# Venezia2021

# Programma di ricerca scientifica per una laguna “regolata”

**Linea 2.1**

***Qualità del sedimento lagunare a supporto della sua gestione sostenibile***

## Deliverable 2.1.4.1

Report sulle attività di monitoraggio di parametri ambientali e fisiologici effettuate mediante “bio-sensori” e sonde multiparametriche

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**Summary**


| | | |
|---|---|---|
| **1.** | **Introduction** | **3** |
| **2.** | **Materials and methods** | **5** |
| 2.1 Area of interest, samples and physiological parameters for the monitoring | | 5 |
| 2.2 Biosensors | | 7 |
| 2.3 Environmental parameters monitoring | | 8 |
| **3.** | **Results and discussion** | **9** |
| 3.1 Manila clam (*R. philippinarum*) monitoring | | 9 |
| 3.2 Analyses of *M. galloprovincialis* farming sites | | 14 |
| **4.** | **Conclusions** | **18** |
| **5.** | **Bibliography** | **19** |

# Abstract

La Vongola filippina (*Ruditapes philippinarum*) e il Mitilo mediterraneo (*Mytilus galloprovincialis*) sono specie di molluschi bivalvi allevati in diverse zone della laguna di Venezia. Nonostante l'elevata tolleranza alle variazioni di parametri ambientali e l'adattabilità a diverse condizioni, lo stato di salute di tali molluschi può essere influenzato dalla presenza fattori di stress, di origine e naturale e antropica.

Ottenere informazioni sullo stato di salute delle vongole e dei mitili allevati e sulla correlazione con le condizioni ambientali richiede misurazioni *in situ* a lungo termine. Per tale motivo, il WP2.1.4 "Valutazione degli impatti della messa in funzione del MOSE sulla produttività delle aree di molluschicoltura della laguna di Venezia" della Linea 2.1 "Qualità del sedimento lagunare a supporto della sua gestione sostenibile" ha svolto due campagne di monitoraggio (maggio 2018-maggio 2019 1° anno; maggio 2019-maggio 2020 2° anno) al fine di registrare i dati biometrici e fisiologici degli organismi e di monitorare continuamente diversi parametri ambientali. Tali monitoraggi servono e serviranno in futuro per determinare le migliori condizioni/siti per l'allevamento di vongole e cozze, nonché a far emergere fattori critici in grado di influenzare lo stato di salute dei molluschi e i potenziali effetti sulle aree di allevamento bivalve del Modulo Sperimentale Elettromeccanico (MoSE).

I parametri chimico-fisici e biometrici analizzati non hanno messo in evidenza variazioni evidenti tra i due anni di monitoraggio. Tuttavia, si sono riscontrate differenze nell'andamento delle mortalità tra le due campagne di monitoraggio.

Alla luce del crescente utilizzo delle barriere del sistema MoSE e dei cambiamenti climatici in atto, lo studio mette in evidenza la necessità di proseguire il monitoraggio delle aree di allevamento nei prossimi anni, al fine di individuare criticità nell'ecosistema lagunare che si possono ripercuotere nelle attività produttive.

# 1. Introduction

Lagoons are heterogeneous environments with gradients of several physico-chemical parameters and typically with high levels of nutrients and consequently high primary productivity. Lagoons host abundant fauna and flora sometimes of socioeconomic importance, either on a permanent or temporary basis (Deheyn and Shaffer., 2007). The Venice Lagoon represents a vulnerable ecosystem and a complex and dynamic transition area at the interface between terrestrial, freshwater and marine habitat. It is prone to high variation of biotic and abiotic factors based on spatiotemporal conditions (Alvarez-Borrengo and Alvarez-Borrengo, 1982; Bertolini et al., 2021) and on intense anthropogenic interventions (Deheyn and Shaffer., 2007). Seasonal variations, adverse climatic conditions, physico-chemical features and anthropogenic stressors can have consequences on the ecology of such a fragile environment as well as on its biology, especially for sessile and highly sedentary organisms, as bivalve mollusks, that cover important ecological and economical roles.

Shellfish, like Manila clam (*Ruditapes philippinarum*) and Mediterranean mussels (*Mytilus galloprovincialis*), are extensively farmed in several areas of the Venice Lagoon. Despite their high tolerance to variations of environmental parameters, such as salinity, temperature, dissolved oxygen, and their adaptability to different conditions (Boscolo Brusà et al., 2013), natural and anthropogenic stressors can have direct and indirect consequences on the fitness of these living organisms.

Gaining insight of the health status of farmed clams and mussels and how it is related to environmental conditions requires long-term *in situ* measurements. The present study carried out a long-lasting monitoring campaign on clams and mussels from several farming stations in the south of the Venice Lagoon. The aim of the present study was to record biometric and physiological data of organisms and to continuously monitor several environmental parameters to determine the best conditions/sites for clams and mussels rearing beyond to underlining critical factors potentially able to influence the fitness of mollusks. Data collected through these analyses are integrated with molecular analyses performed on the same bivalve batches aimed to characterize gene expression profiles and determine host-microbiota interactions. Among other objectives, the two monitoring campaigns (May 2018-May 2019 1st year; May 2019-May 2020 2nd year) allow to characterize potential effects on bivalve farming areas of the Experimental Electromechanical Module (MoSE) entered into operation in autumn-winter 2020.

# 2. Materials and methods

## 2.1 Area of interest, samples and physiological parameters for the monitoring

For the WP2.1.4, two monitoring campaigns (May 2018-May 2019 1st year; May 2019-May 2020 2nd year) were carried out on Manila clam (*R. philippinarum*) and Mediterranean mussel (*M. galloprovincialis*), two species of relevant ecological and commercial importance.

For the two monitoring years, spats of Manila clam, supplied by Satmar Company (France), were placed in different farming areas (5000 clams each) in the south of the Venice Lagoon at gradual distances from the Chioggia inlet. In detail, the experimental sites (see Figure 1) were the outmost 1_VAR, 2_VAR, 3_VAR and 4_SAU. During the first year, high mortality of Manila clams was recorded in several farming sites (see results section). Among the possible factors contributing to these mortality events, high prevalence of *Perkinsus spp*, a parasitic protozoans that infects bivalve species causing disease and mass mortalities, has been proposed. Considering that high salinity and temperature may promote the proliferation and dissemination of *Perkinsus spp.*, an inner site characterized by lower salinity (8_SAU) was added for the second monitoring campaign. In order to detect and quantify *Perkinsus* in Manila clams in the investigated farming sites, a collaboration with Istituto Zooprofilattico delle Venezie (Centro di referenza nazionale per lo studio e la diagnosi delle malattie dei pesci, dei molluschi e dei crostacei; Dott. Giuseppe Arcangeli) has been established during the 2nd monitoring campaign. About *Mytilus galloprovincialis*, natural spats were recruited and then placed in three farming areas at gradual distance from the inlet: 5_SCA, 6_CAM and 7_BLU (Fig.1).

Coordinates of each site are reported in Tab 1.


Figure 1. Area of interest and study sites of clams (▲1 VAR, 2 VAR, 3 VAR, 4 SAU, 8 SAU), mussels (■ 5 SCA, 6 CAM, 7 BLU) and multiparametric probes (● Probe – North, Probe - South A, Probe - South B).

Table 1. Coordinates of each farming site of clams (1 VAR, 2 VAR, 3 VAR, 4 SAU, 8 SAU), mussels (5 SCA, 6 CAM, 7 BLU) and multiparametric probes (Probe – North, Probe - South A, Probe - South B).

| **site name** | **coordinates (Lat Long)** |
|---|---|
| 1_VAR | 45°13,86N 12°16,59E |
| 2_VAR | 45°13,82N 12°15,94E |
| 3_VAR | 45°12,957 N 12°15,62 E |
| 4_SAU | 45°11,59N 12°15,53E |
| 8_SAU | 45°11,655 N 12°14,96 E |
| 5_SCA | 45°14,02N 12°16,89 E |
| 6_CAM | 45°13,82N 12°16,02 E |
| 7_BLU | 45°13,83N 12°15,42 E |
| Probe – North | 45°13,84’N 12°15,99 E |
| Probe - South A | 45°12,07’N 12°15,21 E |
| Probe - South B | 45°11,65 N 12°14,97 E |

Samplings were performed in May 2019 (T0), July 2019 (T1), October 2019 (T2), February 2020 (T3) and May 2020 (T4) during the first year, and in May 2020 (2T0), July 2020 (2T1), October 2020 (2T2), February 2021 (2T3) and June 2021 (2T4) during the second year. For each sampling time and site, clams and mussels were randomly collected, manually and/or using a manual rake, and then transferred to the laboratory. Manila clams were also sampled every one or two months to evaluate and quantify the presence of *Perkinsus spp*. Mortality of clams was detected, biometric parameters were recorded and the Growth rate (GR), Condition Index (C.I.) and Shell Thickness Index (STI) were calculated as follows:

$$GR = \frac{(\text{length final month} - \text{length initial month})}{\text{number of days}} \text{ x } 100$$

$$C.\text{I.} = \frac{\text{soft tissue dry weight}}{\text{shell dry weight}} \text{ x } 100$$

$$\text{STI} = \frac{1000 * (\text{shell dry weight })}{(lenght * (thick^2 + width^2)^{0.5}) * \frac{\pi}{2}} \text{ x } 100$$

To measure the dry weight of clams, soft tissues were separated from shells, washed in distilled water and oven dried at 60°C for 48 h. Shell and meat dry weights were recorded using a scale with 0.01 g sensitivity.

Burrowing capacity of clams grown in different farming sites was also determined in different seasons (summer, winter and spring). To do this, a plastic tank was filled with 5 cm of sand covered in at least 5 cm of water. The suspended sand was let to settle. Once the water reached an adequate transparency, animals from each experimental group were placed on the sand and left for 5 minutes to acclimate. Then, clams were left 1 hour to bury. During this time, animals were observed and the burial time was recorded. At the end of each trial, animals were classified as buried, partially buried (PB) and not buried (NB).

**2.2 Biosensors**

In close cooperation with the Line 5.2 "Impatti vulnerabilità e rischi indotti dal cambiamento del clima" (Prof. Roberto Pastres; Camilla Bertolini), to perform the monitoring of valve-gaping, two adult mussels (>5 cm) were collected at each farm site (5_SCA, 6_CAM and 7_BLU) and therefore 6 biosensors in total were applied. A small magnet (Ø = 3 mm) was glued into each sensor on the mussel shell. Sensors used for the long-term monitoring, developed at the Royal Netherlands Institute for Sea Research (NIOZ), measured the valve-gaping or hydrodynamics (using magnetism/hall sensor -DRV5053VAQLPGM), air/water pressure (pressure sensor - MS580314BA01-00) and temperature (temperature sensor - TSIC506F) over the first year of monitoring as defined in the Line 5.2. In order for the hall sensor to be used for shell gaping, a small magnet (magnet size Ø = 3 mm) was glued on the mussel shell, placed between the inhalant siphon and the mantle (Fig.2).

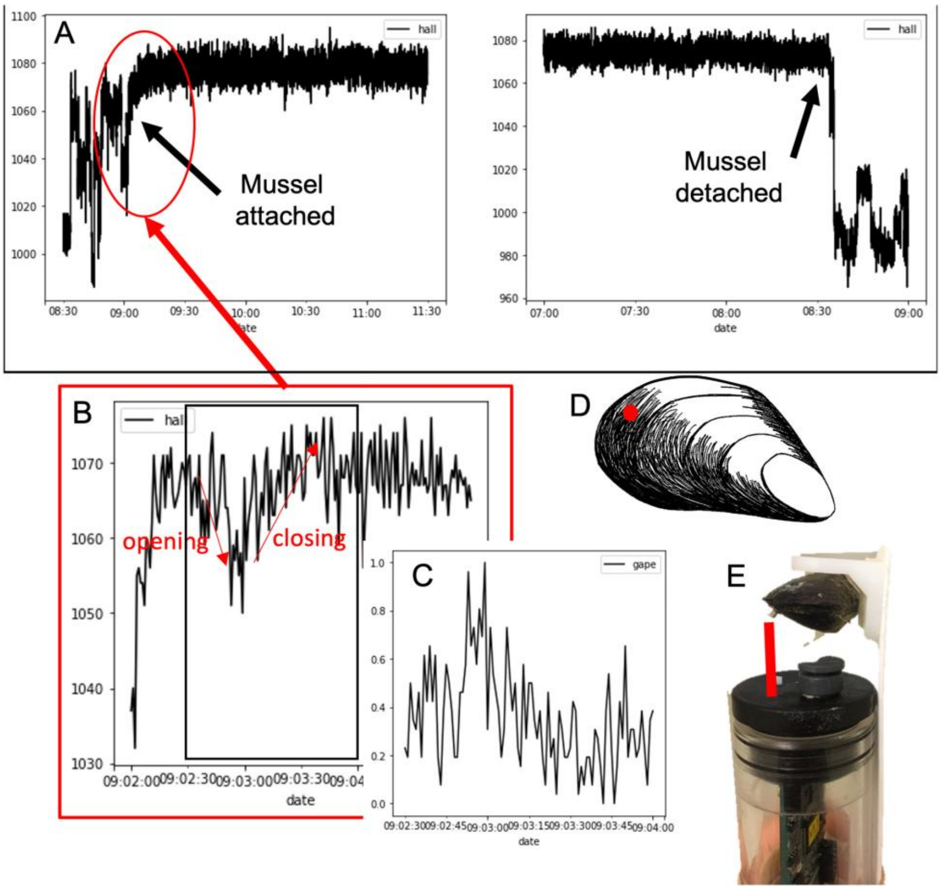


Figure 2. Figure extrapolated from Bertolini et al., 2021. Processing methods for the gaping data. (A) Identification of the moment where mussel was attached to the sensor, jumping occurs because of the presence of the magnet attached to the shell and located directly above the sensor (D, E). Red line in (E) shows the distance being measured to quantify gaping. (B) and (C) represent data series processing.

Data recorded by biosensors were downloaded approximately every month to avoid data loss. The sensors were first deployed on July 16th 2019, and data was retrieved (sensors collected and then redeployed) on August 20th, September 24th, October 24th and December 18th. The valve-gaping measurement is based on the principle described in Ballesta-Artero et al. (2017) and the sampling frequency was set at 1 s for all parameters. Raw data were elaborated and plotted using Phyton 3.6 (Spyder 3.1.4, Anaconda) and to avoid spikes of noise, outliers were removed based on lower and upper quantiles (95%). More details regarding the data management are published in Bertolini et al., 2021. In 2020, sensors were deployed again in January and left until October in order to record all seasons in the same set up. Data are currently being analyzed by Bertolini and Pastres using machine learning algorithms to disentangle influence of environment on valval gap of mussels.

### 2.3 Environmental parameters monitoring

Two multiparametric probes (Tecnos S.a.S) were placed in two strategic sites of the south of the Lagoon (Probe Northand Probe South - A) (Fig.1, Tab.1) to monitor various chemical-physical parameters (temperature (° C), dissolved oxygen (ppm), saturation (%), chlorophyll (mg / l), salinity (ppm), turbidity (NTU) and pH). For the 2$^{nd}$ year, a third probe (Probe South - B) (Fig.1, Tab.1) was installed close to the clams farming site 8_SAU. Furthermore, for the second year of monitoring, in synergy with Line 5.2 Task 1 (Prof. Roberto Pastres and Camilla Bertolini), two thermometers per each clams farming site were placed into the sediment in order to record the sediment temperature along the seasons.

# 3. Results and discussion

### 3.1 Manila clam (*R. philippinarum*) monitoring

Mortality of clams in the investigated farm sites was determined as the increased in mortality in different periods. In detail, mortality values (Table 2) were calculated as the difference between the percentage of mortality in a given sampling time and the mortality observed in the previous sampling time.

Table2. Calculated mortality of clams from each investigated site during different sampling times.

| | Year 1 | | | |
|---|---|---|---|---|
| | **1_VAR** | **2_VAR** | **3_VAR** | **4_SAU** |
| **May - July 2019** | 6.90% | 9.80% | 31.82% | 17.14% |
| **July - October 2019** | 22.82% | 5.08% | 15.80% | 9.69% |
| **October 2019 - February 2020** | 8.03% | 14,79% | 0% | 0% |
| **February - May 2020** | 0% | 0% | 0% | 0% |

| | Year 2 | | | | |
|---|---|---|---|---|---|
| | **1_VAR** | **2_VAR** | **3_VAR** | **4_SAU** | **8_SAU** |
| **May - July 2020** | 3,85% | 3,47% | 5,20% | 5,41% | 3,28% |
| **July - October 2020** | 12,25% | 11,36% | 21,04% | 27,13% | 70,62% |
| **October 2020 - February 2021** | 6,67% | 0,55% | 0,00% | 0,00% | 0,00% |
| **February – June 2021** | 13,9% | 35,9% | 19,8% | 51,8% | n.d. |

In the first monitoring year, the greatest mortality was observed in the early sampling time (from May to October), in particular in 3_VAR and 1_VAR farming sites. A different scenario for the four investigated faming sites has been described for the second year. In the first monitoring campaign high mortality was observed in all farming sites in the warmer season (July-October 2020), whereas the greatest mortality was observed between February and June 2021 in the site 4_SAU and 2_VAR. With reference to 8_SAU, despite the lowest water salinity measured by the Probe South – B (see below) which as a consequence should reduce Perkinsus infections, almost the totality of clams died at the second sampling time. This is probably due to the specific characteristics of the farming area, characterized by very fine sediment granulometry.

Regarding clams shell length (Fig. 3), the maximum length of shells was reached by clams from the innermost site 4_SAU in both monitoring campaigns, followed by the outmost 1_VAR. It should be noted that spats at the beginning of the second year of monitoring (May 2021) were bigger then spats transplanted in the first one (May 2019).

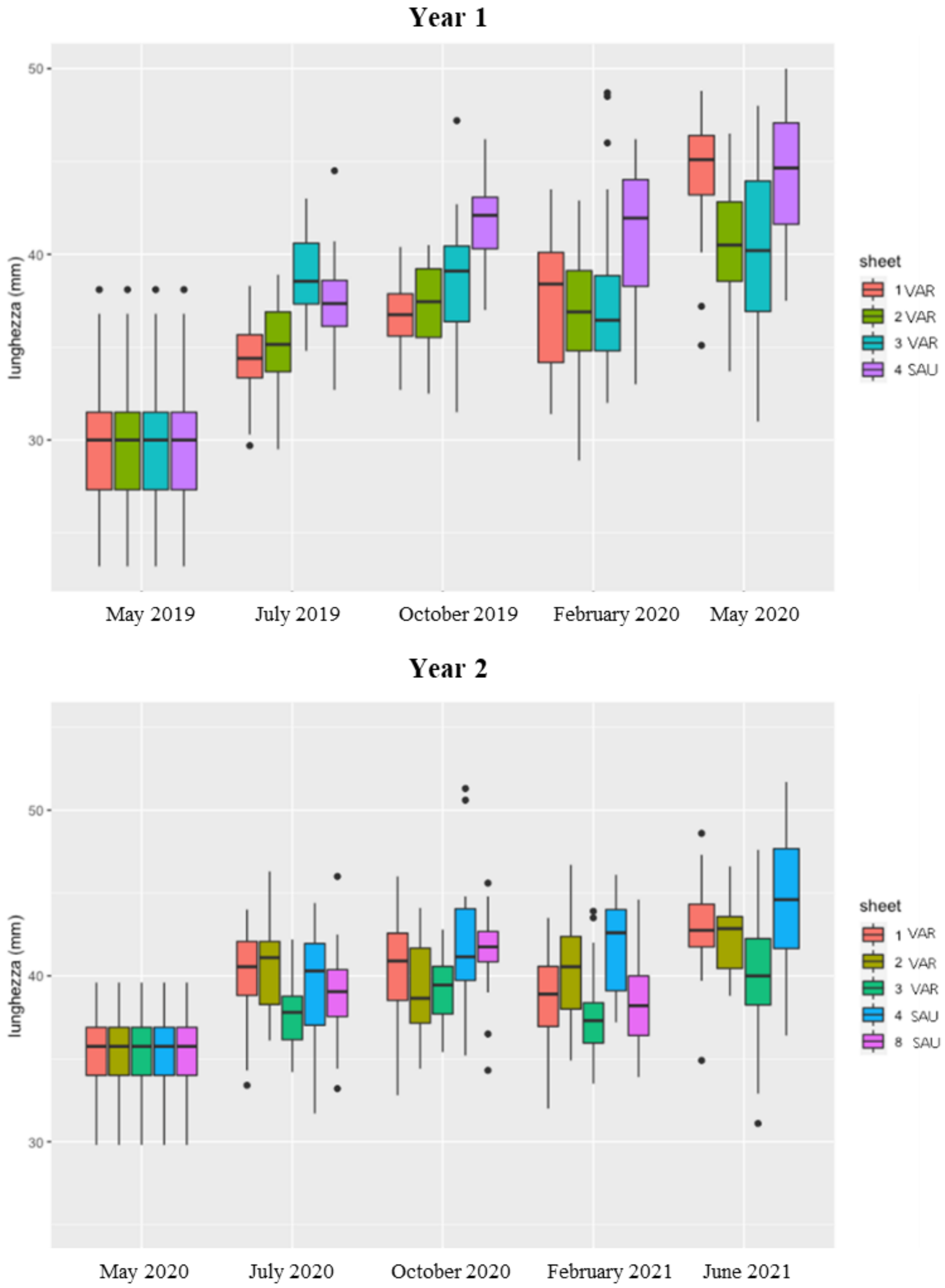


Figure 3. Shell length of clams from each investigated area during the first and second year monitoring campaign.

The growth performance calculated through the GR parameter (Tab.3) showed a higher growth rate in the period May-July both in the first and in the second year of monitoring in all investigated farming sites.

Table 3. Growth rate of clams from each investigated area during each sampling.

| YEAR 1 | Growth rate (GR) | | | |
|---|---|---|---|---|
| | May-July 2019 | July - October 2019 | October 2019 - February 2020 | February-May 2020 |
| 1_VAR | 1,32 | 0,03 | 0,28 | 0,59 |
| 2_VAR | 1,34 | 0,13 | 0,10 | 0,37 |
| 3_VAR | 1,81 | 0,11 | -0,03 | 0,16 |
| 4_SAU | 1,61 | 0,34 | 0,15 | 0,31 |

| YEAR 2 | Growth rate (GR) | | | |
|---|---|---|---|---|
| | May-July 2020 | July - October 2020 | October 2020 - February 2021 | February 2021 - June 2021 |
| 1_VAR | 0,07 | 0,00 | 0,00 | 0,03 |
| 2_VAR | 0,07 | 0,00 | 0,01 | 0,01 |
| 3_VAR | 0,05 | 0,00 | 0,00 | 0,02 |
| 4_SAU | 0,07 | 0,02 | 0,00 | 0,06 |
| 8_SAU | 0,06 | 0,02 | 0,00 | 0,00 |

Regarding the health status of clams, condition index showed the lowest values in clams collected in October and in February in both monitoring years. The highest value of C.I. was found on site 1_VAR in May 2020 during the first year, and in July 2020 during the second year (Fig.4).

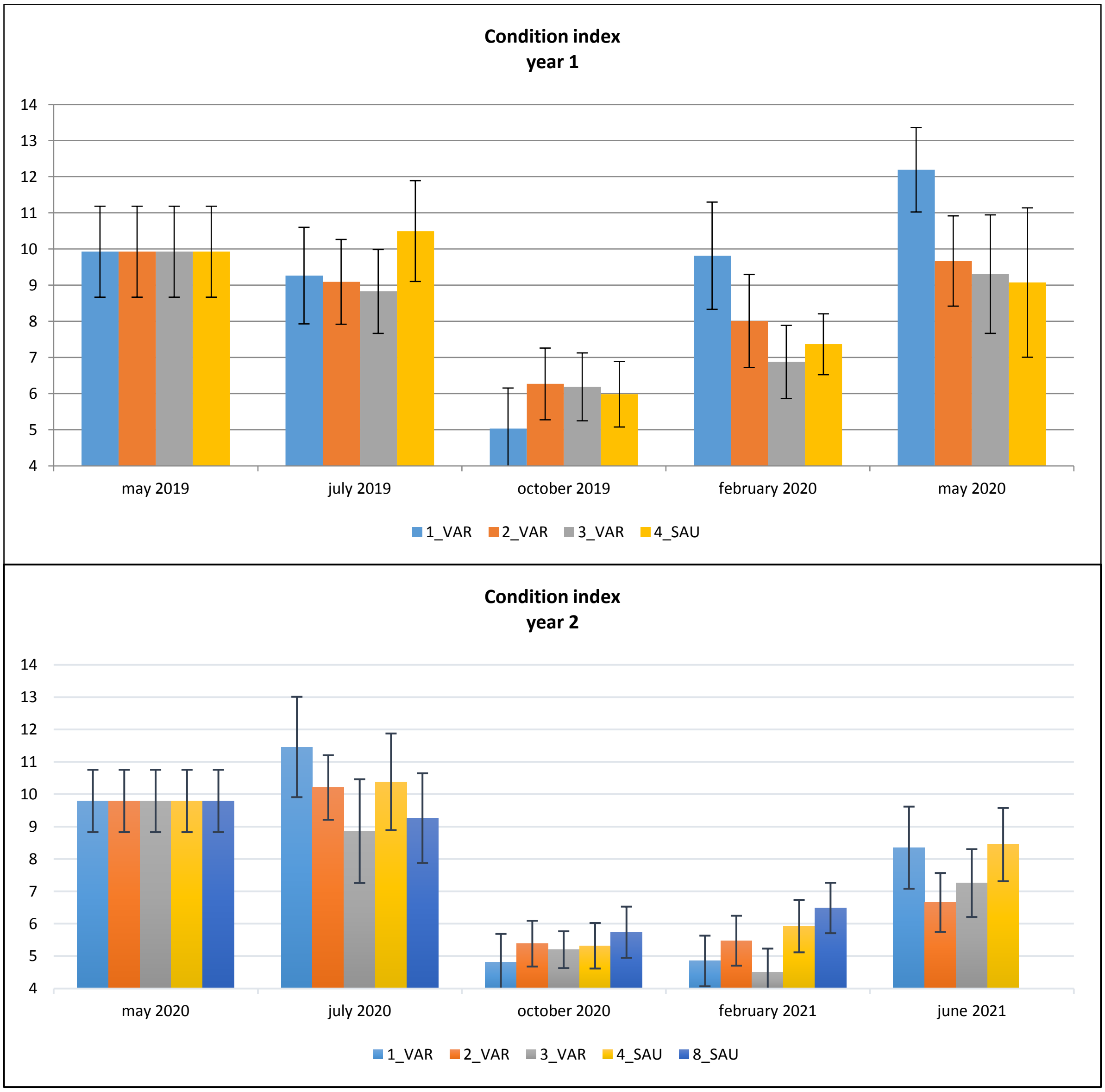


Figure 4. Condition index (C.I) of clams from each investigated area during each sampling.

To implement information regarding the health status and the growth performances, the Shell Thickness Index (STI) was also calculated during the second year. This index reflects the ability to defend from predators, as well as greater shell integrity during harvesting and the improving of the maintenance of fitness-related functions (Babarro et al., 2020; Haider et al., 2018). Results (Fig. 5) showed an increased trend in the value of STI from July 2020 to June 2021 in each investigated site, with the best performance reached in 4_SAU site at the end of the productive cycle.

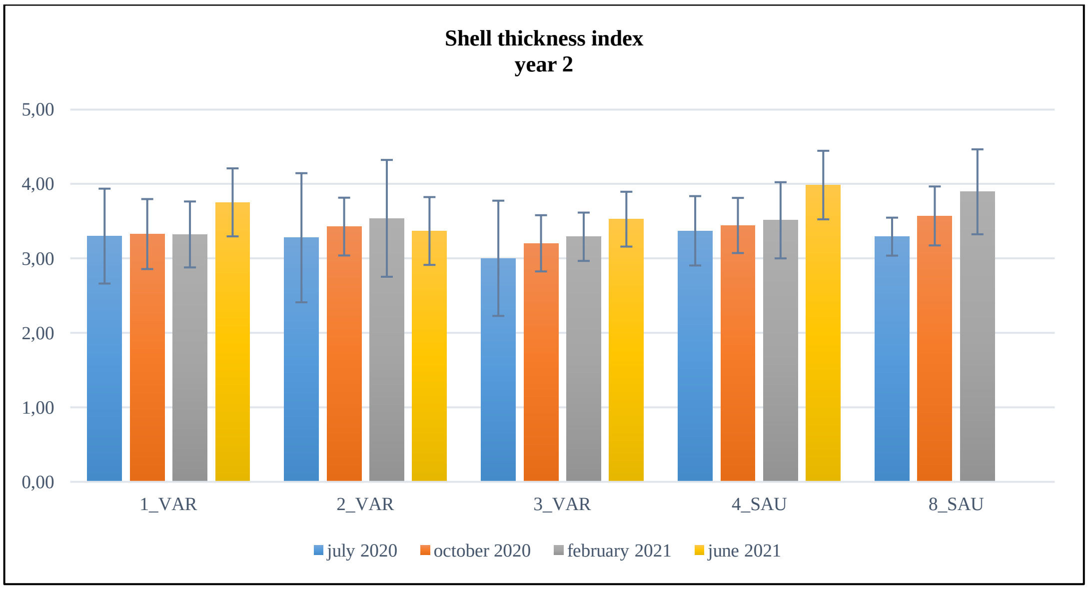


Figure 5. Shell Thickness Index (STI) of clams from each investigated area during each sampling.

In terms of burrowing capacity, it is notable that clams farmed in 8_SAU showed the lowest number of clams completely burrowed in both sampling time (Tab.4). At the end of the monitoring campaign (June 2021) the burrowing capacity was very similar among investigated sites (8_SAU was excluded due to the lack of living animals to perform this test).

Table 4. Burrowing capacity of clams from each investigated area observed in different seasons (summer: August 2020; winter: February 2021; spring: June 2021).

| SUMMER | 1_VAR | 2_VAR | 3_VAR | 4_SAU | 8_SAU |
|---|---|---|---|---|---|
| %B | 30,0% | 20,0% | 16,7% | 10,0% | 3,3% |
| % PB | 23,3% | 23,3% | 20,0% | 16,7% | 13,3% |
| %NB | 46,7% | 56,7% | 63,3% | 73,3% | 83,3% |

| WINTER | 1_VAR | 2_VAR | 3_VAR | 4_SAU | 8_SAU |
|---|---|---|---|---|---|
| %B | 20,0% | 20,0% | 10,0% | 30,0% | 0,0% |
| % PB | 0,0% | 10,0% | 10,0% | 10,0% | 30,0% |
| %NB | 80,0% | 70,0% | 80,0% | 60,0% | 70,0% |

| SPRING | 1_VAR | 2_VAR | 3_VAR | 4_SAU |
|---|---|---|---|---|
| %B | 10,0% | 20,0% | 10,0% | 20,0% |
| % PB | 30,0% | 20,0% | 10,0% | 20,0% |
| %NB | 60,0% | 60,0% | 80,0% | 60,0% |

### 3.2 Analyses of *M. galloprovincialis* farming sites

Shell length in all sampling sites is reported in Fig. 6. It should be highlighted that mussels biometric parameters are affected by high variability due to the high variability in the size of the recruited natural spats.

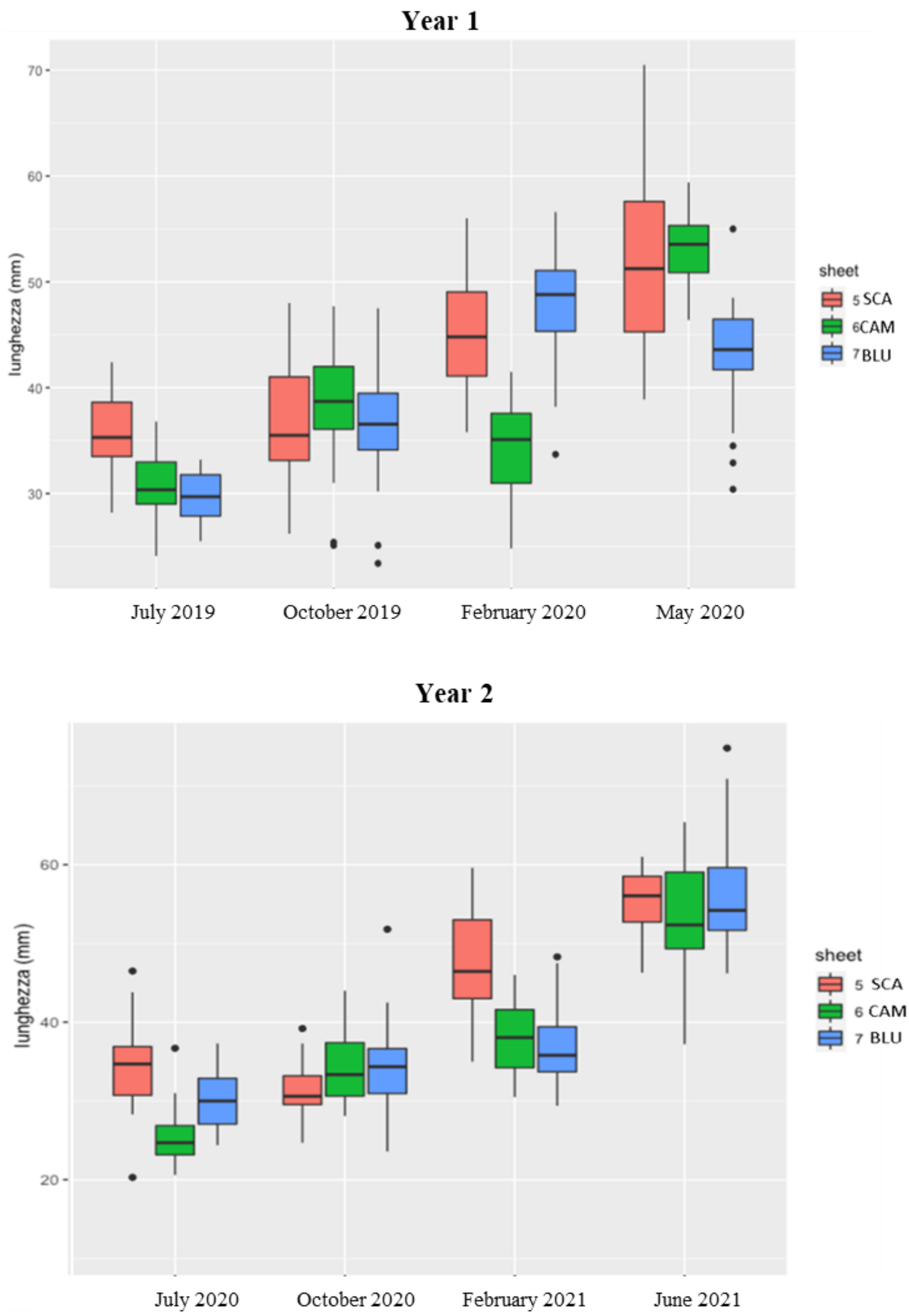


Figure 6. Shell length of mussels from each investigated area during each sampling.

As expected in terms of C.I., the lowest values of C.I. were obtained in both campaigns in the colder season, while the highest values of C.I. were observed in July in both monitoring campaigns (Fig. 7).

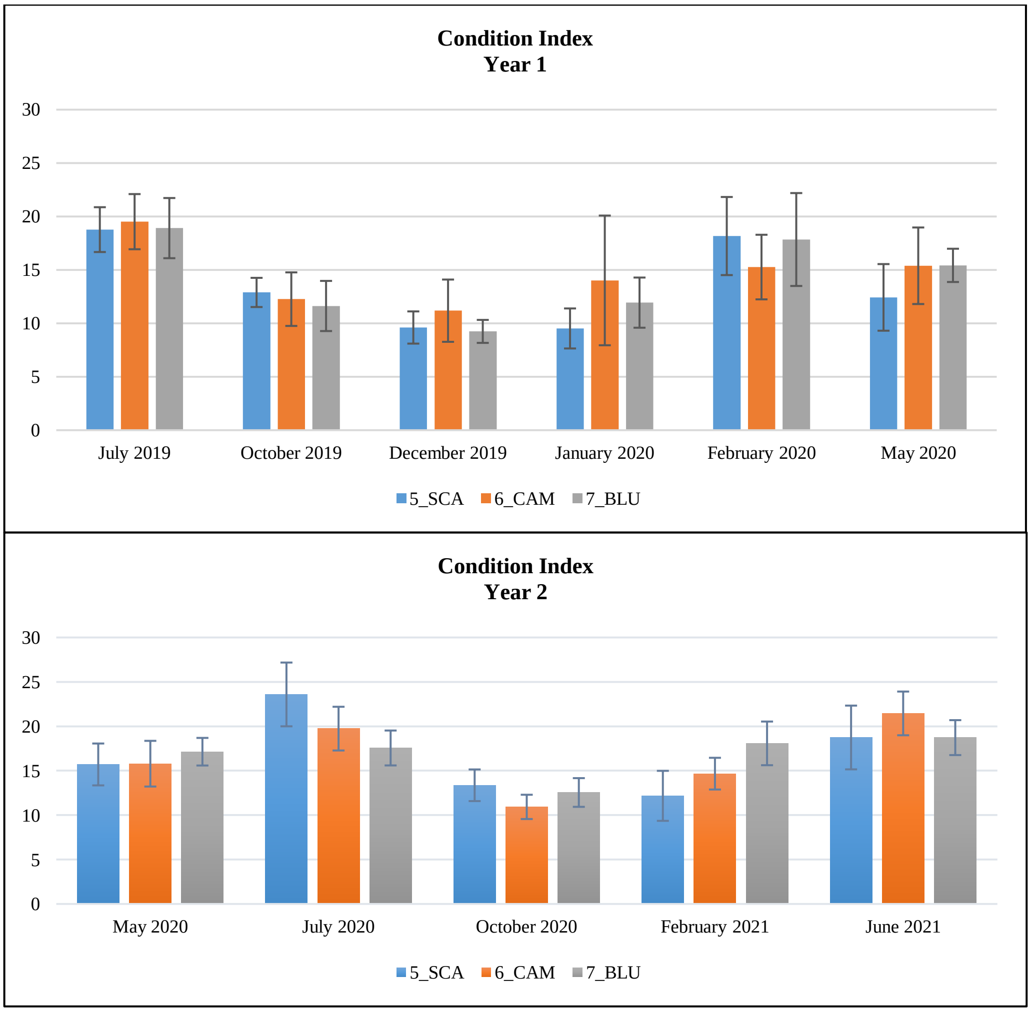


Figure 7. Condition Index (C.I) of mussels from each investigated area during each sampling.

Shell Thickness Index (Fig.8) showed similar trend in 5_SCA and 6_CAM. As for Manila clams, the best performance for this index was recorded in the inner site (6_CAM) at the end of the monitoring campaign.

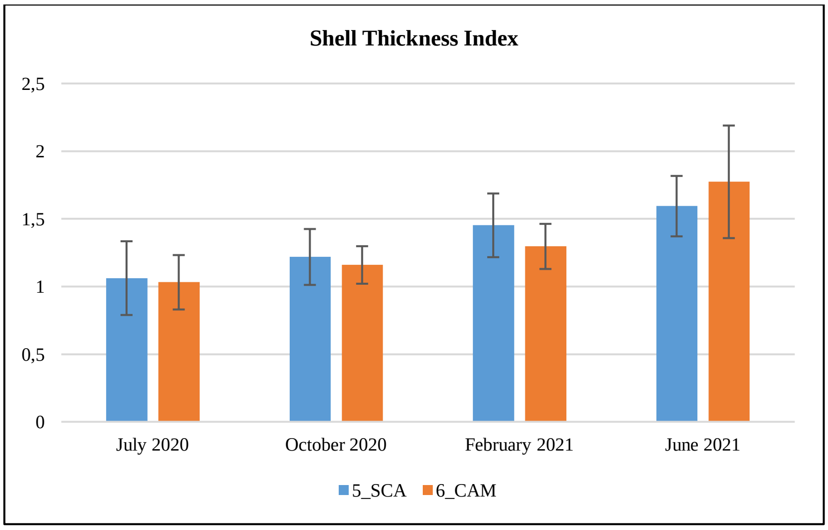


Figure 8. Shell Thickness Index (STI) of clams from each investigated area during each sampling.

### 3.3 Biosensors

The first available results regarding physiological alterations studied through biosensors showed that the opening / closing gap of the mussel valves has periodic rhythms of about 12 and 24 hours. These cycles were more pronounced in mussels reared in the innermost part of the lagoon, especially in the summer months. This first result seems to be essentially influenced by the location position of the farm itself. Daily fluctuations of environmental parameters were found for dissolved oxygen, mainly due to primary productivity, while turbidity has a periodicity due to the tidal cycle. More results are reported in the published paper Bertolini et al., 2021. The elaboration of remaining data is still ongoing and it will performed applying Machine Learning techniques and algorithms, in synergy with the Line 5.2 of this Research Project.

### 3.4 Environmental parameters

The correlation between the response of the organisms and the physical characteristics of the natural environment will play a key role to evaluate the potential effects of MoSE's activation on the ecosystem and on the productive activities related to shellfish farming.

Preliminary data of different chemical-physical parameters are reported below (Fig. 9).

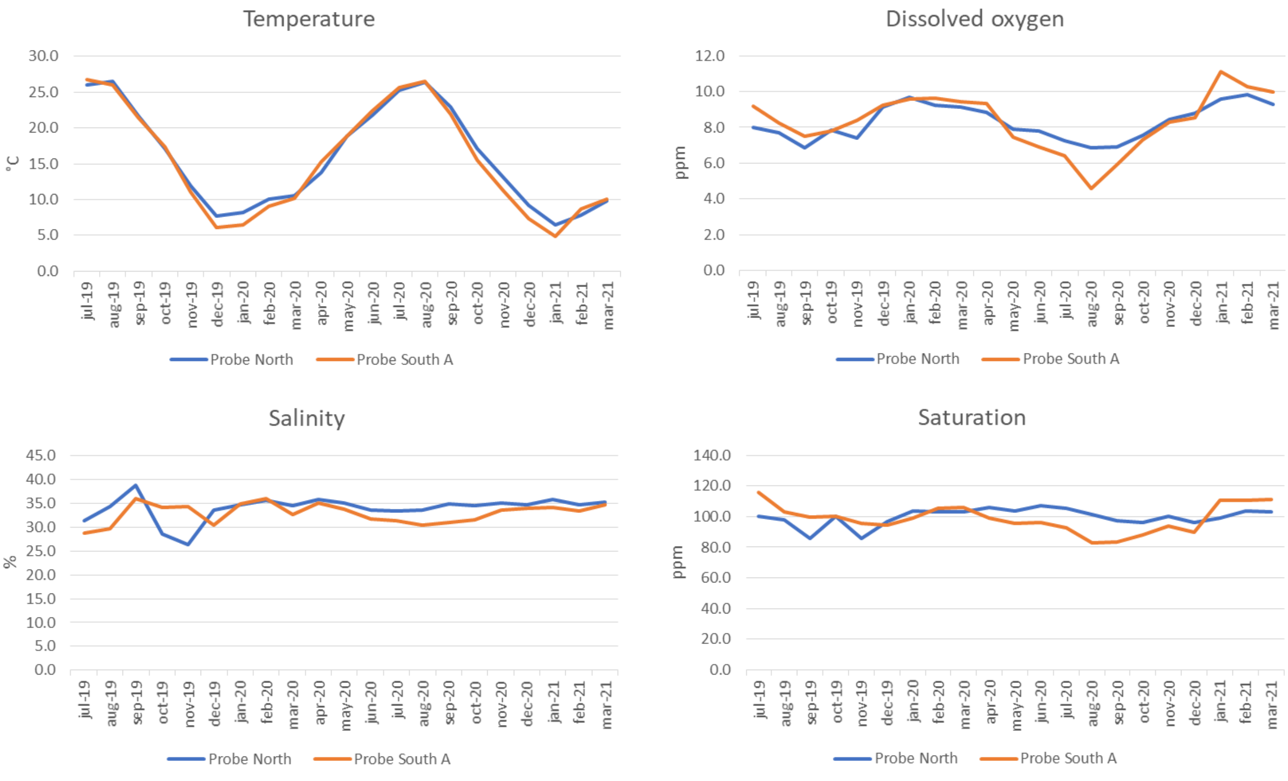


Figure 9. Temperature, dissolved oxygen, salinity and saturation registered by the Probe North and Probe South – A during the years.

Environmental parameters registered by the probes showed greater variability of temperature in the innermost site compared to the outmost farming area. Dissolved oxygen in both monitoring years reached the highest values in winter (January-February). As expected salinity showed the lowest values in the innermost sites.

No particular differences were observed for all investigated parameters before and after the MoSE system inauguration (December 2020). With regard to the Probe South – B, the range and the trend of temperature, dissolved oxygen and saturation were similar to the other two probes, especially to the Probe South – A (Fig. 10).

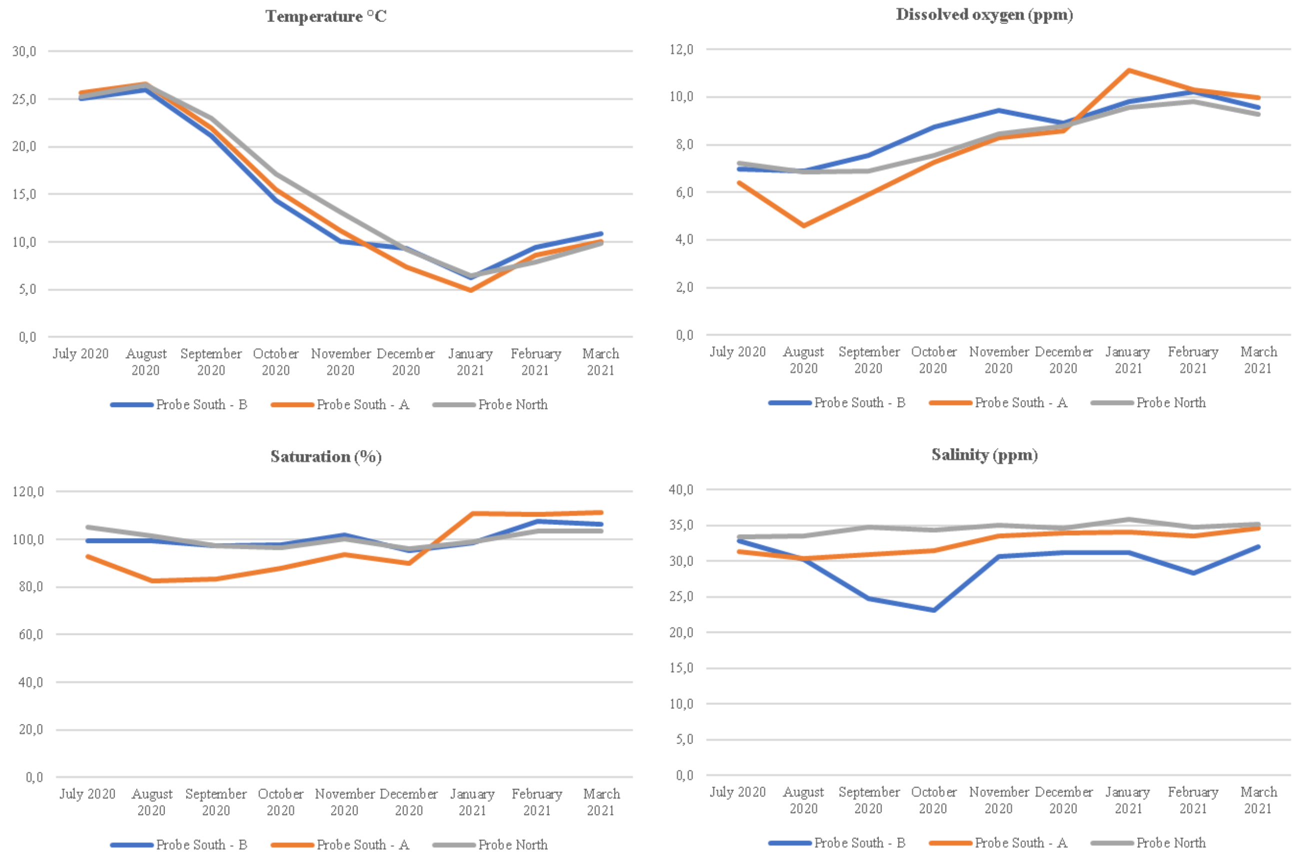


Figure 10. Temperature, dissolved oxygen, salinity and saturation registered by the Probe North, Probe South – A, Probe South - B during the second monitoring year.

It should be highlighted that because of the bad functioning of these probes in few periods of the years, some parameters are not shown in the present deliverable and data elaboration and validation of all parameters, including those not described herein, are ongoing.

## 4. Conclusions

For the first time, different clams and mussels farming sites were monitored through two long-lasting monitoring campaigns. The data here presented will be fundamental to interpret the further results from cellular, biochemical, chemical, molecular and microbiological analyses. Overall, these analyses will enable to obtain an overview about the state of animal health and on the characteristics of the farming areas in a moment of transition due to the entry into operation of the MoSE system. This will be possible thanks to the Weight of Evidence approach that UNIPD and UNIVE-DAIS are currently developing as part of WP2.1.4 activities.

Chemical-physical and biometric parameters here reported do not highlight evident changes between the two years of monitoring. However, mortalities trends of Manila clam were completely different between the two monitoring campaigns, with highest mortality occurring in the inner sites after the activation of MoSE system. It should be highlighted that due to the lack of natural seed recruitment and to mortality events occurring in different periods/sites, the Venice lagoon has experienced in recent years a dramatic decrease in Manila clam annual production, from 40,000 tons produced in 2000 to 3,000 tons in 2019. Accordingly, the mortality trends highlighted in the second year campaign should not be necessarily linked to the functioning of the MoSE system. However, considering the increasing functioning of MoSE in the upcoming years and the ongoing climate changes, in order to identify criticalities in Venice lagoon ecosystem and production activities, it will be crucial to continue the monitoring of these areas.

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