Method Article

A method for bivalve shells characterization by FT-IR photoacoustic spectroscopy as a tool for environmental studies

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**ABSTRACT**

Fourier Transform Infrared Spectroscopy has been employed to investigate the composition of the shells of two marine bivalves Mytilus galloprovincialis and Corbula gibba from four samples collected from the Mar Piccolo of Taranto (Ionian Sea, Southern Italy). Bivalve shells are composed of 95–99.9% calcium carbonate (CaCO\textsubscript{3}), while the remaining portion is constituted by organic matrix, which in some cases may incorporate pollutants from the surrounding environment. Recognizing the role of bivalves in the carbon biogeochemical cycle and their economic importance for aquaculture, we aimed to develop a methodology for shells powder samples preparation and analysis. The main objective of the study was to demonstrate the feasibility of Fourier Transform Infrared photoacoustic spectroscopy to perform a fast sample analysis in order to detect the possible presence of pollutants in the shells.

The results revealed an unbiased differentiation between the shell compositions of the two bivalve selected species. Moreover, the spectra interpretation indicated that C. gibba specimens recorded a shell matrix contaminated by organic pollutants present in the surrounding environment. In conclusion, the described methodology including sample preparation tailored for photoacoustical investigations demonstrated to be a tool for the characterization of bivalve shells contamination enhancing environmental studies of polluted marine areas.

- Bivalve species were collected from sampling stations located in the Mar Piccolo of Taranto (Ionian Sea, Southern Italy).
- Samples preparation stages include: sonication, grinding and fractioning by sieving.
- FT-IR PAS spectral region of interest is in the mid-infrared between 4000 and 400 cm\textsuperscript{-1}.

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A R T I C L E  I N F O
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Introduction

Bivalves (Mollusca: Bivalvia) represent a class of organisms responsible for CO₂ sequestration as active part of the global carbon cycle. Bivalve shells are composed of 95-99.9% calcium carbonate (CaCO₃), the remaining is constituted by organic matrix [1–3]. CaCO₃ is present in the form of calcite, dolomite and aragonite together with inorganic trace elements varying according to species and thermodynamic stability in the surrounding environment [4,5]. Recent studies on ocean acidification have revealed that this class of organisms may be endangered by water pH decrease that causes carbonate dissolution [6–8].

Bivalves provide many ecosystem services for biofiltration, bioturbation, and biodeposition [9]. They are also used as bioindicators to monitor the levels of pollutants in water bodies [9,10]. Moreover, many bivalves like mussels and clams are of economic interest for aquaculture [11–13], while shells are considered of concern as biomaterials in circular economy [4,10].

The CaO extracted by bivalve shells is valuable for: bioremediation as absorbent in water treatment [14], CO₂ capture techniques [15], biofuels catalysis [16], filler in biopolymers [17] and in bactericidal agents [18]. Therefore, bivalve shells characterization is important not only to understand the impacts of ocean acidification but also to investigate their functional properties as biomaterials.

The assessment of carbonate concentration and organic matter in solid marine samples can be performed by Fourier transform infrared spectroscopy (FT-IR) [19–21]. Many studies report FT-IR as a useful technique to quantify carbonate content in marine biogenic sediments [17,19,22–25]. The FT-IR spectroscopic technique exploits vibrations of covalent chemical bonds absorbing infrared energy at specific wavelengths. The typical spectral region of interest is in the mid-infrared between 400 and 4000 cm⁻¹, with modulation frequencies of 50–500 kHz [26]. Within this spectral region infrared absorption is associated to specific functional groups. The spectroscopic technique permits to measure absorption intensity in reflectance or transmittance mode. FT-IR spectroscopy has many advantages with respect to other investigation techniques. A small quantity of sample is required and a short time to perform the analysis is needed. Moreover, many chemical species can be detected including organic, inorganic, crystalline or amorphous compounds [17,19,25].

The FTIR photoacoustic spectroscopy (PAS) is an extension of the IR spectroscopic technique that includes a photoacoustic sensor (PA) [26]. FTIR-PAS is a depth profiling tool providing a non-destructive non-contact material analysis that requires a minimal sample preparation [26]. It exploits the usefulness of interferometry combined with the photothermal technique. Moreover, FTIR-PAS implicates measurements of pressure oscillations that happen in a sealed cell equipped with a very sensible microphone. The sample is inserted into the sealed cell where it is hit by IR light so that a portion of the absorbed energy turns into modulated local heat generating a periodic heat diffusion from the sample in the surrounding gas. At constant volume (as in the PA cell) this generates a pressure wave proportional to temperature following the ideal gas law. Hence, by plotting the intensity of acoustic waves as a function of wavelength the absorption spectrum of the sample can
be obtained thus producing the same information yielded by optical absorption spectra. FTIR-PAS technique also allows to directly measure sample absorption without signal spreading and avoiding any sample manipulation i.e., incorporation in KBr tablets.

This study aims to propose an application of FTIR-PAS method, that includes a tailored sample preparation procedure, to carry out a characterization of bivalve shell powder of interest for environmental monitoring.

**Methodology**

**Bivalve samples selection**

The shells of two marine bivalve species were investigated by FTIR-PAS. The first, well known for its commercial exploitation, was the marine mussel *Mytilus galloprovincialis* Lamarck, 1819. This species lives adhering to substrates at a low depth in the water column and has a life cycle lasting eighteen months [27,28]. For this study we selected mussels that had just started their sessile life stage classified as juveniles [29]. The second selected species was *Corbula gibba* (Olivi, 1792), a bivalve mollusc which populates the soft seabed in unstable environments [30].

*C. gibba* is characteristic of highly polluted areas and mostly lives in environments characterized by carbonate sediments; it is widely diffused in the eastern Mediterranean Sea [30]. This species is characterized by a short life span of about two years and its natural mortality starts in the second year when individuals are about 10 mm long [30].

Bivalve samples (Fig. 1) were collected in the Mar Piccolo of Taranto during routine environmental monitoring surveys (years 2016–2018) Fig. 2.

The Mar Piccolo is a shallow semi-enclosed basin located in the northern sector of the Gulf of Taranto (south-eastern Italy, Ionian Sea) divided into two inlets. The 1st Inlet (maximum depth 13 m) communicates with the larger embayment of the Mar Grande while the 2st Inlet (maximum depth 9 m), is a highly confined marine system and biological area [31,32]. The Mar Piccolo is characterised by limited water circulation with salinity and temperature influenced by seasonal freshwater flows from small surface watercourses and submarine springs (locally called *citri*), mainly located in the northern part, of the Second Inlet [33].

The Mar Piccolo is strongly affected by several anthropogenic inputs, such as industrial pollution, mainly related to the presence of the city of Taranto, harbour activities, aquaculture and commercial fishing. As a consequence, it is one of the most polluted areas in the Mediterranean Sea [34,35].

The basin is one of the most important mussel farming sites in Italy [36,37], now limited to just the 2st Inlet since the 1st Inlet is heavily contaminated by polychlorinated biphenyls (PCBs) and dioxins [38,39]. However, the 2st inlet is characterized by strong eutrophication events, due to the high organic load deriving from the presence of mussels, coupled with the large amount of sewage conveyed by small rivers and channels from nearby towns [40–42].
Table 1
Shell length and weight of the selected specimens. Values are expressed considering the digital calibre sensitivity of ± 0.01 mm and balance sensitivity of ± 0.01 g.

| Specimen ID | Shell length (mm) | Shell weight (g) |
|-------------|-------------------|------------------|
| M1          | 29.00 ± 0.01      | 0.52 ± 0.01      |
| M2          | 23.00 ± 0.01      | 0.44 ± 0.01      |
| C1          | 10.00 ± 0.01      | 0.07 ± 0.01      |
| C2          | 12.00 ± 0.01      | 0.11 ± 0.01      |

Samples preparation

Before carrying out FTIR-PAS measurements, samples were treated and prepared through a simple procedure developed ad hoc. The procedure described below is a modified version of that proposed by [12]. The first phase involves a selection and measurement of the shells. The shell length was measured by a digital calibre with a sensitivity of 0.01 mm. In the case of mussels each pair of valves was labelled, so that one could be used to perform infrared spectroscopy analyses, while the other one was saved for future investigations. Each valve was weighed by a precision balance (model Steinberg Systems SBS-LW-500/10 WS), an important step that allows to detect any loss of the sample after grinding. The measurements were carried out on two mussel individuals labelled as M1 and M2, and on two selected C. gibba individuals, labelled as C1 and C2. The general morphometric data of selected specimens are summarized in Table 1.

The valves were then treated for the removal of any organic pollutants by immersion in a 2.5% sodium hypochlorite (NaClO) solution for 7 min. They were rinsed with deionized water and dried for 10 min at 60 °C using a heating plate. Each valve was then carefully observed by optical microscope (model Zeiss Discovery-V8) to check for encrustation. In case of appreciable encrustations, the sample was immersed for 1 min in an ultrasonic bath (model Bandelin Sondrex Digitec) to definitively remove any residual micro-concretions (e.g., marine microalgae, epifauna) so as not to alter the FT-IR spectra and obtain an accurate cleaning of the surface without damaging the sample with mechanical actions.
Table 2

| Specimen ID | Shell powder weight $\leq 90 \mu m$ (g) | Shell powder weight $\geq 90 \mu m$ (g) |
|-------------|----------------------------------------|----------------------------------------|
| M1          | 0.07 ± 0.01                            | 0.46 ± 0.01                            |
| M2          | 0.06 ± 0.01                            | 0.34 ± 0.01                            |
| C1          | 0.06 ± 0.01                            |                                        |
| C2          | 0.10 ± 0.01                            |                                        |

* not sieved

due to scraping. The sample was subsequently dried again at 60 °C for 10 min. The grinding of each single valve was performed by using an agate mortar and pestle to obtain a very fine powder. Since manual grinding does not always produce homogeneous granulometry, a 90 μm mesh sieve was used (model Retsch Test Sieve 200 × 25 mm) to carry out a granulometric selection. This procedure is particularly important for mussel powder samples which are better dimensionally sorted than the more homogeneous C. gibba powder. Indeed, two main granulometric fractions were obtained for mussel samples: a coarser fraction (labelled as G) over 90 μm and a finer fraction (labelled as F) under 90 μm. Finally, before introducing the samples into the FTIR-PAS sample holder, powders were weighed once again to ascertain any losses in mass (Table 2).

Spectra acquisition

In this study spectra acquisition was performed by a Fourier Transform Infrared Spectrometer, model PERKIN ELMER (SPECTRUM 2000) equipped with a photoacoustic cell MTEC Photoacoustics Model 300. The FTIR-PAS instrumentation was composed by: (i) an IR light source coupled with an intensity modulator (interferometer), (ii) a photoacoustic cell filled with an IR-transparent gas (e.g., helium), (iii) a signal and processing unit including a microphone detector (sensitivity ~ 50 mV/Pa).

The standard procedure for FTIR-PAS spectra acquisition involves some preliminary steps to remove any factor that could influence the spectra interpretation. A first measure is carried out without the sample holder, a second measurement follows with the sample holder containing carbon black, a highly absorbent pigment produced as the result of combustion of heavy petroleum crude oils, to trace the spectrum related to the background. After this background calibration phase each sample is placed into the cell for the sample spectrum analysis.

Data acquisition was performed by the Perkin Elmer software Spectrum v5.0.2. A number of twenty-four scans for each measurement was set. Afterwards, the numerical processing and display of the data was carried out using the software OriginPro v8. Figs. 3 and 4 show the spectra of samples M1, M2, C1 and C2. Each one is compared with reference spectra of the three forms of calcium carbonate (calcite (CaCO$_3$), dolomite (Mg Ca (CO$_3$)$_2$, aragonite (CaCO$_3$)) from the database of the RRUFF™ Project of the University of Arizona [43].

Results

Spectra from specimens of M. galloprovincialis M1 and M2 revealed a good adherence to the characteristic spectra of the three carbonate standards. The spectra of the fine powder samples, M1F and M2F (Fig. 3A and B), actually showed a higher signal resolution in terms of tighter and more resolved peaks when compared to the coarser samples. A band in the region 3400 and 3300 cm$^{-1}$ arises from the stretching vibration of a hydroxy group H-bonded, with a peak outside this band at 3617 cm$^{-1}$ clearly detected in sample M2F (Fig. 3B). Within the range 2900 and 2400 cm$^{-1}$ C-H stretching was detected. In the double bond region, ranging from 1800 and 1600 cm$^{-1}$, two peaks related to stretching C=O and C=C are intense. Important peaks between 1396 and 1413 cm$^{-1}$ are present in compliance with the CaCO$_3$ material. A typical absorption peak of the crystalline structure of aragonite is detected in both samples in correspondence of 1049 cm$^{-1}$ (Fig 3A) and 1052 cm$^{-1}$ (Fig 3B) wavenumbers corresponding to the fundamental mode of the CO$_3^{2-}$ molecular ion. C-H
Fig. 3. Comparison of FTIR-PAS spectra with calcium carbonate reference spectra (calcite (CaCO₃), dolomite (Mg Ca (CO₃)₂), aragonite (CaCO₃)); (A) sample M. galloprovincialis M1 (fine (F) and coarse (G) fractions respectively); (B) sample M. galloprovincialis M2 (fine (F) and coarse (G) fractions respectively).
FTIR-PAS spectra of samples C. gibba C1 (A) and C2 (B) compared with reference spectra of the three forms of calcium carbonate (calcite (CaCO$_3$), dolomite (MgCa(CO$_3$)$_2$), aragonite (CaCO$_3$)).

stretching band was observed around 1100 cm$^{-1}$ wavenumber. In both samples, a peak for the non-planar C-O bond respectively at 850 cm$^{-1}$ (Fig. 3A) and 868 cm$^{-1}$ (Fig. 3B) is assigned to an out of plane deformation band. In correspondence of 700 cm$^{-1}$ wavenumber, an in-plane deformation of C=O band was observed in both samples.

The C. gibba samples, C1 and C2, (Fig. 4 A and B), showed resonances in the region of the triple bonds C≡C and C≡N between 2400 and 2800 cm$^{-1}$, and a wider band in the region between 3200 and 3300 cm$^{-1}$ possibly due to organic linings. This is in correspondence with the stretching of simple NH bonds higher and wider than to those of the stretching of the OH bonds, which are less clear in the band between 3700 and 3600 cm$^{-1}$. In particular, sample C1 showed a peak at 3755 cm$^{-1}$ possibly arising from the stretching vibration of a hydroxy group.

Both samples showed two peaks between 1600 and 1800 cm$^{-1}$ related to stretching C=O and C=C (Fig. 4 A and B). Moreover, in the fingerprint region from 1500 cm$^{-1}$ up to 600 cm$^{-1}$ wavenumbers typical peaks of calcium carbonate were detected [44]. Within this band, the absorption peaks recorded (Fig. 4 A, B) are slightly shifted but highlight a crystalline structure attributable to aragonite [44].
Discussion

Mussels spectra showed a good adherence to the characteristic resonance peaks of the aragonite and calcite standards [45] and differences in the spectra of the two species of marine bivalves confirm the differences in the carbonate composition and structure of their shells. Mussel shells are three-layered. The inner and middle layers consist of nacre sheet separated by pallial myostracal aragonitic, while the outer layer is calcitic with prismatic structure [46,47]. Likewise, this is confirmed by C-H stretching band observed in both samples at 1049 and 1062 cm\(^{-1}\) wavenumbers.

Conversely, the C. gibba shell is aragonitic and composed of two major layers. The inner shell layer is a finely laminated complex cross-lamellar structure, while the outer shell layer shows a simple cross-lamellar structure [48].

The spectra from C. gibba clearly showed characteristic bands related to prevailing aragonite composition. In particular, small differences with the pure spectra in the stretching region of the hydrogen bonds can be observed. Peaks detected within the range 2900 and 2400 cm\(^{-1}\) C-H stretching are the best indicator of the organic matrix in the nacre powder [49]. Sample C1 showed unexpected peak at 1058 cm\(^{-1}\) wavenumber probably due to traces of trapped in mineral composites. Furthermore, in the same sample two other unexpected peaks were detected around the wavenumbers 3624 and 3755 cm\(^{-1}\) leading to hypothesize the incorporation of an organic contaminant from the surrounding sediments.

Additional information provided by the results, is that fine dust (<90 μm) actually recorded an increase in the absorption intensity. This turned directly into a higher degree of signal resolution in terms of tighter and more resolved peaks. Conversely, dust samples dimensionally larger than 90 μm, with heterogeneous and rich micro-fractured grains lead to an attenuation of the signal. Indeed, the spectra obtained from C. gibba samples clearly confirm that a naturally fine and sorted powder produces better signal resolution. Likewise, this is confirmed by recent studies showing that particle size plays an important role in understanding and interpreting the infrared spectra acquired on calcite powder (CO\(_3^{2-}\) anion) and other mineral species [47,50–52].

Conclusions

Bivalve shells spectroscopic investigations can have several purposes, among which (a) the understanding of biomineralization processes in the formation of shells, that vary according to species of molluscs, (b) the assessment of the vulnerability of these organisms to stress factors (i.e., ocean acidification) and (c) the investigation of the chemical composition of these biomaterials.

Aiming to perform quick and accurate characterizations of marine bivalve shell powders of environmental and economic interest, this study developed a method using an optical technique such as infrared spectroscopy, equipped with a photoacoustic-based detection system. This allowed to overcome some limits imposed by traditional spectroscopic techniques, such as time-consuming treatments of the samples and the use of integrating spheres. To this end a simple sample preparation procedure tailored for bivalve shell samples to improve signal resolution was conceived. Novelties in sample preparation steps included shell powder sieving. Moreover, the granulometric fractioning allowed to confirm that particle size plays an important role for photoacoustic infrared spectra interpretation. Results showed a fair differentiation between the shell structures of the two bivalve species. A good adherence to the standards of the carbonate forms of aragonite and calcite was identified for mussels, while the prevailing aragonite composition in C. gibba samples was detected.

Finally, it is worth noting that C. gibba specimens recorded a shell matrix contamination by possible pollutants. Indeed, this species constantly populates polluted bays and harbours and coastal and offshore areas exposed to seasonal or occasional environmental disturbances.

The study demonstrated the advantages of FTIR-PAS technique for bivalve shells investigation aiming at different purposes from environmental studies to biomaterial characterization. Moreover, this methodology could be promising for quick and accurate characterizations of bivalve shells even coupled with other chemical and physical investigation techniques.
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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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Not Applicable

References

[1] J.D. Currey, The design of mineralised hard tissues for their mechanical functions, J. Exp. Biol. 202 (1999) 3285–3294.
[2] E.M. Harper, Are calcitic layers an effective adaptation against shell dissolution in the Bivalvia? J. Zool. 251 (2000) 179–186.
[3] J.P. Morris, Y. Wang, T. Backeljau, G. Chapelle, Biomimetic and bio-inspired uses of mollusc shells, Mar. Genom. 27 (2016) 85–90.
[4] Z. Yao, M. Xia, H. Li, T. Chen, Y. Ye, H. Zheng. Bivalve shell: not an abundant useless waste but a functional and versatile biomaterial, Crit. Rev. Environ. Sci. Technol. 44 (22) (2014) 2502–2530.
[5] K.Y. Chakraborty, M. Joy, High-value compounds from the molluscs of marine and estuarine ecosystems as prospective functional food ingredients: an overview, Food Res. Int. 137 (2020) 109637.
[6] S.C. Fitzner, W. Zhu, K.E. Tanner, V.R. Phoenix, N.A. Kammenos, M. Cusack, Ocean acidification alters the material properties of Mytilus edulis shells, J. R. Soc. Interface 12 (103) (2015).
[7] K. Ramesh, F. Melzner, A.W. Griffths, C.J. Cobbler, C. Rouger, D. Tasdemir, G. Nehrke, In vivo characterization of bivalve larval shells: a confocal Raman microscopy study, J. R. Soc. Interface 15 (141) (2018) 20170723.
[8] H. Kawahata, K. Fujita, A. Iuchi, M. Inoue, S. Iwasaki, A. Kuroyanagi, A. Maeda, T. Manaka, K. Moriya, H. Takagi, T. Toyoshiki, T. Yagishita, A. Suzuki, Y. Togaku, Y. Tsuruoka, T. Yamao, Perspective on the response of marine calcifiers to global warming and ocean acidification-behavior of corals and foraminifera in a high CO2 world “hot house”, Prog. Earth Planet. Sci. 6 (5) (2019), doi:10.1186/s40645-018-0239-9.
[9] A. van der Schatte Olivier, L. Jones, L. Le Vay, M. Christie, J. Wilson, S.K. Malham, A global review of the ecosystem services provided by bivalve aquaculture, Rev. Aquacult. 12 (1) (2018) 3–25.
[10] R. Nisticò, Aquatic-derived biomaterials for a sustainable future: a European opportunity, Resources 6 (2017) 65.
[11] FAO/State World Fish. FAO, 2014 Aqua.
[12] European Commission, Closing the loop – An EU action plan for the Circular Economy COM 614 (2015).
[13] J.P. Morris, T. Backeljau, G. Chapelle, Shells from aquaculture: a valuable biomaterial, not a nuisance waste product, Rev. Aquacult. 11 (1) (2018) 42–57.
[14] J.H. Shariffuddin, M.J. Jones, D.A. Patterson, Greener photocatalysts: hydroxyapatite derived from waste mussel shells for the photocatalytic degradation of a model azo dye wastewater, Chem. Eng. Res. Des. 91 (2013) 1693–1704.
[15] M.A. KW, H. Teng, CaO powders from oyster shells for efficient CO2 capture in multiple carbonation cycles, J. Am. Ceram. Soc. 93 (2010) 221–227.
[16] I. Boro, P. Deka, A. Thakur, A review on solid oxide derived from waste shells as catalyst for biodiesel production, Renew. Sustain. Energy Rev. 16 (2012) 904–910.
[17] X. Liu, S.M. Colman, E.T. Brown, E.C. Minor, L. Hongyu, Estimation of carbonate, total organic carbon, and biogenic silica content by FTIR and XRF techniques in lacustrine sediments, J. Paleolimnol 50 (2013) 387–398.
[18] K. Okawa, T. Asada, K. Yamamoto, H. Wakabayashi, M. Sasaki, M. Sato, J. Matsuda, Antibacterial activity of calcined shell calcium prepared from wild surf clam, J. Health Sci. 46 (2) (2000) 98–103.
[19] M. Mecozzi, E. Pietrantonio, M. Amici, G. Romanelli, Determination of carbonate in marine solid samples by FTIR-ATR spectroscopy, Analyst 126 (2001) 144–146.
[20] S. Veerasingam, R. Venkattachalamathy, Estimation of carbonate concentration and characterization of marine sediments by fourier transform infrared spectroscopy, Infrared Phys. Technol. 66 (2014) 136–140.
[21] G. Sreenivasulu, N. Jayaraju, B.C. Sundara Raja Reddy, T. Lakshmi Prasad, K. Nagalakshmi, B. Lakshmanna, Organic matter from bentic foraminifera (Ammonia beccarii) shells by FT-IR spectroscopy: a study on Tupilipalem, South east coast of India, MethodsX 4 (2017) 55–62.
[22] T.D. Herbert, B.A. Tom, C. Burnett, Precise major component determinations in deep-sea sediments using fourier-transform infrared-spectroscopy, Geochim. Cosmochim. Acta 56 (1992) 1759–1763.
[23] N. Kovac, J. Fraganeli, O. Bajt, B. Orel, A.S. Vuk, Investigation of sediment samples from the gulf of Trieste (northern Adriatic) by FTIR spectroscopy, Mater. Geoenviron. 52 (2005) 81–85.
[24] M. Tatzer, M. Stemmer, H. Spiegel, K. Katzberger, G.F. Haberhauer, M.H. Gerzabek, An alternative method to measure carbonate in soils by FTIR spectroscopy, Environ. Chem. Lett. 5 (2007) 9–12.
[25] V. Bruckman, K. Wiessneg, Improved soil carbonate determination by FT-IR and X-ray analysis, Environ. Chem. Lett. 11 (1) (2013) 65–70.
[26] R. Kizil, J. Irudayaraj, G.C.K. Roberts, Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS), Encyclopedia of Biophysics, Springer, Berlin, Heidelberg, 2013.
[27] V. Ceccherelli, R. Rossi, Settlement, growth and production of the mussel Mytilus galloprovincialis, Mar. Ecol. Prog. Ser. 16 (1/2) (1984) 173–184.
[28] L. Giordano, G. Portacci, C. Caroppo, Multidisciplinary tools for sustainable management of an ecosystem service: the case study of mussel farming in the Mar Piccolo of Taranto (Mediterranean, Ionian sea), Ocean Coast. Manag. 176 (2019) 11–23.

[29] Y.M. Abada-Boudjema, J.C. Dauvin, Recruitment and life span of two natural mussel populations Perna Perna (Linnaeus) and Mytilus galloprovincialis (Lamarck) from the Algerian coast, J. Mollus. Stud. 61 (4) (1995) 467–481.

[30] M. HRS-Benito, The haddock shell, Caruba gibba (Olivi), 1792 (Bivalve Mollusks) as a species resistant to environmental disturbances: a review, Acta Adriatica 4 (1) (2006) 49–64.

[31] G. Belmonte, I. Vaglio, F. Rubino, G. Alabiso, Zooplankton composition along the confinement gradient of the Taranto sea system (Ionian sea, south-eastern Italy), J. Mar. Syst. 128 (2013) 222–238.

[32] L. Ferraro, F. Rubino, M. Belmonte, S. Da Prato, M. Greco, F. Frontalini, A multidisciplinary approach to study confined marine basins: the holobenthic and merobenthic assemblages in the Mar Piccolo of Taranto (Ionian sea, Mediterranean), Mar. Biodivers. 47 (2017) 887–911.

[33] A.R. Vatova, La produttività delle acque del Mar Grande e del Mar Piccolo di Taranto (1962–1969), Boll. Pes. Idrobiol. 27 (1) (1972) 81–103.

[34] L. Spada, C. Annicchiario, N. Cardellicchio, S. Giandomenico, A. Di Leo, Mercury and methylmercury concentrations in Mediterranean seafood and surface sediments, intake evaluation and risk for consumers, Int. J. Hyg. Environ. Heal. 215 (2012) 418–426.

[35] N. Cardellicchio, C. Annicchiario, A. Di Leo, S. Giandomenico, L. Spada, The Mar Piccolo of Taranto: an interesting marine ecosystem for the environmental problems studies, Environ. Sci. Pollut. Res. 23 (2016) 12495–12501.

[36] C. Caroppo, L. Giordano, N. Palmieri, G. Bellio, A.P. Bisci, G. Portacci, P. Sclafani, T.S. Hopkins, Progress toward sustainable mussel aquaculture in Mar Piccolo, Italy, Ecol. Soc. 17 (3) (2012) 10.

[37] A. Franco, T. Cibic, R. Auriemma, P. Del Negro, Benthic ecosystem functioning in the severely contaminated Mar Piccolo of Taranto (Ionian Sea, Italy): focus on heterotrophic pathways, Environ. Sci. Pollut. Res. 23 (2016) 12645–12661.

[38] S. Giandomenico, L. Spada, C. Annicchiario, G. Assennato, N. Cardellicchio, N. Ungaro, A. Di Leo, Chlorinated compounds and polychlorinated diphenyl ethers (PBDEs) in mussels (Mytilus galloprovincialis) collected from Apulia region coasts, Mar. Pollut. Bull. 73 (2013) 243–251.

[39] L. Spada, C. Annicchiario, N. Cardellicchio, S. Giandomenico, A. Di Leo, Heavy metals monitoring in mussels Mytilus galloprovincialis from the Apulian coast (Southern Italy), Mediterr. Mar. Sci. 14 (2013) 99–108.

[40] G. Alabiso, M. Cannalire, D. Ghionda, M. Milillo, G. Leone, O. Cacciorgna, Particulate matter and chemical-physical conditions of an inner sea: the Mar Piccolo in Taranto. A new statistical approach, Mar. Chem. 58 (1997) 373–388.

[41] G. Alabiso, M. Giacomini, M. Milillo, P. Ricci, The Taranto sea system: 8 years of chemical–physical measurements, Biol. Mar. Medit. 12 (2005) 369–373.

[42] C. Caroppo, N. Cardellicchio, Preliminary study on phytoplankton communities of Mar Piccolo in Taranto (Jonian Sea), Oestivalia XXI (1995) 61–76.

[43] B. Lafuente, R.T. Downs, H. Yang, N. Stone, T. Armbruster, R.M. Danisi, The power of databases: the RRUFF project, in: Highlights in Mineralogical Crystallography, De Gruyter W, Berlin, Germany, 2015, pp. 1–30.

[44] S.J. Galfey, Spectral reflectance of carbonate minerals in the visible and near-infrared (0.35–2.55 microns) calcite, aragonite, and dolomite, Am. Mineral. 71 (1986) 151–162.

[45] N.V. Vagenas, A. Gatsoul, C.G. Kontoyannis, Quantitative analysis of synthetic calcium carbonate polymorphs using FT-IR spectroscopy, Talanta 59 (2003) 831–836.

[46] J.G. Carter, R.A. Lutz, M.J.S. Tevesz, J.G. Carter, Shell microstructural data for the Bivalvia; Part VI. Orders Modiomorphoidea and Mytilioida, in: Skeletal Biominalization: Patterns, Processes and Evolutionary Trends, Van Nostrand Reinhold, New York, 1990, pp. 391–411.

[47] L. Addadi, D. Joester, F. Nudelman, S. Weiner, Mollusk shell formation: a source of new concepts for understanding biominalization processes, Chemistry 12 (2006) 980–987.

[48] Z. Lewy, C. Samtleben, Functional morphology and palaeoecological significance of the conchilin layers in corbulid pelecypods, Lethaia 12 (1979) 341–351.

[49] J. Balmain, B. Hannoyer, E. Lopez, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction analyses of mineral and organic matrix during heating of mother of pearl (nacre) from the shell of the mollusc Pinctada maxima, J. Biomed. Mater. Res. 48 (5) (1999) 740–754.

[50] M.R.R. Hamester, P.S. Balzer, D. Becker, Characterization of calcium carbonate obtained from oyster and mussel shells and incorporation in polypropylene, J. Mat. Res. 15 (2012) 204–208.

[51] P Kristova, L.J. Hopkinson, K.J. Rutt, The effect of the particle size on the fundamental vibrations of the [CO3\(^{2–}\)] anion in calcite, J. Phys. Chem. A 119 (2015) 4891–4898.

[52] I. Kontopoulos, S. Presslee, K. Penkman, M.J. Collins, Preparation of bone powder for FTIR-ATR analysis: the particle size effect, Vib. Spectros. 99 (2018) 167–177.