an actual work day at sea. The Black Sea Shells Ltd. farm is situated in the vicinity of the town of Kavarna, Bulgaria. With the aim of rearing black mussel *M. galloprovincialis* production, cutting-



edge New Zealand technologies have been introduced, approved on a world scale as the most effective ones with the highest quality characteristics. The trainees took part in a typical workday at sea, which involved collecting seed mussels from one long-line and transferring them by socking to another long-line, for final development until harvesting to be released on the market. It was demonstrated the operating part of the large-scale mussel farm operation (1 x 2 km) including specifications on the equipment and working boat design.

After the completion of the one-week training course, the trainees were awarded graduation certificates (Fig. 4) and encouraged to transfer the acquired knowledge to the aquaculture sector (both research and market) in their countries.



**Fig. 4.** Awarding ceremony of the Shellfish Pathology Training Course, Constanta, Romania, 6-10 May 2019 (*original photo*).

## CONCLUSIONS

The programme was super-intensive and very diverse, as it had to cover the requested tasks by the legislation for production and marketing of the bivalves (certified microbiological and zoo-sanitary zones, monitoring programs). Despite that bivalve shellfish industry is a rather small sector in the Black Sea, the requested standards are higher than those of the farmed fish sector due to health and safety purposes.

The main outcomes of the training course can be summarized as follows:

- The main conclusion of the training course was that there is potential for shellfish aquaculture development in the Black Sea, mainly by joint work and collaboration between riparian countries. The most important factors



identified by participants are environmental peculiarities (limitative factors of developing mussel farms along the Black Sea coast), as well as the legislative framework. Legal/classification aspects are crucial for potential investors willing to develop profitable businesses. Future trainings should also include some marketing notions/market analysis etc.;

- Overall, participants were satisfied with the structure of the training course and the information they have acquired during the one week period.

- The field visit at the mussel farm was highly instructive and indicative on the potential of developing such businesses/activities in the Black Sea. The active contribution of the Bulgarian mussel farmers through hosting S-ADC participants gave an extra advantage for the S-ADC as it strengthens the collaboration links between two countries-partners (Romania-Bulgaria) for the operation of the S-ADC and gives in practice the multinational approach of this effort.

The second S-ADC training course focused on shellfish pathologies and monitoring. It was aimed to bring together people from different levels of the decision making in order to fill the gap between them so as to support the sector development from the “farm to the fork” of the consumer. The industry development has multi-scale and multidimensional levels, as a socioecological system depending on the captured-based approach of aquaculture. Similar interest for the further development of the post-harvesting shellfish quality management systems could be referred on the mussel depuration demonstration, as well as on the exploitation and handling of the *Rapana* sp., another high value shellfishery product in the Black Sea.

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## REFERENCES

- Anon. (2017), Microbiological monitoring of bivalve mollusk harvesting areas - Guide to good practice: Technical Application, 6, [www.eurlcefas.org](http://www.eurlcefas.org)
- European Community (2004), Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. Off. J. Eur. Union L **226**, 25.6.04: 83.

- European Community (2019), Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls. Off. J. Eur. Union: L 131/51, 17.5.2019.
- EN ISO 16649-3, Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* -- Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide. International Organization for Standardization, Geneva.
- Nenciu M.I., Niță V., Țoțoiu A., Hamza H. (2019), Framework for Setting-Up a Classification and Monitoring Program for Shellfish at the Romanian Black Sea Coast. *J Environ Prot Ecol (in press)*.
- Niță V., Theodorou J., Nicolaev S., Maximov V., Nenciu M.I. (2018), Capacity building and expert training in the frame of the Constanta Shellfish Aquaculture Demonstrative Center. *Cercetări Marine/Recherches Marines*: **48**: 92-99.
- Niță V., Theodorou J., Nicolaev S., Nenciu M.I. (2019), Advancing shellfish aquaculture as a sustainable food procurement option in emerging Black Sea riparian countries: Romania country report. *Scientific Papers, Series D, Animal Science*, Vol. LXII, No. 2, USAMV Bucharest, ISSN 2285-5750, ISSN CD-ROM 2285-5769, ISSN-L 2285-5750, ISSN Online: 2393 - 2260: 364-370.