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Potential to produce brown mussel integrated to a net-cage fish farm in a Mediterranean bay

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ABSTRACT

In this study, we investigated the potential exploitation of the brown mussel *Perna perna* (Linnaeus, 1758) in an aquaculture farm (Monastir Bay, Mediterranean Sea, Tunisia). The quality of seawater as well as performance indicators such as Condition Index, length, weight, growth and frequency distribution of the mussel were determined over an annual cycle; from July 2017 to August 2018. Interestingly, *P. perna* has an extended sexual cycle during the year with two main spawning periods, the first in spring and the second in autumn, when suspended matter and Chlorophyll *a* were at their maximum concentrations in seawater. Spat mussels and young mussels showed significant growth rates from 1 July 2017 to 31 October 2017. Adult mussels exhibited negative allometric growth and a modal size of 41 to 50 mm. Results showed that several potentially toxic microalgae species developed at low densities in this marine zone. By using Solid Phase Adsorption Toxins Tracker (SPATT); lipophilic toxins including Okadaic acid and yessotoxins were detected at low levels in seawater and may be involved in the positive LSTs (lipophilic shellfish toxins) mouse bioassays in *P. perna* during the autumn. No other toxins such as paralytic shellfish toxins or amnesic shellfish toxins nor pathogenic bacteria or viruses were detected in *P. perna.* Altogether, our results suggest that finfish farms in Monastir Bay could be suitable sites for farming *P. perna.*

1. Introduction

Brow mussels *Perna perna* (Linneaus, 1758) have been reported in south Mediterranean coastal waters (Morocco, Algeria and Tunisia) as *Mytilus africanus* (Chemnitz, 1785) or *Mytilus pictus* (Born, 1778). Lubet (1973) determined that there were no differences between the species *P. perna* of South America and *M. africanus* from North Africa. These populations therefore appear to belong to a single species, *P. perna* (Linneaus, 1758).

The reproduction and population structure of *P. perna* have been studied by Abada-Boudjema (1981) and Boukroufa (1987) at Bordj El Kiffan (Algeria) and by Chaoui (1993) and Kerdoussi et al. (2017) in Annaba Bay (Algeria). In Tunisia, Zaouali (1973) documented the presence of *P. perna* close to ports in the north of Tunisia. Recently, this species was observed in Monastir Bay (eastern coast of Tunisia), associated with floating cages of aquaculture farms. Since 2008, the bay of Monastir has become a major zone of offshore aquaculture production with around 6,579 tons of fishes in 2015 (Challouf et al., 2018). Two species are reared in this area, sea bass *Dicentrarchus labrax* (Linneaus, 1758) and sea bream *Sparus aurata* (Linneaus, 1758) (Challouf et al., 2018). In the Mediterranean Sea, in addition to the oyster *Crassostrea gigas*, the mussel *Mytilus galloprovincialis* (Lamarck, 1819) is the main

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cultured shellfish in coastal waters along both the northern and southern shores (Andreu, 1958; Cáceres-Martínez and Figueras, 1997; Ferreira 1988, Figueras 1976; 1989; Figueras et al., 1991; Gosling, 2003; Lutz et al., 1991). In Tunisia, Bizerte Lagoon (north of Tunisia) is the only area for shellfish (mussels and oysters) farming (Beji, 2000). It has a national economic value with a production of 260 t/year of both the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) and the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819) (Turki et al., 2014).

With the increase of urbanization along the Mediterranean coast and the lack of wastewater treatment facilities, marine coastal ecosystems could be contaminated by human faecal matter containing pathogenic bacteria (*Salmonella* and *Escherichia coli)* and enteric viruses (*Norovirus* and hepatitis A virus) (Bellou et al., 2013; Fouillet et al., 2020; Meghnath et al., 2019; Miranda and Schaffner, 2019; Nasheri et al., 2019; Romalde et al., 2017). As shellfish filter large volumes of seawater for food, those pathogenic viruses and bacteria could accumulate in their tissues. Human consumption of raw or undercooked shellfish contaminated by these pathogenic microorganisms can be harmful to human health (Froelich and Noble, 2016; Richards, 2006). *Salmonella* and *E. coli* are responsible of gastroenteritis, enterocolitis, enteric fevers such as typhoid fever and septicaemia with metastatic infections in humans (Gantzer et al., 1998; Abad et al., 1997). Norovirus (NoV) and hepatitis A virus (HAV) are the causative agents of gastroenteritis, meningitis and hepatitis (Macaluso et al., 2021).

Aquatic pathogens and viruses can lead to the closure of collection areas and can cause high economic losses due to health risks for human consumption and to and increase in disease and mortality in aquaculture species (Sanches-Fernandes et al., 2022; Borrego et al., 2017; Rodger, 2016; Lafferty et al., 2015). As for other Mediterranean countries, the contamination of shellfish by pathogenic bacteria and viruses remains a recurrent problem that negatively impacts the sustainable exploitation of shellfish in Tunisia (Zormati et al., 2018; El Amri et al., 2006; Amri et al., 2011).

The main food source for bivalves is phytoplankton. When they feed on toxin-producing species, these toxins build up in their tissues, thereby increasing the risk to human health. Several human diseases have been reported to be associated with toxin-producing dinoflagellates, diatoms or cyanobacteria. Marine algal toxins are a major problem in shellfish farming as they may concentrate in shellfish that are subsequently eaten by humans, causing syndromes including lipophilic shellfish poisoning (LSP), paralytic shellfish poisoning (PSP) and Amnesic shellfish poisoning (ASP), among others (Visciano et al., 2016). These responsible toxins could impair shellfish aquaculture and require constant and costly monitoring by regulatory agencies to assess human health-related risks (Lee et al., 1989; Reguera et al., 2012). Over the last three decades, the occurrence of toxin-producing microalgae has increased around the world (Van Dolah, 2000; Hallegraeff, 1993; 2010; Hallegraeff, 2003; Wells et al., 2015) with important economic losses for the aquaculture industry (Álvarez et al., 2019; Ibrahim, 2007). Since 1994, shellfish production along the Gulf of Gabès' coasts of Tunisia have been impaired by gymnodimines produced by the toxic dinoflagellate *Karenia selliformis* (Marrouchi et al., 2010; Medhioub et al., 2010; Ben Naila et al., 2012; Feki et al., 2013). Since 1990, many blooms of the PSTs producer *Alexandrium minutum* have been recorded in the same area (Abdennadher et al., 2012), thus contributing to hampering the exploitation of molluscs.

The brown mussel, *P. perna*, is an ecologically important species with great potential for aquaculture in Tunisia as it is harvested for local consumption in Monastir Bay (Tunisia). However, as its cultivation requires careful consideration of environmental parameters to ensure proper growth and survival capability of the stock through harvest for safe human consumption (Ait Chattou et al., 2019), the lack of knowledge on the environmental conditions of Monastir Bay favourable to the growth of *P. perna* stops the management of its exploitation. It is necessary to develop sustainable farming and provide valid information for the sustainable management of this resource not only in our ecosystem but also in other similar Mediterranean areas. For this reason, priority has been given to diversifying production sites through the development of mussel offshore farming techniques to create a new income opportunity for fishermen and fish farmers.

In this context, our study aimed to evaluate the potentiality of exploiting mussels in an aquaculture finfish farm in Monastir Bay (Mediterranean, Tunisia). Our main objectives were to gain more insight into the growth and reproductive performance of *P. perna* and to investigate the environmental parameters that are important for the health status of this mollusc. We also looked for the presence of toxic microalgae, toxins, pathogenic bacteria and viruses in the water and in mussels.

2. Materials and methods

2.1. Environmental samples

This study was conducted at the Aquaculture of Sahel (AS) farm (35°45. 902 N. 10°55. 548 E) located of the Monastir Bay on the Monastir-Kuriates Islands (15 km from the Tunisian Mediterranean shore with a water depth of 28 meters).

Every month from July 2017 to August 2018 wild *Perna perna* mussels (200–250 individuals) were randomly picked by scuba divers on the *Sea bream* and *Sea bass* of all 16 floating cages (Figs. 1 and 2). Then, mussels were placed in sterile bags and transported to the laboratory at 4 °C, where the Condition Index (CI), biometric measurement, sex ratio, maturation stage and contamination by phycotoxins and microorganisms (bacteria and viruses) were determined.

Temperature, dissolved oxygen (DO), suspended matter (SM), seawater transparency and Chlorophyll *a* were measured directly *in situ* once per season from February 2018 to August 2018 at four stations. S1, S2 and S3 were inside the AS farm while S4 was the control sampling site situated outside of the AS farm (Fig. 1).

For Chlorophyll *a* (Chl *a*) measurement, two litres of seawater were collected, placed on ice at 4° C, then transported to the laboratory until extraction and analysis. For phytoplankton, once per season one litre of seawater was sampled with a 5 L Niskin bottle at integral depth levels at each station (Fig. 1), then 1 mL of Lugol was added. The preserved samples were protected from light and high temperatures until the phytoplankton taxa were identified. For lipophilic toxins in seawater, bags were used in each of the eight sampling events from February 2018 to November 2018. Moreover, one litre was sampled six times from May 2017 to September 2018 then transported in sterile bottles on ice at 4° C test for total coliforms and *E. coli* and *Enterococci* in seawater.

2.2. Mussels biometry

Maximum length of mussels along of the anterior-posterior axis was measured using a Vernier caliper KS TOLLS® with an accuracy of ± 0.01 mm. Total wet weight (live mussel weight) was determined using the electronic balance KERN PCB® (0.01 g precision) after washing of sediment particles adhering to the shell and using a tissue to get rid of the attached water.

The length-weight relationship of *P. perna* population was determined by linear regression analysis (Microsoft Excel® software) using the following equation: $W=a*L^b$, where W is the weight in g, L the length in mm, a is the condition factor and b is the growth coefficient (Quinn and Deriso, 1999).

2.3. Condition index

To calculate the Condition index (CI), which reflects the physiological status of individuals in a population (Lucas and Beninger, 1985), estimation of the share of organic matter emitted during reproduction (Bodoy and Massé, 1979; Bodoy, 1980) was previously determined according to the method of Beninger and Lucas (1984). Briefly, the dry

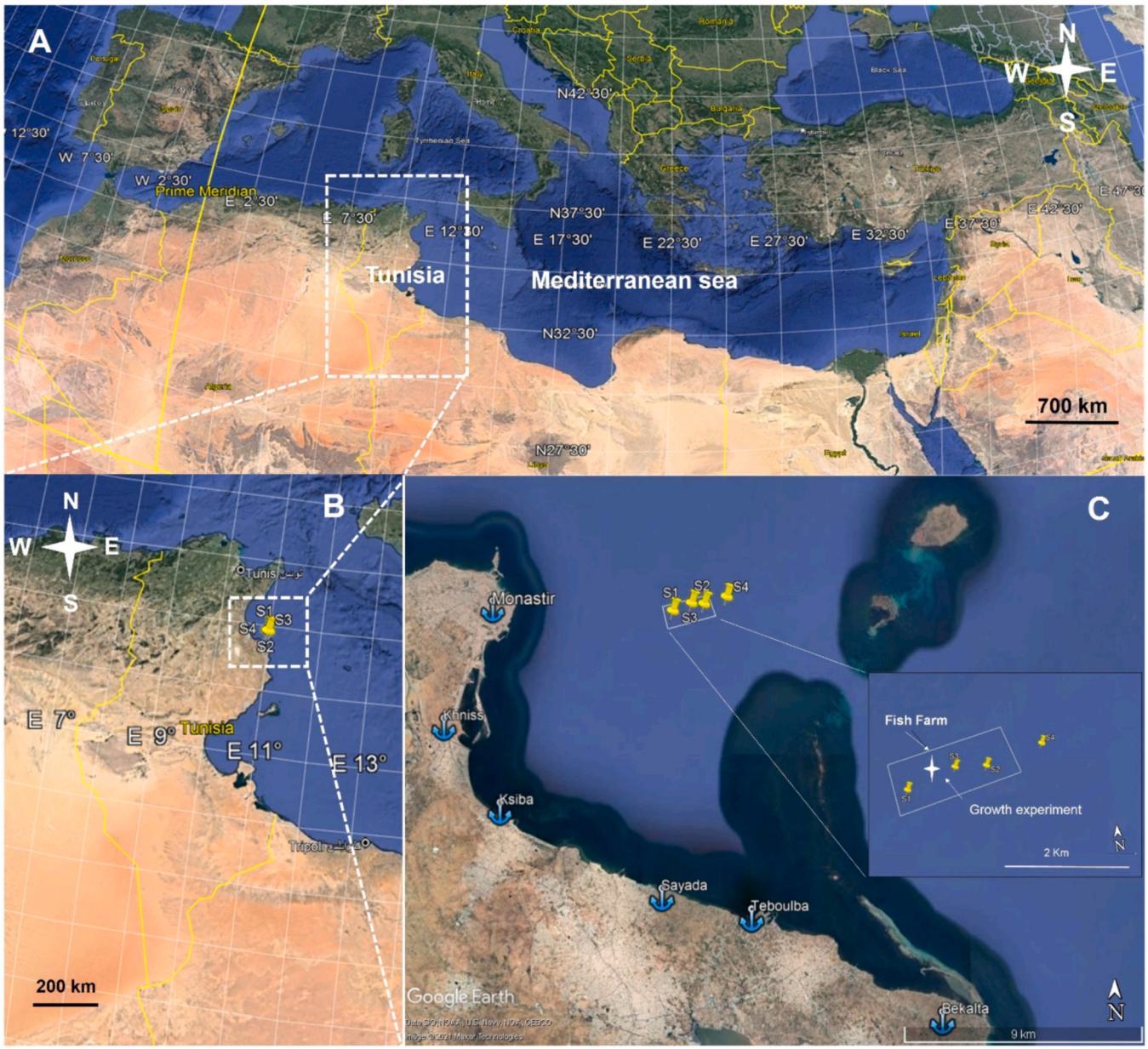

Fig. 1. The geographic location of the study area in Tunisia, Central Mediterranean Sea (A), The geographic location of the study area in Monastir Bay (Eastern Tunisia, Central Mediterranean), Farm spatial layout and sampling stations S1 (35° 45. 907 N. 10° 55. 008E), S2 (35° 45. 902 N. 10° 55. 548E), S3 (35°45 903 N. 10°55. 250E) and S4 (35°46.060 N. 10°56.150E). S1, S2, and S3 are inside the aquaculture farm while S4 is outside the farm. (C). Scales bars A, 700 km; B 200 km; C 9 km.
Source Google Earth® 2022.

weight of soft tissue and dry weight of shell were kept in a Memmert® dryer at 80 °C until total desiccation. The CI was then calculated as follows: CI (%) = [dry weight of soft tissue (g) / dry weight of shell (g)] X 100.

2.4. Gonad developmental stage and sex ratio

Among randomly collected wild mussels only adults (up to 40 mm of length) were dissected, then gonads were observed using a photonic microscope (Zeiss Primostar 3®). The development and maturity state of mussels' gonads were determined by the histological method described by Rolland et al. (2014). Accordingly, 5 cm cross-sections including the gonad tissues of mussels (males and females) were fixed in Davidson's solution (Shaw and Battle, 1957) for 48 h. Tissues were dehydrated in ascending ethanol solution (80°, 95° and 100°), cleared with xylene and embedded in paraffin. Then, 5 μm thick sections were cut by using a microtome (Leica® RM 2125RT) mounted on glass slides. Paraffin was removed with xylene then coloured by haematoxylin eosin. Tissues were observed under a photonic microscope (Zeiss Primostar 3®) and photographs were taken using an Axiocam 105 Color camera. Stages of the sexual cycle were determined according to the classification of Lubet (1959) modified by Wilson and Seed (1974).

2.5. Environmental growth survey

An *in situ* growth experiment was carried out from 1 July 2017 to 31 October 2017 at the floating cage situated in the middle of the farm (Fig. 2A). Wild spat and young mussels collected in floating cage were placed separately in two 1m-length nylon bags (3 mm mesh size) up to total weights of 1 kg (~1000 individuals) and 5 kg (~ 1250

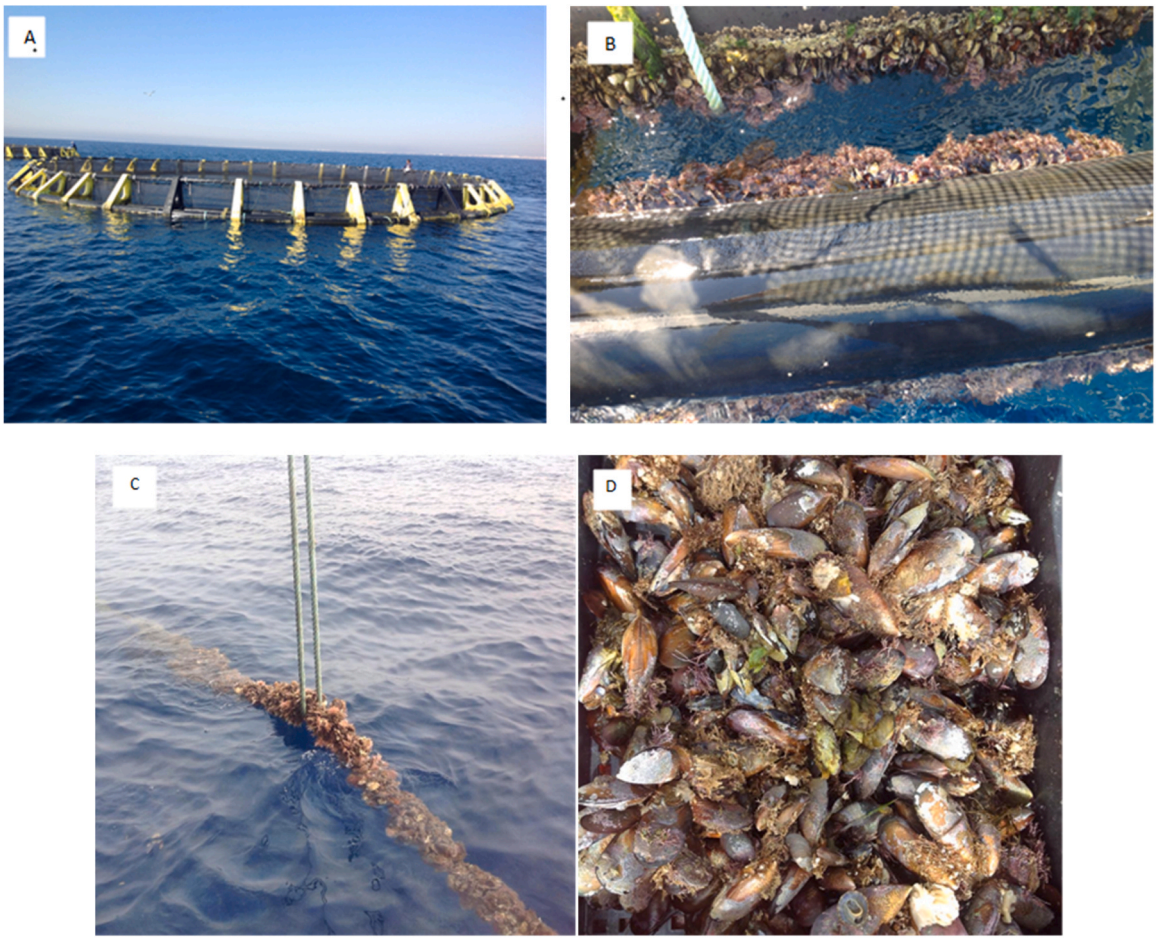

Fig. 2. Brown mussels *Perna perna* integrated at finfish offshore cage in the Mediterranean Sea (Bay of Monastir, Tunisia), (A) Finfish cage of sea bass *(Dicentrarchus labrax*) and sea bream (*Sparus aurata*), (B) mussels *P. perna* integrated at finfish offshore cage, (C, D) Sampled mussels *P. perna* showing different sizes from spat to adults.

individuals), respectively. All mussels were maintained at 2 m depth attached to the structure of the fish cages (Fig. 2A). This depth was chosen to ensure mussels exposure to the maximum Chl *a* concentration (proxy of phytoplankton biomass) that occurs in Monastir Bay (Challouf et al., 2017). Spat and young mussels were randomly collected after 3 months of environmental exposure. At the laboratory, mussels were rinsed to remove debris and their lengths and weights were measured using KS TOLLS® digital callipers and a KERN PCB® electronic balance, respectively. Statistical analyses were performed for the growth results of spat mussels and young mussels using Student's t-tests and XLSTAT® software.

2.6. Seawater parameters measurement

Seawater temperature and dissolved oxygen (DO) were measured directly *in situ* using an electronic thermometer (LUTRON®BC-4308) and a DO meter (PCE®-WO2 10), respectively. To determine the concentration of SM, 1.5 liters of seawater was collected and filtered on pre-weighed GF/C 0.45 μm filters (WHATMAN®). Subsequently, filters were dried in an oven at 100 °C for 24 h and total SM weight (mg L^{-1}) was estimated according to the differential weighing method of Aminot and Chaussepied (1983). Transparency of the water (in meters) was measured using a Secchi Disk as described by Testa et al. (2019)

For Chlorophyll *a* (Chl *a*), two liters of seawater were filtered through 200–250 μm pore size membrane filters to remove large particulate matter and zooplankton. The eluate was filtrated on GF/C 0.45 μm filters (WHATMAN®). Chl *a* was extracted with 10 ml of acetone 90% for 24 hours at 4 °C then the absorbance at two wavelengths (665 μm and 750 μm) were determined before and after acidification using 100 μl of HCL 0.3 M according to Aminot and Chaussepied, (1983).

2.7. Monitoring of toxic microalgae in seawater

Toxic microalgae density was estimated (cells L^{-1}) using an inverse phase photonic microscope and method described by Utermöhl (1931) and Sournia (1987). Taxa were identified using the determination keys of Trégouboff and Rose (1957), Huber-Pestalozzi (1968) and Balech (1988).

2.8. Lipophilic toxins analysis in seawater

SPATT (Solid Phase Adsorption Toxins Tracking) (MacKenzie, 2010; Zendong et al., 2014; Roué et al., 2018; Fux et al., 2009) were used collectors containing, into a 30 μm nylon mesh, 3 g of suspended resin HP20 (Diaion® HP20) as adsorption agents, were placed in an open box suspended at 3–5 m depth in seawater and fixed to the aquaculture structures. After three weeks, collectors were retrieved then placed on ice for transport. Back to the laboratory they were stored at -20 °C until toxin extraction and analysis. SPATT collector was taken from the freezer then the resin was rinsed with Milli Q (500 mL) water and transferred to a 12 mL tube for vacuum drying. The elution was carried out with 24 mL of MeOH. The recovered volume was evaporated under nitrogen, then taken from the dry extract in 500 mL of MeOH 50% and

filtered at 0.2 μm. The analyses were carried out by Liquid Chromatography Mass Spectrometry tandem (LC-MS/MS) using a UPLC Shimadzu® coupled with an AB-SCIEX® API 4000QTRAP mass spectrometer. Toxins were eluted in a Kinetex® 2.6 μm PFP 100 Å, LC Column 150 ×2.1, Phenomenex) at 25 °C with a linear gradient fixed at 0.8 ml min^{-1}. The analyses were performed using the MRM method (Multiple Reaction Monitoring) in positive and negative ionization modes. The two most intensely produced ions per compound were selected.

2.9. Toxin analysis in mussels

2.9.1. Lipophilic shellfish toxins (LSTs) analysis by mouse bioassay

The analyses of LSTs were carried out using the method of Yasumoto et al. (1978) for the detection of LSTs by mouse bioassay. Briefly, 20 g of mussel hepatopancreas was crushed in 50 ml of acetone then filtered. Eluate was collected in a 500 ml flask. The residue remaining in the filter was mixed with 50 ml of acetone, then filtered. This step was repeated one time. All eluates were recovered in the same flask. Acetone was evaporated at 42±2 °C in a rotary steamer. The residue obtained was mixed with 50 ml of dichloromethane. The organic phase (containing lipophilic toxins) was mixed with 50 ml of dichloromethane. This step was repeated to obtain a final volume of 150 ml. The dichloromethane was evaporated at 42±2 °C in a rotary steamer. Finally, the obtained residue was dissolved in 5 ml of tween 60 (1%), of which 1 ml was injected into the intraperitoneal cavity of an albino Swiss mouse. After 24 h of incubation the death of at least 2/3 mousses was considered positive for LSTs in shellfish.

2.9.2. Paralytic shellfish toxins (PSTs)

The PST analysis was carried out by mouse bioassay (MBA) according to AOAC Official Method 959.08 (AOAC, 1990). Briefly, 100 g of mussel meat homogenate was mixed with 100 mL of 0.1 M hydrochloric acid and pH was adjusted to 3–4. The mixture was boiled gently for 5 min then centrifuged. The supernatant was used for the analysis. Sample toxicities were calculated from the median death times of the mice and expressed in terms of μg Saxitoxin (STX) di-HCl eq Kg^{-1} shellfish meat. The limit of detection (LOD) of the bioassays was approximately 370 μg STX di-HCl eq Kg^{-1} mollusk meat (EFSA, 2009). The sanitary threshold is 800 μg STX di-HCl eq Kg^{-1} mollusc meat.

2.9.3. Amnesic shellfish toxins (ASTs)

The AST analysis was carried out by HPLC-UV using the method of Quilliam et al. (1995). Domoic acid (DA) was extracted from 20 g of mussel meat homogenates with 50% aqueous methanol followed by a strong-anion exchange, solid-phase extraction. The sample was analysed using the following chromatographic conditions: Column C_{18} (5 μm, 250 mm×4.6 mm), mobile phase flow rate of 1 ml min^{-1}, detector wavelength of 242 nm, injection volume of 20 μL and an oven temperature for the column of 40 °C. Determination of domoic acid concentration was performed by liquid chromatography with ultraviolet absorbance detection. The determination of DA content in samples was done with a detection limit of 0.05 mg kg^{-1}.

2.10. Bacteria and virus identification

Mussels were scrubbed under running tap water to remove shell debris and attached algae. Then, they were dried and aseptically opened using a sterilized scalpel. Half of each mussel was used to test for *Salmonella* and *E. Coli* according to the method of Brenner et al. (2009), and the other half of each mussel for virus research (NoV G1, NoV GII and VHA) according to RT-PCR methods (Elamri et al., 2006; Bigoraj et al., 2014).

Escherichia coli and total coliforms in water tests were performed according to Rubini et al. (2018). Briefly, 1 ml of each seawater sample collected was plated on a nutrient agar culture medium then incubated at 37 °C or 22 °C. Colonies that formed units were counted and *E. coli* and total coliforms were enumerated as culturable microorganisms.

3. Results

3.1. Grow and reproduction performance of Perna perna mussel

3.1.1. Size distribution and allometry analysis

During the survey, the lengths of 743 individuals corresponding to the whole population of wild mussels including spat (151 individuals) and adults (592 individuals) were measured. The sizes of mussels were between 20 and 123 mm with an average size between 41 and 50 mm (Fig. 3) which was considered as a modal commercial size. Based on the analysis of the relationship between the total length and total wet weight of mussels, we determined the following equation $W=0.0003L^{2.61}$ (Fig. 4). Through this equation, we obtained a b value of 2.61. The observed b value was less than three, implying negative allometry of the sampled specimens meaning a faster increase of the mussels in size then weight.

3.1.2. Two seasonal synchronization phases of reproduction

During one year of monitoring, 225 adult mussels were collected to determine the CI index and 120 adult mussels for sex ratio (Table 1). The examination of histological sections of female and male gonads showed short gamete emission and gonad restructuration between summer and fall from July 13, 2017 to October 31, 2017 (Table 1, Fig. 5).

CI Increases from 6.5±2.5 to 9.7±1.2 from July 13, 2017 to October 19, 2017, followed by a decrease to 7.4 ± 0.6 on November 22, 2017 (Fig. 6). Then CI increased a second time to a maximum of 12.8 ± 1.3 on March 28, 2018 then decreased to 7.5 ± 1.2 on August 8, 2018 (Fig. 6). The gametogenesis and sexual maturity of *P. perna* was observed in winter on January 17, 2018 and in spring on May 16, 2018 (Fig. 5; Table 1). From July 13, 2017 to December 13, 2017 a second phase of gametogenesis and sexual maturity was observed (Table 1). This result is in agreement with the decrease of CI observed previously (Fig. 6). Moreover, histological analysis of the gonads showed a synchronization of the phases of the gonadal development cycle between males and females. Furthermore, the sex ratio stayed stable across the years with a dominance of males (> 1, Table 1), except on October 31, 2017 and December 13, 2017, with sex ratios of 0.4. Altogether, these results showed that *P. perna* mussel was able to reach its sexual maturity and make gametogenesis.

3.1.3. Growth

Spat and young mussels (150 individuals each) were harvested after

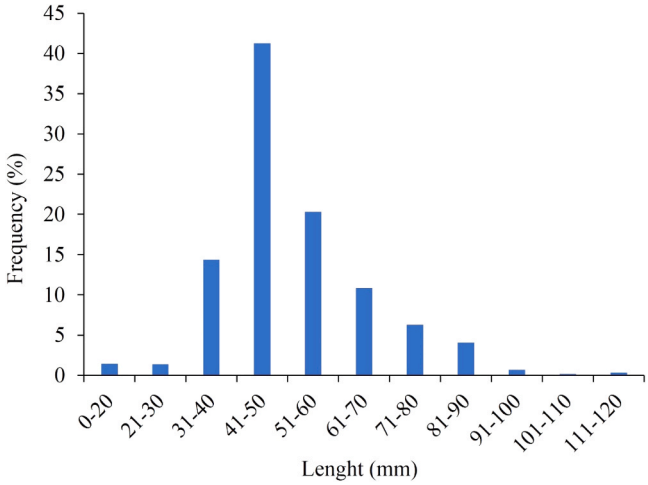

Fig. 3. Size distribution of mussels *Perna perna.* (n=743).

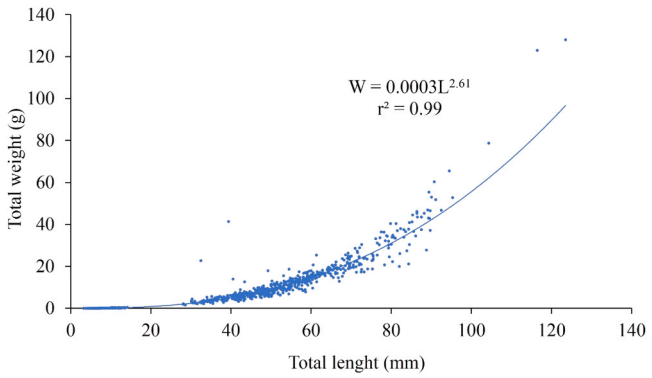

Fig. 4. Regression analysis of the relationship between length and weight of brown mussels *Perna perna* considering a total of 743 individuals sampled during our survey with a mean of 106 individuals for each sampling date.

Table 1
Seasonal variation of Condition Index (n=15), sex ratio (n=15) and states of the males and females gonads development of the mussel *Perna perna* (n=6). 1: Stage of gametogenesis and sexual maturity, 2: Stage of gamete emission and restructuring of the gonad.

Seasons	Date	Condition index	Sex ratio	Stage of male's gonadal development	Stage of female's gonadal development
Spring	28/03/2018	12.8± 1.3	2.7	1	1
	16/05/2018	6.6±0.8	1.5	1	1
Summer	13/07/2017	6.5±2.5	1.5	2	2
	08/08/2018	7.5±1.2	2.3	2	2
Autumn	18/09/2017	7.8±0.9	2.3	2	2
	31/10/2017	8.2±0.9	0.4	1	2
Winter	13/12/2017	9.1±1.0	0.4	2	2
	17/01/2018	9.9±2.3	2.3	1	1

four months of exposure in the floating cage of the AS farm located in Monastir Bay. Before exposure on July 1, 2017, the average spat length was 10.3±6.6 mm for an average weight of 0.2±0.1 g (Fig. 7A and B). On October 31, 2017, after four months of deployment in the aquaculture farm of Monastir Bay, the Student's t-test showed significant ($P \leq 0.05$) growth differences between mussel spat and young mussels. Mussel spat length reached 34.7±5.6 mm and an average weight of 4.2 ±1.9 g (Figs. 7A and 7B), which corresponds to growth rates of 8.1 mm month^{-1} and 1.3 g month^{-1}. Similarly, on 1 July 2017, the average young mussel length was 36.7±6.6 mm for an average weight of 4.5±1.3 g (Figs. 7A and 7B). After four months on October 31, 2017, young mussel length and weight increased to reach 48.7±10.2 mm and 10.2±4.3 g, respectively (Figs. 7A and 7B), which correspond to growth rates of 4.0 mm month^{-1} and 1.9 g month^{-1}. Altogether, these results showed that *P. perna* was able to grow an offshore Aquaculture farm.

3.2. Seawater parameters

3.2.1. Temperature, dissolved oxygen, suspended matter, turbidity and chlorophyll a

During our survey, four samples were used to determine the environmental parameters of Monastir Bay at the Aquaculture of Sahel (AS) farm. The temperature in the aquaculture farm in Monastir Bay ranged from a minimum of 14.1 °C in the winter on 20 February 2018 to a maximum of 28.3 °C in the summer on 8 August 2018 (Table 2). Dissolved oxygen values ranged from 7 to 10 mg L^{-1}, with the highest values recorded in winter. For suspended matter, values ranged from a minimum of 6.5 in the winter on 20 February 2018 to a maximum of 33.2 mg L^{-1} in the summer on 8 August 2018. Water transparency was low in the autumn on 26 October 2017 and high in the summer on 8 August 2018 (Table 2). Chl *a* concentration showed a seasonal fluctuation (Fig. 8), from a minimum of 0.12 μg L^{-1} recorded at the S2 station in the summer on 8 August 2018 to a maximum of 1.1 μg L^{-1} at the S3 station in the autumn on 26 October 2017 (Fig. 8). There was no significant difference in temperature, DO, SM, water transparency and Chl *a* concentration in water inside and outside the farm (Table 2).

3.2.2. Toxic phytoplankton

During the survey, 138 phytoplankton taxa were identified by optical microscopy in the water samples (one per season) taken from the four stations studied (S1, S2, S3 and S4) of the Monastir Bay at the Aquaculture of Sahel (AS) farm. They were mainly dinoflagellates with 83 species, which represented 50–60% of the taxa observed, followed by diatoms with 45 taxa, representing 30–40% of the taxa (Fig. 9). The other phytoplankton taxa such as Cyanobacteria, Coccolithophorides, Euglenophyceae and Dictyophyceae represented around 10% of the taxa (Fig. 9). Among the taxa, 10% correspond to 14 taxa potentially toxic (Table 3), a diatom (*Pseudo-nitzschia* spp.), three cyanobacteria (*Anabaena* spp., *Microcystis* spp. and *Oscillatoria* spp.) and ten dinoflagellates (*Alexandrium* spp., *Coolia monotis, Coolia* spp., *Phalacroma rotundata, Dinophysis tripos, Karenia papilionacea, Karenia selliformis, Karlodinium veneficum, Prorocentrum lima and Prorocentrum minimum*) (Table 3). These potentially toxic species were observed at varying densities over the year both inside and outside the farm. The highest densities were recorded in summer with the diatom *Pseudo-nitzschia* spp at site S4 and in spring with the dinoflagellate *Prorocentrum lima* at site S3 and the cyanobacterium *Anabaena* spp. at site S2 (Table 3).

3.2.3. Toxins

Lipophilic shellfish toxins were determined in eight samples of seawater from February to November 2018, which represent one sample in winter, two samples in spring, three samples in summer and two samples in autumn. Analysis by LC-MS/MS of SPATT evidenced the presence of several toxins (Okadaic acid, yessotoxins, gymnodimine-A, pectenotoxins, pinnatoxin-G and Portimine) in the water column (Fig. 10). Okadaic acid was present at high concentrations ranging from 21.6 ng g^{-1} in autumn to 48.4 ng g^{-1} in winter. Yessotoxins were detected at a maximum concentration of 10.2 ng g^{-1} in winter and 4.5 ng g^{-1} in autumn (Fig. 10). Gymnodimine-A and pectenotoxins showed low concentrations across the years, ranging from 0.6 ng g^{-1} to 5.9 ng g^{-1}. Pinnatoxin-G and portimine toxins were detected at low concentrations under 1.3 ng g^{-1} (Fig.10) all over the year.

3.2.4. Pathogenic bacteria and viruses

Human intestinal *Enterococci* were detected in seawater in the spring season. 1 Enterococci /100 ml on May 24, 2017 and 6 Enterococci /100 ml on April 25, 2018 (Table 4). No pathogenic *E. coli* and no coliforms were detected in seawater during the years of the survey.

3.3. Pathogenic microorganisms and toxins in mussels

During our survey, 360, 540 and 1620 adult mussels were collected

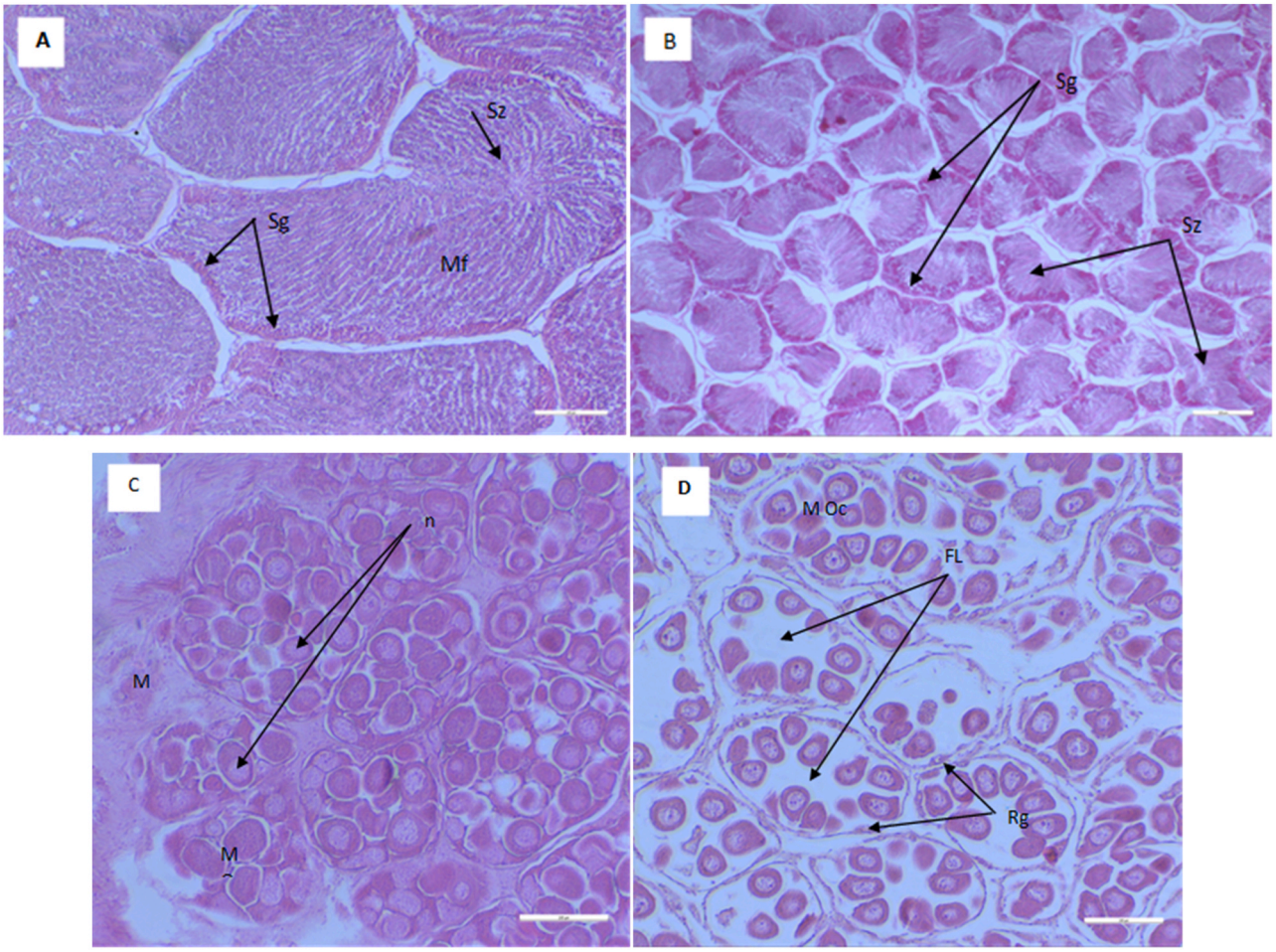

Fig. 5. Hematoxylin-eosin stained paraffin Section (5 μm) showing some stages of gonad's development of the males (A and B) and females (C, D) of *Perna perna* (n=6). A and B correspond to stage 1, showing spermatozoa (Sz), spermatogonia (Sg) and mature follicle (Mf). C and D correspond to female's gonads showing gonad maturation (Stage 1) (C) and gamete emission (Stage 2) (D). oocyte (Oc), Mantle (M), nuclei (n), follicular lumen (Fl), MOc (mature oocyte), Residual gonia (Rg). Photos were taken with light microscope, GRX40. Scales bars A, C, D 100 μm and B, 200 μm.

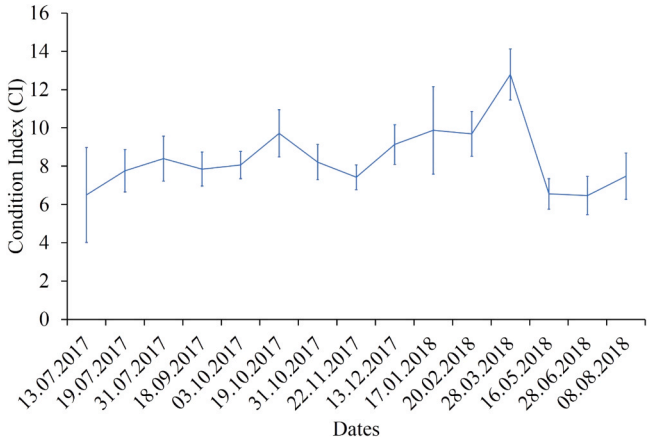

Fig. 6. Variation of the Condition Index of *Perna perna* (n=15).

from the Monastir Bay at the Aquaculture of Sahel (AS) farm for pathogenic bacteria and viruses and toxins analysis respectively. No pathogenic bacteria or viruses were detected in mussels (Table 5). Among toxins, only LST was detected in adult mussels in autumn on October 31, 2017 and November 21, 2018 (Table 3). These results showed a low level of contamination of mussels, which live and develop in the aquaculture farm of the bay of Monastir at concentrations below the sanitary threshold.

4. Discussion

4.1. Reproduction and growth performance of P. perna mussels in the Bay of Monastir

The annual variation of the CI refers to the gonad state and the progress of the reproductive cycle of mussel may determine the period of emission of gametes (spawning). In the present study, the calculated CI was at its maximum (12.8) in early spring. Different studies reported that high CI could be explained by high phytoplankton consumption by mussels (Fang Sing and Ransangan, 2019) and by the water temperature that reached the physiological temperature that would promote better metabolization of the ingested microalgae (Romeo et al., 1993). This is in agreement with the elevation of the temperature and the increased phytoplankton biomass (maximum Chl *a*) observed in Monastir Bay in early spring and corroborated with the observation reported by Kerdoussi et al. (2017), which show that *P. perna*'s maximum CI (5.47) was in spring along the coast of Cap de Garde and Hnaya in Algeria.

Although a single spawning period of *P. perna* has been reported in the Tunisian environment (Zaouali, 1973), Algeria (Abada-Boudjema and Mouëza, 1981; Kerdoussi et al., 2017), CI indicate that two periods of reproduction occurred in aquaculture farm of the Monastir Bay. The first period was observed in autumn from October to December and the

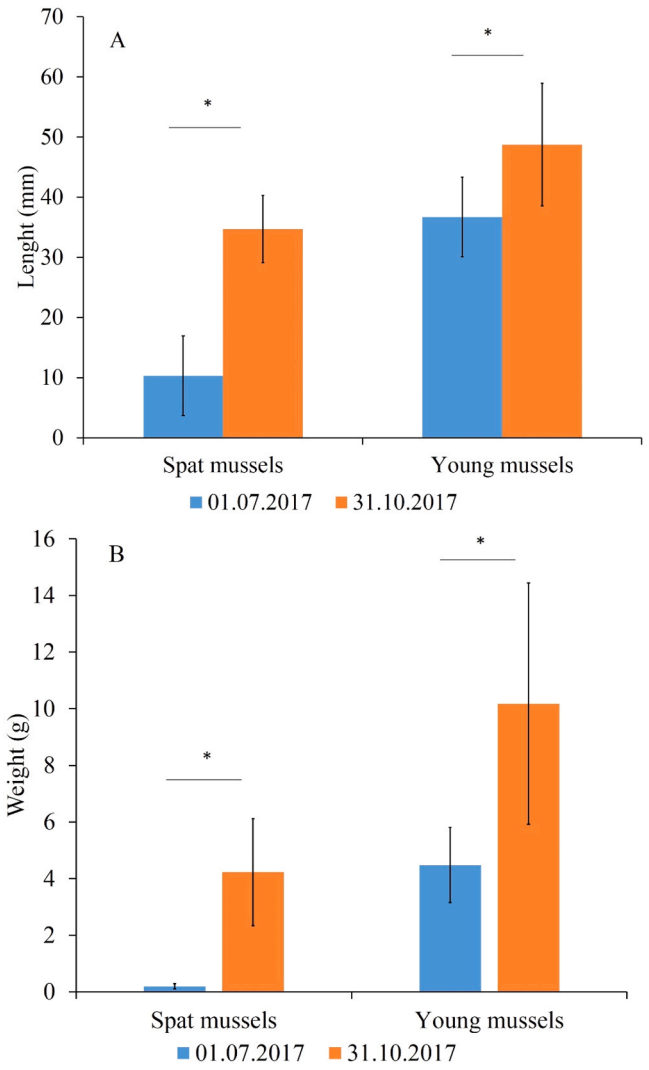

Fig. 7. Temporal variation of length and weight of spat (A) and young (B) *Perna perna mussels* during a growth trial (n=150). *A statistically significant test result ($P \leq 0.05$).

Table 2
Environmental parameters for different seasons and sampling stations inside (S1, S2 and S3) and outside (S4) the aquaculture farm in Monastir Bay, South Western Mediterranean. (-): Missing data.

Parameters	Station	Autumn	Winter	Spring	Summer
		26/10/17	20/02/18	15/05/18	08/08/18
Temperature (°C)	S1	23.5	14.7	19.0	27.9
	S2	23.7	14.5	19.2	28.0
	S3	23.8	14.5	19.1	28.1
	S4	23.5	14.5	18.7	28.3
Dissolved Oxygen (mg L^{-1})	S1	7.8	10.0	-	7.0
	S2	7.2	9.9	-	7.2
	S3	8.2	9.6	-	8.8
	S4	7.8	9.9	-	9.0
Suspended matter (mg L^{-1})	S1	28.6	14.2	22.0	18.8
	S2	26.4	15.0	23.0	17.5
	S3	20.1	8.2	16.2	33.2
	S4	28.2	6.5	13.5	18.7
Transparency (m)	S1	8.0	14.0	11.0	17.0
	S2	7.0	9.0	12.0	14.0
	S3	7.0	8.0	11.0	14.0
	S4	8.0	9.0	12.0	17.0

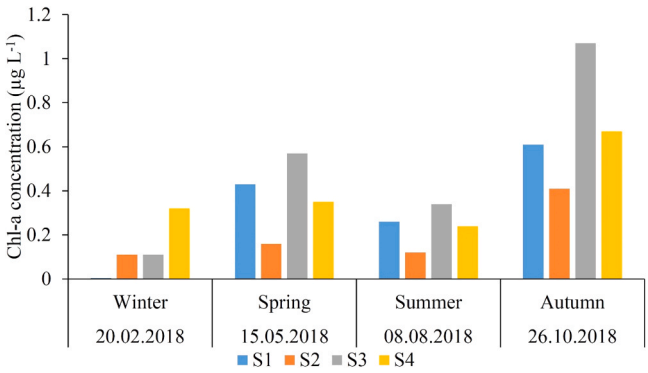

Fig. 8. Temporal variations of Chlorophyll *a* concentration at study station (µg. L^{-1}).

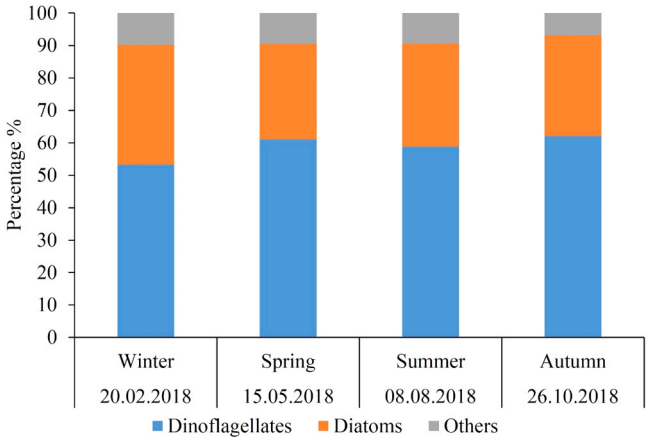

Fig. 9. Percentage of major classes of phytoplankton community identified.

second one took place in spring between March and August. This reproduction trend was in agreement with observations made on the southern coast of South Africa, where *P. perna* showed a summer and winter spawning period (Zardi et al., 2007). In Brazil, Lunetta (1969) also showed two periods of spawning for *P. perna*, one in April–May and July and the second in September. In Congo, Cayré (1978) showed that the most important spawning occurred during the cold season from June to October and the less important took place in December. *P. perna* has a long sexual emission and its genital activity varies according to climatic conditions (Schurink and Griffiths, 1991).

We showed a predominance of *P. perna* males during the winter, spring and summer seasons. This has already been observed in the Mediterranean areas: in El Kala, Algeria (Kerdoussi et al., 2017) and in Anza, Morocco (Id Halla et al., 2004; 2018). In autumn, this sex ratio is turned to a predominance of females. This was similar to *Perna viridis* mussels in Marudu Bay in Malaysia with a sex ratio lower (Soon et al., 2016) or equal to one (Al-Barwani et al., 2013).

The statuses of mussel populations can be estimated using the sex ratio and the shifts in the gender composition of artificial and natural mussels (Chelyadina et al., 2021) and can reflect the influence of environmental factors on molluscs in specific habitats (Dekhta and Katalevsky, 2000). In this study, the evaluation of the sex ratio revealed a predominance of males during the winter, spring and summer seasons. The predominance of *P. perna* males was previously reported in the Mediterranean Sea, in El Kala, Algeria (Kerdoussi et al., 2017) and in Anza, Morocco (Id Halla et al., 2004; 2018). In autumn, the sex ratio was reversed with a predominance of females. This was previously observed with *P. viridis* populations in Marudu Bay in Malaysia with a sex ratio less than one (Soon et al., 2016) or a sex ratio equal to one (Al-Barwani

Table 3
Abundances in cells L^{-1} of potentially toxic microalgae taxa in the investigated stations of Monastir Bay, Mediterranean (Tunisia). (-) absence of the taxa.

	Autumn 26/10/2017				Winter 20/02/2018				Spring 15/05/2018				Summer 08/08/2018			
Taxa	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Dinoflagellates																
Alexandrium spp.	1800	200	-	500	200	-	-	-	-	-	-	-	-	400	-	200
Coolia monotis	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
Coolia spp.	-	-	-	-	-	-	-	-	-	-	100	100	-	100	-	-
Dinophysis rotundata	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-
Dinophysis tripos	-	-	100	200	-	-	-	-	-	-	-	-	-	-	-	-
karenia papilionacea	-	-	-	-	100	-	100	-	-	-	-	-	-	-	-	-
Karenia selliformis	-	-	-	1800	-	-	-	-	-	-	-	-	300	-	-	200
Karlodinium veneficum	-	-	-	-	-	200	-	-	-	-	-	-	-	-	-	-
Prorocentrum lima	-	-	100	400	100	300	400	-	-	-	100	600	-	-	3800	200
Prorocentrum minimum	-	200	1200	500	100	300	300	-	100	-	600	-	-	600	100	-
Diatoms																
Pseudo-nitzschia spp.	-	200	-	1800	1800	-	900	1200	-	-	-	-	200	1200	-	2400
Cyanobacteria																
Anabena spp.	-	–	200	-	1200	-	600	-	-	31500	200	200	-	200	-	700
Microcystis spp.	-	-	-	-	-	200	200	-	-	-	-	-	-	-	-	2400
Oscillatoria spp.	-	-	-	-	-	-	-	-	-	200	-	-	-	-	-	-

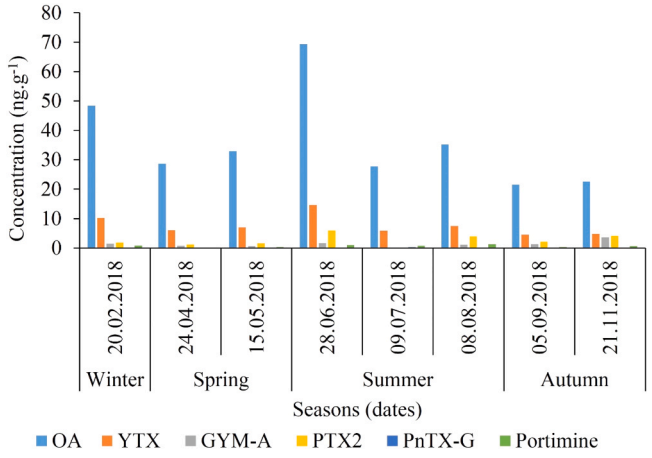

Fig. 10. Temporal variation of lipophilic toxins expressed (ng g^{-1}) by using SPATTs method. OA (okadaic Acid); YTX (Yessotoxins); GYM-A (Gymnodimines-A); PTX-2 (Pectenotoxins-2); PnTX-G (Pinnatoxins-G) and Portimine.

Table 4
Temporal detection of total coliforms and *Escherichia coli* and *Enterococci* in sea water. The temperatures of incubation (22°C and 37°C) are indicated.

Seasons	Dates	Total Coliform/ml		*Escherichia coli*/ 100 ml	Enterococci /100 ml
		22°C	37°C		
Spring	24/05/ 2017	-	-	0	6
	25/04/ 2018	-	-	0	1
Summer	10/07/ 2017	-	-	0	0
Autumn	06/09/ 2018	-	-	0	0
Winter	13/12/ 2017	-	-	0	0
	20/02/ 2018	-	-	0	0

et al., 2013). The inversion of sex structure in one direction from females to males can have economic implications in aquaculture as well as ecological significance. Chelyadina et al. (2021) showed that under the influence of certain environmental factors during the post-spawning period, mussels reared near the Crimean coast (Black Sea) can invert sex direction.

In our experiment, the length of *P. perna* grown in Monastir Bay varied from 20 to 120 mm with the most common size from 41 to 50 mm. This result is in agreement with the profiles expected for a normal population of shellfish. The maximum length of *P. perna* grown in Algiers Bay (Abada-Boudjema and Mouëza, 1981) and in the Tunisian Sea (Zaouali,1973) does not exceed 66 mm and 35 mm, respectively. When they grew on the northeast coast (Kerdoussi et al., 2017) and in the port of Algiers in Algeria (Asso, 1982) they reached 120 mm.

The difference in the maximum size of *P. perna* mussels could be explained in part by the variation in the age of the mussels. However, other studies reported that age only explained 30% of the variation observed in terms of growth; as for the origin of the spat it explained 27% of the differences, while the habitat (rearing site) explained 40% of the difference (Sukhotin and Kulakowski, 1992).

In this study, the evaluation of the growth of *P. perna* mussels over two four-month periods was informative for the length of the rearing cycle and the growth rate of each rearing phase. The size of the spat used in the growth experiment was suggested to be 2.5–3.5 months for spat at a size of 10.3 mm, while it was suggested to be 6.5–7.5 months for young mussels at a size of 34.7 mm. During four months of mussel growth, the results showed that size increased from 34.7 to 48.7 mm. This suggests that 10.5–11.5 months was necessary to reach the commercial size of 50 mm.

It should be noted that the time needed to reach commercial size depends on the geographical area, the rearing method and the release period (Id Halla et al., 2004). As an example, 6.5–7.5 months were necessary to reach a commercial size of 6 mm for *P. perna* mussels in Agadir Bay (Id Halla et al., 2004). However, in Brazil, 6 months of rearing were necessary for mussels released in spring to reach the commercial size (50 mm), whereas the necessary period was 7 months for mussels released in autumn and 8 months for mussels released in winter and summer (Marques et al., 1998).

The length-weight relationship showed that *P. perna* grows faster in size than in weight. Benchikh (2009) previously reported that the relationship between the length and the weight of *P. perna* was negative (allometry $b<3$). The growth of mussels was affected by the variation of abiotic factors such as temperature, pelagic and benthic habitat and biotic factors such as age, sex or the maturity stage of gonads, explaining the elongated shape of the shell according to the anteroposterior axis (Burgeot and Galgani, 1998; Diouf et al., 2020; Grangnery, 2001).

In the present study, the mean *P. perna* growth rate was 8.1 mm length $month^{-1}$ and 1.3 g weight $month^{-1}$ for spat mussels and 4.0 mm length $month^{-1}$ and 1.9 g weight $month^{-1}$ for young mussels. This was in agreement with those obtained over three months of rearing by Id Halla

Table 5
Temporal detection in *Perna perna* of bacteria (*Salmonella* and *Escherichia coli*), viruses (*Norovirus* and *Hepatitis* A virus) and toxins contamination (Lipophilic Shellfish Toxins: LSTs, Paralytic Shellfish Toxins: PSTs determined by Mouse Bioassay method and Amnesic Shellfish Toxins: ASTs measured by HPLC-UV).

		Bacteria n=60		Viruses n=60		Biotoxins n=60		
Seasons	Dates	*Escherichia coli*/g	*Salmonella*/25 g	VHA	NoV GI and NoV GII	LSTs	PSTs	ASTs
Spring	19/04/17	<20	-	na	na	-	-	-
	24/05/17	<20	-	-	-	-	-	-
Summer	19/06/17	<20	-	-	-	-	-	-
	19/07/17	na	na	-	-	-	-	-
	09/07/18	<20	-	-	-	-	-	-
Autumn	21/09/17	na	na	-	-	na	na	na
	20/10/17	na	na	-	-	+	-	-
	21/11/18	<20	-	-	-	+	-	-
Winter	13/12/17	na	na	-	-	-	-	-
	20/02/18	<20	-	-	-	-	-	-

na: not analysed; (-) Negative; (+) positive; VHA: Virus of Hepatit A; NoV G1: Norovirus GI; NoV G11: Norovirus GII.

(2004; 2018), who showed that the mean length in *P. perna* increased by 5.2 mm length month^{-1} weight and 9.7 g weight month^{-1} in an offshore submerged longline system in Agadir in Morocco.

The gain in biomass and length of *P. perna* was probably linked to the higher growth rate of spat mussels. It was especially high during the first four months. Indeed, the young mussels have a low growth rate, probably due to the reduction of metabolic activity (Cheung, 1993), the filtration rate (Bayne et al., 1976), the rate of food ingestion (Seed and Suchanek, 1992) and the increase in gamete production (Hilbish, 1986). Several other factors could influence the growth rates of the mussels including the availability, quantity and quality of food (Seed and Suchanek, 1992; Župan and Šarić, 2014), physiological parameters of these bivalves (Bayne, 1999), genetic parameters (Riisgård et al., 2013) and physicochemical parameters such as temperature, water depth (García-March et al., 2019), population density (Gosling, 2003) and DO levels (Taib et al., 2016).

Based on their growth performance and their ability to reproduce, our results showed that *P. perna* could be cultured in an aquaculture farm located in the Mediterranean bay of Monastir.

4.2. Environmental characteristics

The environmental characteristics of the rearing area, directly and indirectly influence the growth and development of the somatic and reproductive tissues of marine bivalves (Griffiths and Griffiths, 1987; Lodeiros and Himmelman, 2000). Bautista (1989) indicated that mussel growth is a function of several environmental parameters, mainly food and temperature. Regarding temperature, our results showed three distinct periods in Monastir Bay: low temperature in the winter (14.1 °C), moderate temperatures in the spring and autumn periods (between 18.7 and 23.7 °C) and high temperature in the summer (28 °C). These thermal fluctuations characterize the Mediterranean environment where the contrast between cold and hot seasons is high. Similar results were reported in the Gulf of Annaba by Khati Hadj Moussa, (2009) and the Mediterranean coastal waters of Morocco (Rijal Leblad et al., 2020). Environmental results showed that the rearing experiment was conducted at a temperature within the range recommended for the development of *P. perna* (Henriques et al., 2006; 2007). The availability and quality of food can be considered important factors since they affect the physiological processes linked to growth and sexual maturation (Bayne and Newell, 1983). Suspended food particles for bivalves vary in quality and quantity and, in general, are composed of seston which itself is a complex mixture of pelagic organisms and suspended detritus (Navarro and Thompson, 1995; Cranford and Hill, 1999). The maximum Chl *a* content (1.2 μg L^{-1}) in our trial was recorded during the autumn period and could be explained by the optimal temperature, light intensity and nutrient conditions in seawater, promoting phytoplankton development (Trombetta et al., 2019). The DO level is considered a fundamental parameter that controls the respiration rate (Trigos et al., 2015) and mortality of shellfish (Katsanevakis, 2007). It is also a good indicator of the health and suitability of an ecosystem. The high oxygenation of the environment observed in cold periods (~10 mg L^{-1}) was may be linked to low temperatures and mechanical agitations caused by the winds.

Suspended Matter (SM) showed high values in the autumn (28.6 mg L^{-1}) and low values (6.5 mg L^{-1}) in the winter period. The increase in SM levels during the autumn period could be explained by the significant flux generated by the excess of feed and feces in the fish farming cages (Challouf et al., 2017). The high-water transparency observed in summer in this area is a characteristic of oligotrophic waters but was not indicative of low food availability for mussels, whereas the highest turbidity was observed during the autumn period as described by Zaafrane et al. (2019).

4.3. Low concentration of toxic phytoplankton taxa

Dinoflagellates and diatoms are the major components of the phytoplankton community in the investigated aquaculture farm of Monastir Bay, representing 60% and 30% of the total identified taxa, respectively. These results are comparable to those of Challouf et al. (2017) who showed the predominance of dinoflagellates and diatoms in Monastir Bay. Similar results were reported in other Mediterranean ecosystems such as Mahdia (eastern Tunisia) by Mabrouk et al. (2011), in Turkey by Yucel-Gier et al. (2008) and in the southern Alboran Sea by Idmoussi et al. (2022). Our results clarified knowledge on phytoplankton assemblages as natural diets available to mussels *P. perna* in a fish farm of Monastir Bay which was comparable to other zones of bivalve production in a Mediterranean coastal site (Caroppo and Giordano, 2022). The most dominant potentially toxic taxa observed in the aquaculture farm of the Monastir Bay were *Alexandrium* spp., *K. selliformis, P. lima, Dinophysis* sp. and *Pseudo-nitzschia*. These microalgae were previously observed in the Mediterranean Sea (Lounas et al., 2021; Feki et al., 2016; Zmerli-Triki et al., 2016; Ben Gharbia et al., 2016; Abdennadher et al., 2012; Dhib et al., 2013; Feki et al., 2013; Fertouna-Bellakhal et al., 2015; Sahraoui et al., 2009; Drira et al., 2008; Aboualaalaa et al., 2022). In Tunisia, *A. minutum* has been recorded in Bizerte Lagoon (Bouchouicha Smida et al., 2012), and since 1990 in the Gulf of Gabès (Abdennadher et al., 2012; Feki et al., 2013) at concentrations of up to 7×10^5 cells L^{-1} and 9×10^3 cells L^{-1}, respectively. Blooms of *K. selliformis* have been recorded since 1990 in the Gulf of Gabès at concentrations of up to 12×10^8 cells L^{-1} (Feki et al., 2008; Abdennadher et al., 2014; Feki et al., 2013). This dinoflagellate has been considered the most problematic species in Boughrara Lagoon (Gulf of Gabès) for several years and was incriminated for the contamination of the most important exploited shellfish species, the grooved carpet shell *Ruditapes decussatus* by gymnodimine toxins (Medhioub et al., 2009; 2010; Marrouchi et al., 2010; Ben Naila et al., 2012; Abdennadher 2014; Feki et al., 2013). Other harmful microalgae, *Dinophysis* spp. and *Pseudo-nitzschia* spp., were observed in Bizerte Lagoon at high concentrations of 6×10^4

Cells L^{-1} (Aissaoui et al., 2014) and 20×10^5 Cells L^{-1} (Bouchouicha Smida et al., 2012; Sahraoui et al., 2009), respectively. Recent studies showed the proliferation of benthic toxic dinoflagellates such as *Ostreopsis cf. ovata* and *Prorocentrum lima* in several Tunisian marine ecosystems (Ben Gharbia et al., 2016; 2019). However, compared to those found in the other exploited Tunisian areas such as the Bizerte Lagoon (north of Tunisia) and the Gulf of Gabès (southwestern of Tunisia), these species were present in lower densities in the aquaculture farm of Monastir Bay.

4.4. Low contamination of mussel by pathogenic bacteria and virus

Over the one-year's survey no human pathogens (enterovirus, *Salmonella* and *E. coli*) were evidenced in mussels. In the south Mediterranean, especially in Tunisia, data on *in situ* contamination of shellfish by viral and bacterial pathogens are scarce and investigation methods are heterogenous including site characteristics, sampling and detection. Despite this, El Amri et al. (2006) reported the presence of HAV, *Salmonella* and *E. coli* in *Mytilus galloprovincialis* and *Ruditapes decussatus* sampled from Bizerte Lagoon and the Gulf of Gabès, but no *Salmonella* or *E. coli*. Zomati et al. (2018) showed microbial contamination of clams in the production areas of the Sfax region (southern Tunisia) over four consecutive years with prevalence of 36% for *E. coli*, 11% for *Salmonella,* 19% for NoV and 3% for HAV (Zormati et al., 2018). The absence of microbial contamination of *P. perna* in our studies could be explained by the relatively large distance of the study site from the coast (e.g., 15 km), which is probably linked to a dilution effect reducing the microbial load. As suggested by Brenner et al. (2009), this increased distance to shore would further reduce parasite infestation rates and most likely lead to minimal microbial and viral infestations. However, the results from this study suggest that the exploitation of the oyster *P. perna* in an aquaculture farm in Monastir Bay presents a limited biological risk for consumers and human health compared to other Tunisian environments.

4.5. Low contamination of mussel by toxins

Biotoxin analyses of wild *P. perna* mussels of the aquaculture farm of the Monastir Bay, revealed no contamination by PSTs and ASTs and low contamination by LSTs. The contamination of shellfish by LSTs was evidenced in various marine ecosystems in the Mediterranean Sea. The most important shellfish farming area in Alfacs Bay in Catalonia (Spain, NW Mediterranean Sea) showed concentrations of LSTs in SPATT devices of up to 94 ng OA g^{-1} resin, 42 ng PTX2/g resin and 7 ng PTX2-sa/g resin (García-Altares et al., 2016).

By using SPATT, this work revealed the presence of Yessotoxins in seawater suggesting the presence of *Ligulodinium polyedrum* or *Gonyaulax spinefera* in the water column. It revealed also for the first time the presence of Pinnatoxins and Portimine suggesting the emerging of the benthic dinoflagellate *Vulcanodinium rugosum* (Abadie et al., 2016) never observed to date in southern Mediterranean waters. As these dinoflagellates have not been observed in the phytoplankton assemblage examined by photonic microscopy, this result suggests that SPATT could be good tool to survey the expansion of toxic species in environment. Especially in the context of global warming affecting the Mediterranean and the introduction of invasive dinoflagellate species through ballast waters and the translocation of molluscs from one breading area to another (Ben Gharbia et al., 2016).

During the investigated year, we had evidenced the presence of several toxins in seawater but at low concentrations (<50 ng.g^{-1}) below the concentration that triggers mouse bioassay positive, low contamination of natural mussels by LSTs and low concentration of toxic phytoplankton taxa in water. These results suggest that the exploitation of the oyster *P. perna* in an aquaculture farm in Monastir Bay represents a limited toxic risk for consumers and human health when compared to aquaculture activities in other Tunisian environments.

5. Conclusion

Although further studies on *P. perna* mussels are needed to confirm their growth, reproduction and health status, this work undoubtedly shows for the first time that rearing of the mussel *P. perna* in aquaculture farms of the Bay of Monastir should be considered. The surrounding seawater did not show high contamination with pathogenic bacteria or viruses, suggesting that rearing in aquaculture farms could significantly reduce the biological risk of human contamination. While toxic species of phytoplankton have indeed been detected in seawater, only LSTs have been found in mussels, but at concentrations below the sanitary threshold, which doesn't impact their commercialisation. In conclusion, studies on the culture of mussels in aquaculture farms should be considered to open up a new horizon and participate in the development of integrated multitrophic aquaculture (IMTA) in Tunisia.

CRediT authorship contribution statement

Walid Medhioub: Writing original paper and final approval of the version to be published; Conceptualization; Methodology; Shellfish and environmental sampling and data analysis; SPATTs deployment, extraction and analysis. **Rafika Challouf:** Environmental sampling and analysis. **Youness Limayem, Sondes Bchir, Wissem Slimeni:** Laboratory analysis (shellfish sampling, storage and biometry analysis). **Dorsaf El Amri:** Bacteria and viruses analysis. **Asma Hamza, Mabrouka Mahfoudhi:** Toxic Microalgae identification. **Mohamed Laabir, Eric Abadie:** LC/MS/MS analysis and review the manuscript. **Benlahcen Rijal Leblad, Jean Luc Rolland:** Critical revision of the manuscript. **Mohamed Salah Azaza, Mohamed Néjib Medhioub:** Supervision and review the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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