

TOPIC A]

Exploring biodiversity in microbial ecosystems along the food chain

PRELIMINARY RESULTS ON BACTERIOLOGICAL AND VIRAL INVESTIGATION COMBINED WITH PHYTOPLANKTON AND ALGAL BIOTOXINS FROM A COASTAL LAGOON IN THE WESTERN MEDITERRANEAN (SARDINIA, ITALY)

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INTRODUCTION

Coastal lagoons are among the most productive and diversified ecosystems of the whole ecosphere, for their connection to the continent and the sea, the limited dimensions and the shallow waters. They are characterized by abiotic gradients that influence the biotic components, making them extremely interesting from a both naturalistic and scientific point of view. Nevertheless, most Mediterranean lagoons are subjected to eutrophication processes due to different natural and anthropogenic pressures, such as urban, agricultural and industrial discharges.

The Calich Lagoon is a Mediterranean coastal lagoon located along the north western coast of Sardinia in the middle of the western Mediterranean Sea, in an area of strong touristic impact. It is inserted in the Regional Natural Park of Porto Conte and plays a central role in contributing to biodiversity and protecting habitats. The lagoon is connected to the sea through a natural channel and a tourist harbour is in this channel. Two natural fluvial tributaries and one artificial canal deliver freshwater to the lagoon. The contribution of marine and fresh water, rich in nutrients, determines a high productivity of this lagoon. Despite its great potential and presence of growing natural beds of bivalve molluscs, the lagoon has not been yet classified for shellfish production.

In this study, through a multidisciplinary approach, it was evaluated the presence of several microbiologic (*Escherichia coli*, *Salmonella* spp, *Vibrio* spp) and viral (*Hepatitis A virus*, *Norovirus* GI and GII) pathogens, in addition to phytoplankton composition and associated algal biotoxins (Paralytic and Diarrhetic Shellfish Poisoning) in mussels and lagoon waters. The aim was to provide useful data to improve the knowledge on their spread and to assess the potential risk for public health as well as to implement rational conservation and management strategies in support of future economic and productive activities.

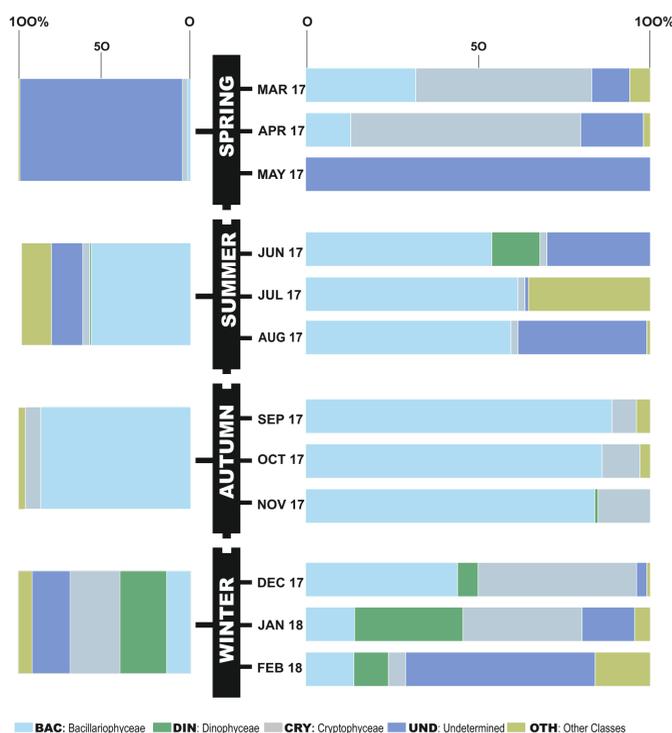
RESULTS

DETECTION OF BACTERIA, VIRUS AND BIOTOXINS IN MUSSELS AND WATER FROM CALICH LAGOON.

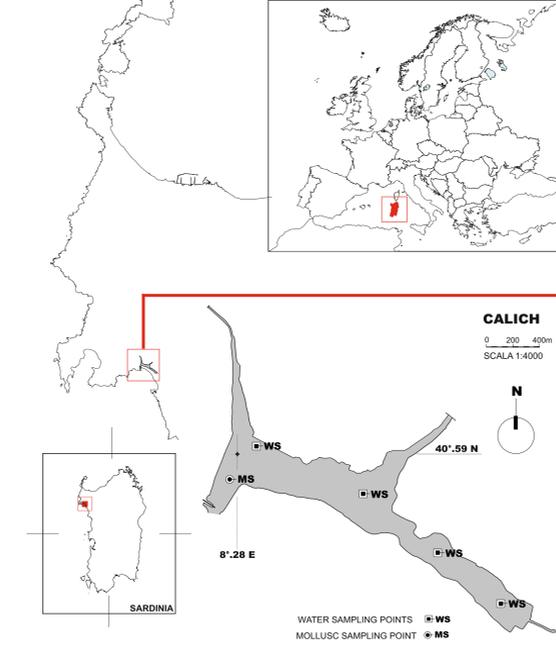
	SPRING	SUMMER	AUTUMN	WINTER
BACTERIOLOGIC PATHOGENS				
<i>Escherichia coli</i>	130 MPN	-	270 MPN	330 MPN
<i>Salmonella</i> spp	+	-	-	-
<i>Vibrio parahaemolyticus</i>	-	-	+	+
VIRAL PATHOGENS				
Norovirus GI	-	-	-	+
Norovirus GII	-	-	-	+
Hepatitis A virus	-	-	-	-
BIOTOXINS				
PSP	-	-	-	-
DSP	-	-	-	-

PSP: PARALYTIC SHELLFISH POISONING; DSP: DIARRHETIC SHELLFISH POISONING

MONTHLY (RIGHT) AND SEASONALLY (LEFT) CONTRIBUTION (%) OF PHYTOPLANKTON CLASSES TO THE TOTAL ABUNDANCE.



STUDY AREA

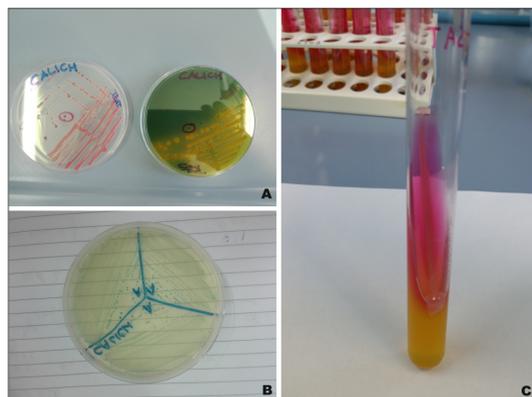


MAIN FEATURES OF THE CALICH LAGOON

LAGOON AREA	0.87 km ²
CATCHMENT AREA	432 km ²
MEAN DEPTH	1.20 m
MAX DEPTH	2.10 m
NUMBER OF INLETS	3
NUMBER OF OUTLETS	1
WATER INPUT	28.6 x 10 ⁶ m ³ y ⁻¹
RESIDENCE TIMES	8 d
MEAN SALINITY	17.4 ‰
TIDAL REGIME	Nanotidal
TYPE OF SEA CONNECTION	Permanent



IDENTIFICATION AND GROWTH OF BACTERIA



A] red and green colonies of *V. parahaemolyticus*, respectively on TSA and TCBS after 24h of incubation at 37°C
B] blue-green colonies of *E. coli* glucuronidase + on YBX agar after 24h of incubation at 44°C
C] typical growth of *V. parahaemolyticus* on TSI-S after 24h of incubation at 37°C

In February 2017, some macroalgae (mainly *Ulva* sp), favoured by mild temperature values and the nutrient presence, proliferated massively both in surface and on the bottom, forming the so-called "green tide". Samplings began in late March (spring 2017), coinciding with the first stages of the decomposition of the algae, which then continued until May. This caused a hypoxic crisis, resulting in a mass mortality of cultured fish as Flathead grey mullets and Sea breams. In addition, numerous individuals of the *Aplysia depilans* mollusc were present, likely attracted by the bloom of *Ulva* used as food.



A-B] Green tide in Calich Lagoon
C] *Aplysia depilans*

MATERIAL AND METHODS

The study was conducted from March 2017 to February 2018. A monthly water sampling was carried out for phytoplanktonic community and virus analysis, while *M. galloprovincialis* samples were collected seasonally from growing natural beds for microbiological, viral and biotoxin investigation. Four water sampling points representative of different hydrological features, including depth and salinity, were included in the study. A total of 48 monthly water samples and 4 of seasonally shellfish samples from a single mollusc sampling point were examined.

All microbiological and viral parameters and the biotoxins were analysed with official reference methods according to European legislation.

PSP: AOAC 959.08 (2005)
DSP: Regulation EC No 15/2011
Escherichia coli: ISO 16649-3
Salmonella spp: ISO UNI EN 6579:2002
Vibrio spp: ISO/TS 21872-1:2007
Viral analyses: ISO/TS 15216-2: 2013

Phytoplankton: Utermöhl's method

DISCUSSION

The Calich Lagoon is a very productive habitat and for this reason it is used for fishing and fish farming. Nevertheless, it could be better exploited implementing different forms of aquaculture.

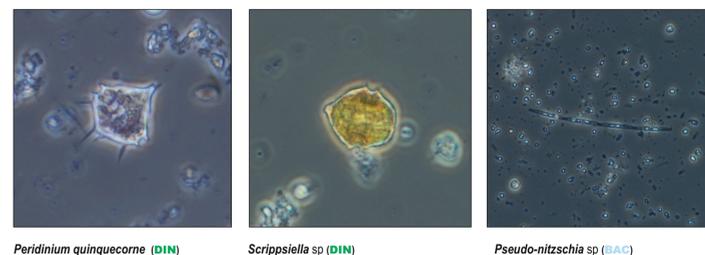
The Calich Lagoon plays a central role in contributing to birds' biodiversity and protecting scrublands and wetlands habitats. The scientific framework, through an integrated approach that consider how many biotic factors (viral, microbiological, phytoplanktonic) influence the water quality of the lagoon and the potential risk for public health connected to food resources (natural or bred), must be implemented.

The presence of microbiological and viral pathogens in autumn 2017 (*V. parahaemolyticus*) and winter 2018 (*V. parahaemolyticus*, *NoV* GI and *NoV* GII), positively correlated with the presence of *E. coli* above the legal limits for commercial molluscs, constituted a serious risk to the consumer's health.

Elevate phytoplanktonic abundance was detected in summer in water samples, with Bacillariophyceae as dominant group (60%). The interaction between mussel population and microalgae as food source (Bacillariophyceae in particular) is well known to favor the growth and the production of bivalves.

Although positive shellfish for DSP and PSP were recurrent and a relevant number of potentially toxic HAS were observed in the past years along the Sardinian coasts, no biotoxins have ever been detected in this study. *Pseudo-nitzschia* was the only toxic taxa documented, confirming that it is the most important one in terms of distribution.

PHYTOPLANKTONIC SPECIES DETECTED IN CALICH LAGOON DURING THE STUDY PERIOD



CONCLUSIONS

Several data are necessary for understanding the health-related problems connected with the consumption of seafood in complex ecosystems as the coastal lagoons. Water conditions can suddenly and quickly vary due to particular weather conditions or environmental changes. This vulnerability imposes the development of accurate and multidisciplinary sampling strategies that take into account possible bacteriological and viral contaminations, together with the presence of potentially toxic algal species. In order to ensure the classification of shellfish harvesting areas and their accurate and adequate management, only an alert monitoring system can guarantee the protection of consumers, especially where the risk of shellfish toxicity is so real.