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New Insights on Phytoplankton Communities as a Tool for the Sustainable Management of the Musselculture in a Mediterranean Coastal Site

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Abstract: A simulation of phytoplankton dynamics in the coastal shellfish farming site of Mar Piccolo in Taranto (Ionian, Mediterranean Sea) is here reported. This simulation is an element of a greater ecological model produced taking into account the System Approach Framework. The model represents a refinement of a previous phytoplankton tool aimed to better understand the complex mosaic of the ecosystem functioning by providing additional information to support mussel farming. In particular, throughout the years the increase of water temperature and intensive musselculture caused a phytoplankton size and biomass reduction. Results of our simulation evidenced that the phytoplankton tool reproduced the size-fractions (micro-, nano- and picophytoplankton) biomass growth and the mussel (*Mytilus galloprovincialis*) grazing with a good level of performance. This newly calibrated phytoplankton model aims to provide new insights on modeling scenarios for the sustainable production of mussels.

Keywords: coastal zone; indicators; model; phytoplankton; grazing; sustainability; mussel farming; *Mytilus galloprovincialis*; Mar Piccolo in Taranto; Mediterranean Sea

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1. Introduction

The growth of mollusk bivalves depends largely on food availability [1–3]. Bivalves feed on microalgae, zooplankton, bacteria, organic particulate and dissolved matter [4]. However, due to the high absorption efficiency, phytoplankton is usually considered the main food source for filter-feeder bivalves, mussels comprised [5–7].

Even if mussel condition and phytoplankton biomass are linked [8,9], size structure and nutrient composition, are also important [10,11]. The ingestion and assimilation of mussels is determined by the phytoplankton dimensions and measurements on the retention efficiencies evidence that small (<5 µm) and very large phytoplankton (>200 µm) are often not usable as a food source by bivalves [12]. Moreover, several studies demonstrated that the most suitable size range for the larval growth is 4–45 µm (e.g., [13]). As regards the nutritional value, each phytoplankton species or group has a characteristic one, mainly due to the content of lipids, which are the principal source of energy for bivalves [14,15].

Mussel (*Mytilus galloprovincialis* Lamarck, 1819) farming has been performed in the Mar Piccolo (MP) of Taranto (Ionian Sea, Mediterranean) since the early 1900s [16–18]. In the past, urban development and intensive land cultivation determined an increase in nutrient concentrations as well as in phytoplankton biomass [19–21]. In addition, the quality of mussels, defined in terms of condition index values, was high [22]. The environmental quality of the Mar Piccolo worsened over time, as pressures on this ecosystem increased [20]. To overcome this problem, between 2000 and 2005 the political authorities imposed

the closure of numerous sewers. Coincident with the reduction of the sewer discharge, an expansion of the farmed area was allowed by a more liberal policy. As a result, the mussel production is today characterised by a high biomass variability and decline in the quality. The combined effect of these changes appears to have also influenced the phytoplankton community physiognomy. In the past, autotrophic communities were dominated by micro-sized diatoms, which have been largely replaced by nano-sized flagellates [23]. More recent studies on the phytoplankton size-fractions in Mar Piccolo evidenced the important role of the smallest components (pico- and nano-sized autotrophs) in phytoplankton and food webs dynamics [21,24,25].

MP has been one of the 18 European case studies of the Integrated Project SPICOSA (Science and Policy Integration for Coastal Systems Assessment, <http://www.spicosa.eu>, accessed on 18 March 2022). The aim of this project was to deliver fine-tuned system-based models including science-based decision-support tools to tackle challenging issues in coastal zones [26,27]. Furthermore, a combination of System Approach Framework (SAF)-based tools was designed to reproduce dynamic systems following a scenario-based approach in order to investigate policy issues [22,28–30]. The SAF application designed for MP aimed to improve both the assessment of coastal systems and managing the mussel farming. The general ESE (Ecological-Social-Economic) model is extensively illustrated in Caroppo et al. [22].

This paper reports some refinements on the phytoplankton tool as part of the MP ESE model previously developed during the SPICOSA project. Particularly, the phytoplankton model formerly described in Caroppo et al. [22] and calibrated only using data on the First Inlet of MP has been improved by exploiting new field datasets. In this paper, an eight-year environmental dataset and phytoplankton size-components (Pico-, 0.2–2.0 μm ; Nano- 2.0–20.0 μm ; Micro-Phytoplankton, 20.0–200.0 μm) biomass data for MP were used to recalibrate the phytoplankton model as well as to improve its predictive power. Starting from the annual mussels Carbon budget a top-down calculation was exploited to check the deviation between simulated biomasses and field data. All physical constraints as well as boundary conditions were reproduced by the MP ecosystem model [22].

The general objective was to improve the predicting ability of the mussel growth model described in Giordano et al. [30] reproducing the link between mussel biomass and phytoplankton dynamics in an anthropogenically stressed ecosystem.

2. Materials and Methods

2.1. Study Area

The Mar Piccolo in Taranto (40°28′–40°30′ N, 17°13′–17°19′ E) is a semi-enclosed sea located in the northern Ionian Sea (Mediterranean) (Figure 1). Its climate is typically characterised by mild and wet winters, and hot summers with heatwaves becoming very common since 2012 [30]. In July and August of recent years (2012, 2015, 2017, 2019, 2020 and 2021) for a period of about 3–4 weeks, average temperatures of 30.5 ± 1.8 °C (up to 32.7 °C) were detected in MP.

MP is divided into the First (FI) and Second Inlet (SI), which have maximum depths of 13 and 10 m, respectively. It is connected to the larger bay of Mar Grande by the Navigabile and the Porta Napoli channels. Mar Grande opens into the Gulf of Taranto and the Northern Ionian Sea. The scarce hydrodynamism and the low water exchange with Mar Grande make the SI more confined than the FI. Moreover, SI is characterized by fluctuations in nutrient content as well as by eutrophication phenomena in summer, with hypoxia in the lower water layers [22].

The flushing through the two channels is generally weak and varies seasonally in relation to the density difference with the Mar Grande [22]. The low velocity currents (about 5–10 cm s^{-1}) are driven by the sea tides [31]. Two dominant currents at 1 and 6 m depth have been observed [32]. At subsurface (–1 m), the current moves from the SI to the

FI towards the Mar Grande. At 6 m depth, the current moves from Mar Grande through the two channels into the FI and then into the SE [32].

In MP salinity is influenced by the input of freshwater deriving from small tributary rivers (the most important is Galeso, with a mean flow of $50,000 \text{ m}^3 \text{ day}^{-1}$), runoff from the surrounding agricultural soils, and from 34 freshwater springs (locally called “Citri”). The most important are Citro Galeso in the FI ($0.6 \text{ m}^3 \text{ s}^{-1}$) and Citro Le Copre in the SI ($0.1\text{--}1.2 \text{ m}^3 \text{ s}^{-1}$) [31].

The exchange with Mar Grande has been modified in 1985 by the installation of a water-scooping machine ($0.15 \text{ mil m}^3 \text{ d}^{-1}$) to provide cooling water for an iron and steel industry (ex ILVA, actually ArcelorMittal Italia). A more important disturbance has been the presence of the discharge from 14 sewages that, starting from 2000 have been progressively reduced to five (one at the FI and four at the SI), actually all subjected to depuration. Before 2000, these wastes were equivalent to about $18,272 \text{ m}^3 \text{ d}^{-1}$ (of which 85% at the SI), with organic matter equal to 6767 kg d^{-1} of BOD_5 , whereas N_{tot} and P_{tot} were of 17.2 and 0.3 t d^{-1} , respectively [20]. These wastes have actually the capacity of about $3000 \text{ m}^3 \text{ d}^{-1}$ and the levels of nitrogen and phosphorus have been reduced to 8.0 and 0.12 t d^{-1} , respectively [33]. The strong reduction of nutrient input changed the trophic characteristics of MP from being relatively eutrophic before 2001, to moderately oligotrophic after 2001 [34].

An intensive mussel commercial fishery is carried out in MP with an annual production of $40,000 \text{ t y}^{-1}$ [30]. Shellfish farming is actually performed using polyethylene floats (‘long lines’) that since 1990 have almost completely replaced the traditional method, which involved suspending mussels from wooden stakes driven into the sea bottom. Other human activities consist of the moorage for the region’s fishing fleet and the largest Italian naval base. A vast heavy-industrial area comprising the most important steel production plant in Europe, an oil refinery, two thermoelectric power plants and three waste incinerators is located immediately adjacent to the basin.

2.2. Sampling and Environmental Factors

Surface seawater samples were collected monthly with a 5-L Niskin bottle from January 2007 to December 2008 for phytoplankton analyses and from January 2007 to October 2009 for nutrient and chlorophyll *a* determination. Samplings were carried out at two stations located in the FI (1E, $40^\circ 29' 01''$ $17^\circ 14' 46''$) and in the SI (2C, $40^\circ 28' 57''$ $17^\circ 17' 41''$), respectively (Figure 1). Temperature, salinity, dissolved oxygen and pH were measured by using the multiparametric probe Idromar IM5260. Nutrient (N-NH_4 , N-NO_2 , N-NO_3 , P-PO_4 , and Si-Si(OH)_4) concentrations were determined by the spectrophotometric method according to Strickland and Parsons [35]. Unfiltered samples digested by peroxodisulfate in pressure vessels and heated in a microwave oven were used to determine Total Nitrogen (TN) and Total Phosphorus (TP). Final analysis was carried out by spectrophotometry as already described for other nutrients.

Chlorophyll *a* was evaluated by the spectrophotometric method [36]. All these parameters are described in detail by Kralj et al. [34].

Annual abiotic and chlorophyll *a* data from July 2010 to December 2014 were obtained from the WFD 2000/60/EC monitoring program carried out by the Regional Agency for the Environmental Prevention and Protection [37]. Moreover, other data derived from the monitoring activities of the Flagship RITMARE project (<https://maritime-spatial-planning.ec.europa.eu/projects/la-ricerca-italiana-il-mare>, accessed on 18 March 2022) (seasonal, from June 2013 to April 2014) [34] and the ILVA project (monthly, from September to December 2014) were incorporated [38].

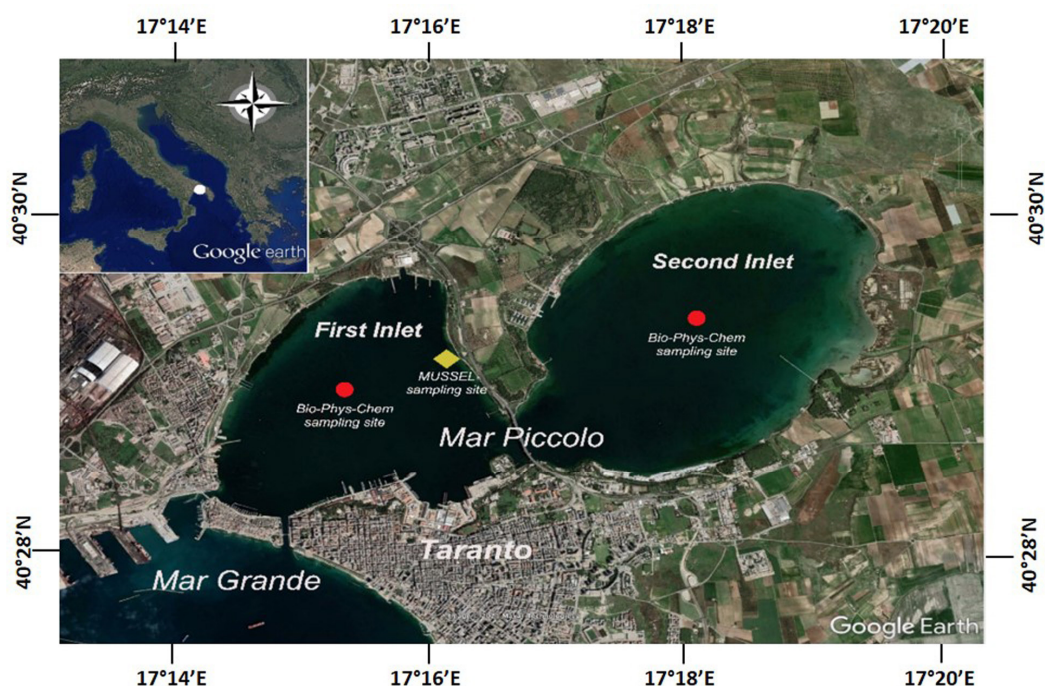


Figure 1. Map of sampling stations in the Mar Piccolo (Taranto, Northern Ionian Sea).

2.3. Phytoplankton Abundances and Biomass

A total of 72 samples (24 for each phytoplankton fraction) were collected during the survey.

Water samples for Pico-Phytoplankton (PPP) were preserved with formaldehyde (2%) and kept at 4 °C until their analyses (within four weeks). Cell counts were performed by using a Zeiss Standard Axioplan epifluorescence microscope equipped with a halogen (Hg 100) lamp. Duplicate slides were prepared from each sample by filtering 10 mL of seawater through 0.2 µm (pore size) Millipore black membranes. A BP 485/20 exciter filter, a FT 510 chromatic beam splitter and a LP 520 barrier filter were used. A minimum of 200 cells were counted for each filter to ensure ±10% confidence levels. Cell number was converted into Carbon biomass using a factor of 250 fg C cell⁻¹ [39].

Water samples for Nano-Phytoplankton (NPP) and Micro-Phytoplankton (MPP) were preserved with an acid Lugol's iodine solution and stored at 4 °C until analyses. Identification and counting were performed by using an inverted microscope (Labovert FS Leitz) equipped with phase contrast and following the Utermöhl method [40]. The minimum value of counted cells has been 200 cells per sample, by accepting a confidence limit of 14% [41]. Biovolume was calculated by applying standard formulae according to Hillebrand et al. [42]. The obtained biovolumes were converted to Carbon content using the conversion factors introduced by Menden-Deuer and Lessard [43]. Details on NPP and MPP are reported in [23].

Seasonal data of phytoplankton abundances and species composition from July 2010 to December 2014 were obtained from the reports of ARPA Puglia [37] and converted into MPP and NPP biomass according to the previous procedure. Moreover, MPP, NPP and PPP biomass derive from the RITMARE project (seasonal, from June 2013 to April 2014) [25].

2.4. Statistical Analyses

The mean data in the text are reported as averaged values \pm standard deviation.

To test for significant differences between and within groups of environmental and biotic data, one-way ANOVA tests were used. Moreover, correlation analysis was applied to evaluate relationships between in situ observations and simulated data.

2.5. The Mar Piccolo Ecological Model

The general features of the MP Ecological Model allow simulating the key ecological relationships and biogeochemical processes affecting mussel growth dynamics and consequently mussel farming in a double-layered semi-enclosed basin. The model features and applicability are explained in Caroppo et al. [22].

The ecosystem dynamics is schematized by five embedded models each for two water-layers, mimicking deterministically physical and bio-chemical variables in the FI and SI of MP [22].

Ecological processes are coupled following a cause-effect principle existing among the principal ecosystem components such as primary producers and filter feeding bivalves (i.e., mussels) and some of the key biogeochemical fluxes representing larger and discrete functions within the modelled system. Particulate organic Carbon (POC) and biomasses accumulation are represented in terms of Carbon budget affecting the local ecosystem productivity.

In order to predict mussel biomass dynamics the MP ecological model includes an individual based model constructed as calculation of the Scope for Growth [44,45] dynamically linked with a Mussels Farm Model (MFM) tailored for MP as formerly explained in Giordano et al. [30].

The MP ecological model blocks were developed using the ExendSim™ simulation tool (<https://extendsim.com>, accessed on 18 March 2022) that allows improving computational box-model very suitable for dynamic processes modelling.

2.6. The Phytoplankton Growth Model

The simulation of phytoplankton as designed in Caroppo et al. [22] was recalibrated in this work using new data available for the period 2007–2014 characterised by a sharp decline in the production of mussels in the MP. The parameters relating to the assimilation coefficients of nutrients, grazing and other biomass losses were simulated using the coefficients in Caroppo et al. [22].

Three box-models simulate the phytoplankton community dynamics that characterize the real MP ecosystem (e.g., each for three major plankton fractions: PPP, NPP and MPP). The basic equations describing basically the growth processes follow a revised formulation of the Villefranche Bay model [46].

The growth calculation at the time-step (t) for each phytoplankton fraction (PP) is parameterized in the model as follows:

$$PP_{(t)} = (PP_{(t-1)} * (Mu + KLight) - (Km + Kr) * PP_{(t-1)} - Kg * PP_{(t-1)}) * dt + PP_{(t-1)} \quad (1)$$

Fundamentally, the two driving variables, such as nutrients uptake (Mu) and light (KLight), are added linearly while the losses include mortality (Km), respiration (Kr), and a grazing parameter (Kg).

Some approximations on light and growth coefficients have been included in (1), aiming to reproduce the real seasonal succession that characterizes the ecosystem as a replication of the changes among groups' maximum nutrients absorption rates (Mu). The Mu parameter includes, for each phytoplankton size fraction, the uptake capacity both on new and regenerated nitrogen whose concentration is locally simulated in the basin by the Ecosystem Model.

Therefore, the total nitrogen uptake (Mu) is reproduced by the Michaelis-Menten kinetics as a simple sum accounting, respectively, for the new and regenerated nitrogen:

$$\text{Mu} = \text{Mu}_{\text{Nnew}} + \text{Mu}_{\text{Nreg}} \quad (2)$$

The reliance between light and growth (KLight) is reproduced considering the light attenuation that happens in the basin top layer, as:

$$\text{KLight} = \text{Ligth}(t) * \text{KI} \quad (3)$$

where the coefficient KI varies in function of the Total Suspended Matter (TSM) concentration mimicked by the ecosystem model at the simulation time (t).

With respect to the original phytoplankton model described in Caroppo et al. [22] some novelties were introduced to replicate the feedback between mussel grazing and phytoplankton dynamics.

In fact, the mortality rate (Km) and respiration loss (Kr) are considered both population dependent and group-specific, while the grazing coefficient (Kg) originally described in Caroppo et al. [22] as a simplified time-delayed predator-prey dynamics has been reformulated.

Furthermore, the grazing coefficient is expressed as a sum of two terms (Kg_m and Kg_o) differentiating the mussel grazing and other grazers action on phytoplankton dynamics. The Kg_m parameter is reliant on the mussel population dynamic simulated by the MFM and reproducing a real-time predator-prey population response. The other grazing parameter (Kg_o) is a constant rate due to other grazers and it is expressed as a function of the population dynamics including a time delay that allows simulating a simplified predator-prey population response.

The phytoplankton biomass growth and losses were simulated for each of three size components (PPP, NPP and MPP) of the MP ecosystem. Group-dependent parameters are shown in Caroppo et al. [22].

Likewise, the top-down control on phytoplankton due to grazing by mussels is simulated by the MFM block [30], while the bottom-up is due to nutrients budget process reproduced by MP ecological model [22].

In order to improve the phytoplankton dynamic predictions and to overcome the limitations imposed by scarce data availability as previously discussed in Caroppo et al. [22], we compared the simulated data with a newly available observational biomass and chlorophyll *a* dataset for the years 2007–2014 directly collected by sampling activities or obtained from literature, and local Authorities. This facilitated the mussel model calibration to improve the simulation of Carbon stocks for the farming scenarios and carrying capacity evaluations. Top-down calculation for mussel Carbon budget for years 2007–2008 and 2013–2014 is presented in Table 1.

The mussel Carbon budget with respect to condition index values served in the phytoplankton model as a term of closure for mussel grazing on phytoplankton biomass.

The condition index is a measure of the quality of mussels and it is calculated by the mussel individual based model [30] as a ratio between the Carbon content of flesh and shell data reference for 2007–2014.

Table 1. Top-down calculation for mussel Carbon budget estimated from annual harvesting data (modified from Caroppo et al. [22]) in the First and Second Inlet (FI and SI) of MP. Mussel physiological data for the years 1990 and 2007–2014 served as proxies to estimate the values of flesh dry weight (B) and the relative percentage of Carbon content values (C). The total Carbon ingested by mussels (E) is expressed as the sum of the Carbon content of flesh dry weight (C) and the quote of Carbon due to mean assimilation efficiency (D, percentages are from Brigolin et al. [47]) together divided by 0.2 representing the trophic efficiency parameter [48,49].

Mussels Carbon Budget for year 2007 (CI = 0.07)			FI (t)	SI (t)	Total (t)
A	Annual Mussel Crop (wet weight)		14,104	28,208	42,312
B	Mussel Flesh (dry weight)	4% of A	564	1128	1692
C	Flesh Carbon content (dry weight)	40% of B	226	451	677
D	Carbon gain due to assimilation efficiency	25% of C	56	113	169
E	Total Carbon ingested by Mussels	(C + D)/0.2	1410	2821	4231
Mussels Carbon Budget for year 2008 (CI = 0.11)			FI (t)	SI (t)	Total (t)
A	Annual Mussel Crop (wet weight)		11,520	23,040	34,560
B	Mussel Flesh (dry weight)	7% of A	806	1613	2419
C	Flesh Carbon content (dry weight)	40% of B	323	645	968
D	Carbon gain due to assimilation efficiency	25% of C	81	161	242
E	Total Carbon ingested by Mussels	(C + D)/0.2	2016	4032	6048
Mussels Carbon Budget for year 2013 (CI = 0.12)			FI (t)	SI (t)	Total (t)
A	Annual Mussel Crop (wet weight)		1329	2658	3987
B	Mussel Flesh (dry weight)	7% of A	93	186	279
C	Flesh Carbon content (dry weight)	40% of B	37	74	112
D	Carbon gain due to assimilation efficiency	25% of C	9	19	28
E	Total Carbon ingested by Mussels	(C + D)/0.2	233	465	698
Mussels Carbon Budget for year 2014 (CI = 0.09)			FI (t)	SI (t)	Total (t)
A	Annual Mussel Crop (wet weight)		1198	3594	5390
B	Mussel Flesh (dry weight)	4% of A	60	180	296
C	Flesh Carbon content (dry weight)	40% of B	24	72	119
D	Carbon gain due to assimilation efficiency	25% of C	6	18	30
E	Total Carbon ingested by Mussels	(C + D)/0.2	150	449	741

3. Results

3.1. Environmental Data

The physico-chemical and biotic characteristics of the two stations during the period 2007–2014 are reported in Table S1. Temperature, salinity, pH and percentages of oxygen saturation showed comparable values at the two stations, without any significant differences (ANOVA, $p > 0.05$). In addition, nutrient concentrations showed significant differences in the two Inlets. Particularly, ammonia ($p < 0.001$), nitrites ($p < 0.05$), silicates ($p < 0.05$), TN ($p < 10^{-4}$) and TP ($p < 0.001$) were significantly more abundant in the SI than in the FI.

Further, chlorophyll *a* concentrations ranged between 0.45 to 12.02 $\mu\text{g L}^{-1}$ with values slightly higher in the SI ($1.58 \pm 1.01 \mu\text{g L}^{-1}$) than in the FI ($2.21 \pm 2.07 \mu\text{g L}^{-1}$) (Figure 2), even if not significantly different ($p > 0.05$).

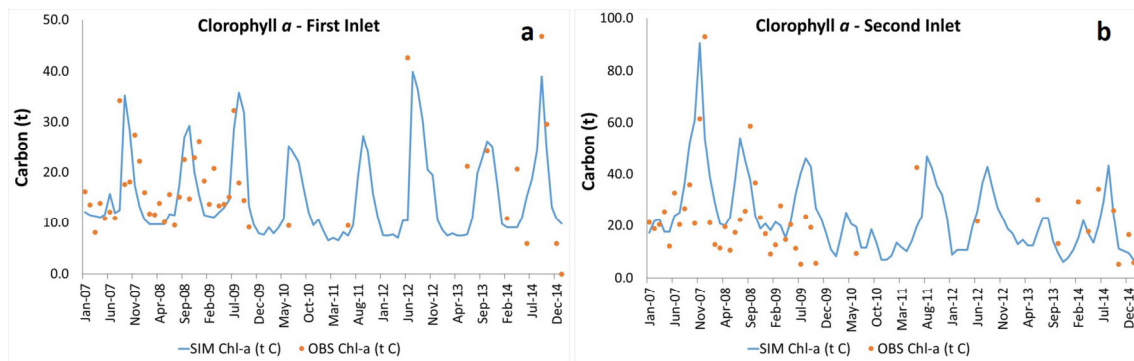


Figure 2. Chlorophyll *a* concentrations (t of Carbon) simulated (line) and field data (dots) in the First Inlet (a) and in the Second Inlet (b) of Mar Piccolo.

3.2. Phytoplankton Abundance and Biomass

Abundance and biomass values of PPP, NPP and MPP fractions at each station in the whole sampling period are reported in Table S1.

Total phytoplankton abundances displayed values ranging between 1.9×10^6 and 6.0×10^8 cells L^{-1} . In particular, values of each fraction showed the following ranges: 1.58×10^6 – 5.81×10^8 cells L^{-1} (PPP), 3.25×10^4 – 1.2×10^7 cells L^{-1} (NPP), 14.6×10^3 – 5.8×10^6 cells L^{-1} (MPP). PPP represented the most abundant fraction accounting on average for 95.2% of the total abundances. Despite their wide variability, values did not show significant differences in all the dimensional components between the two Inlets ($p > 0.05$).

Total phytoplankton biomass (TPB) values ranged between 5.5 and 562.8 $\mu g C L^{-1}$, with average values of 49.5 ± 30.5 and $94.3 \pm 141.5 \mu g C L^{-1}$ in the FI and SI, respectively. The seasonal trend of phytoplankton biomass was characterized by marked temporal variations (as evidenced by the high standard deviation values) and peaks were detected throughout the year. Higher values were usually observed in the summer and autumn months in both the Inlets.

Statistical analyses did not show significant differences in terms of total phytoplankton biomass between the two Inlets (ANOVA, $p = 0.13$), even if slightly higher values were monitored in the SI (Figure 3). In addition, the biomass values of PPP, NPP and MPP were not significantly different ($p > 0.05$).

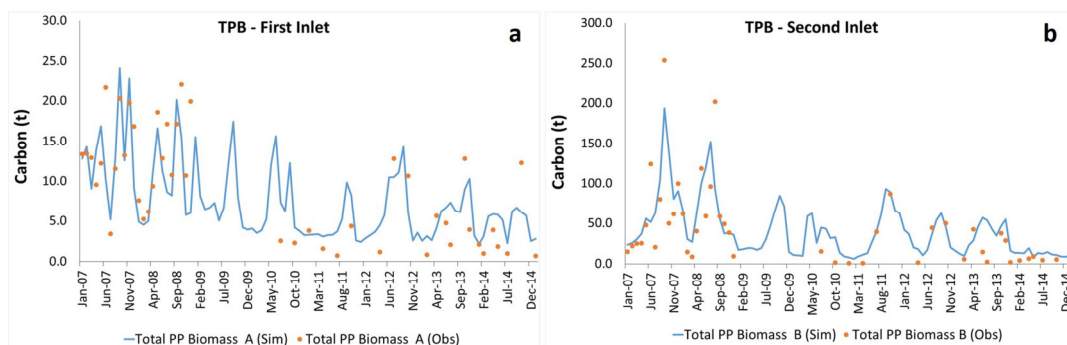


Figure 3. Total phytoplankton biomass (TPB) (t of Carbon) simulated (line) and field data (dots) in the First Inlet (a) and in the Second Inlet (b) of Mar Piccolo.

MPP was the most important component of the community (on average up to 46.5% of TPB) throughout the year and, mainly in the SI, they gave rise to blooms due to diatoms (up to $442.3 \mu g C L^{-1}$ in July 2008) (Figure 4).

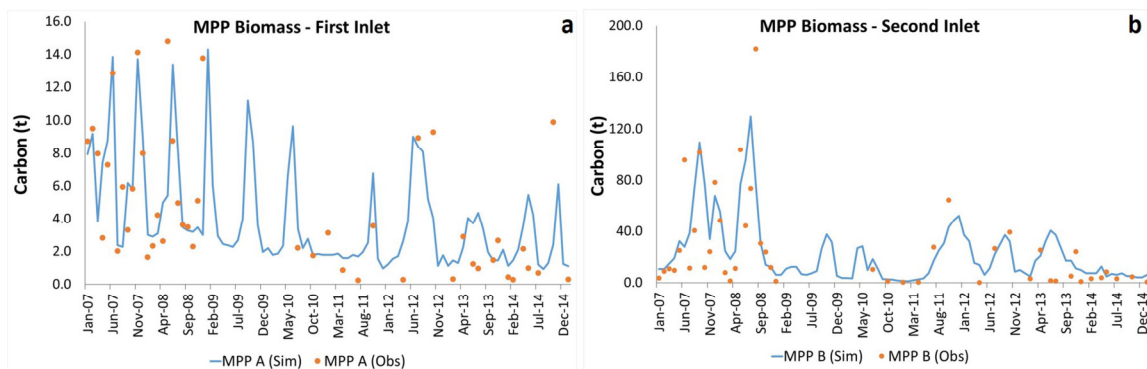


Figure 4. Micro-Phytoplankton (MPP) biomass (t of Carbon) simulated (line) and field data (dots) in the First Inlet (a) and in the Second Inlet (b) of Mar Piccolo.

NPP biomass (on average up 31.7% of TPB) was characterized by slight temporal variations and a peak due to cryptophyceans was detected in December 2007 (SI, 306.7 $\mu\text{g C L}^{-1}$) (Figure 5).

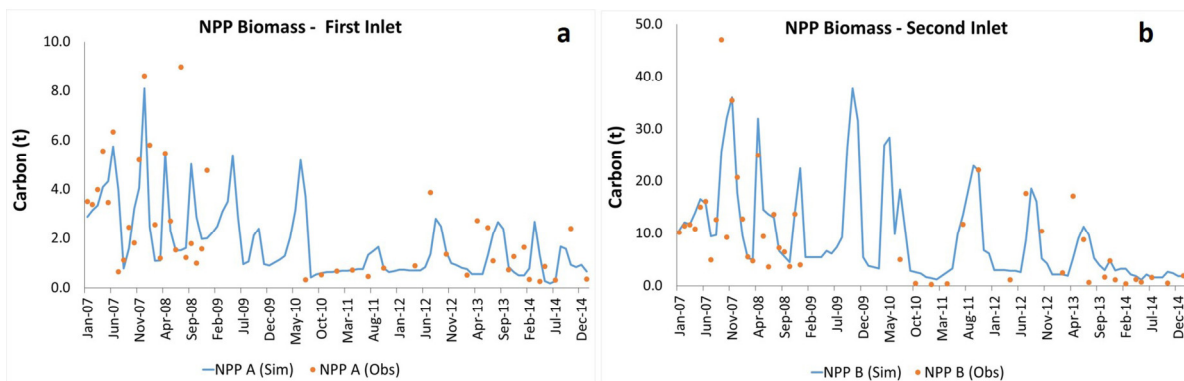


Figure 5. Nano-Phytoplankton (NPP) biomass (t of Carbon) simulated (line) and field data (dots) in the First Inlet (a) and in the Second Inlet (b) of Mar Piccolo.

PPP biomass was usually negligible (on average up 21.8% of TPB), except in summer when they reached the maximum value of 92.5 $\mu\text{g C L}^{-1}$, representing up to 47.5% of TPB (September 2007, SI) (Figure 6).

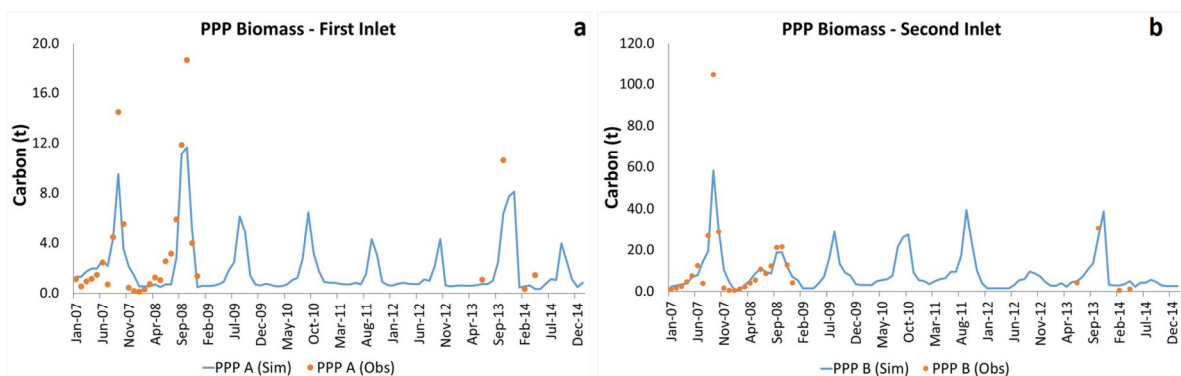


Figure 6. Pico-Phytoplankton (PPP) biomass (t of Carbon) simulated (line) and field data (dots) in the First Inlet (a) and in the Second Inlet (b) of Mar Piccolo.

To summarize these results, in Figure 7 are represented the ratio trends of the three phytoplankton size-fractions with respect to the total biomass.

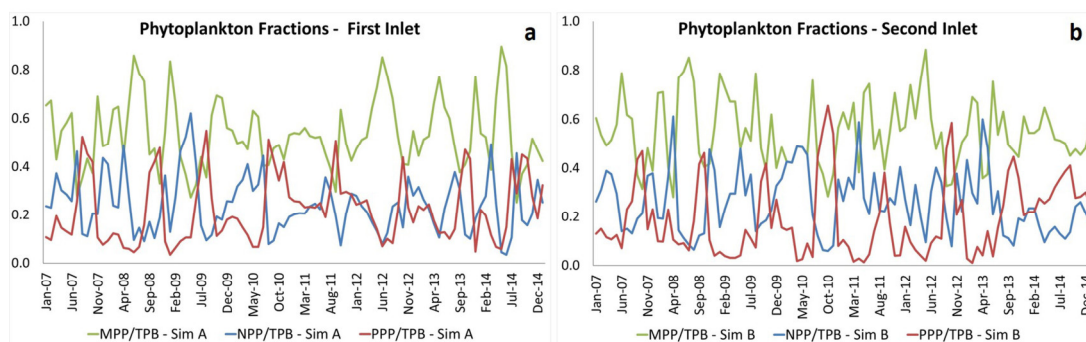


Figure 7. Phytoplankton fractions: Micro-Phytoplankton/Total Phytoplankton Biomass (MPP/TPB), Nano-Phytoplankton/Total Phytoplankton Biomass (NPP/TPB) and Pico-Phytoplankton/Total Phytoplankton Biomass (PPP/TPB) ratios in the First Inlet (a) and in the Second Inlet (b) of Mar Piccolo.

The results related to the simulation of the dynamics of the TPB (Figure 3) and the phytoplankton fractions are compared with in situ observations in Figures 4–6. The model shows a good capacity of predicting the phytoplankton dynamics in MP and the model and in situ observations are in good agreement ($R^2 = 0.95$ for MPP and PPP, $R^2 = 0.96$ for NPP and $R^2 = 0.85$ for TPB).

Simulation outputs of phytoplankton biomass (TPB, PPP, NPP, and MPP) evidenced a total annual overestimation of 8% (2007) and of 11% (2008) for the expected phytoplankton biomass in MP. Particularly, in 2007 a comparison between observed and simulated evidence showed an overestimation of about 7% for the FI and of about 9% for the SI. In 2008, the average biomass overestimation was comparable with the 2007 value, but it was lower for the FI (6%) and higher for the SI (12%). Since no phytoplankton biomass data were available for 2009, no data comparison was possible. Moreover, the simulation data in this period comply with the expected dynamics for the MP ecosystem. In fact, in 2010–2014 there is again a good overlap with the observed data.

Between 2010–2012 simulation outputs for TPB, NPP and MPP showed a good accordance with field data. Particularly, in 2013 output data showed an overestimation of TPB in the FI (12%) and in the SI (15%), while in 2014 the TPB is underestimated in the FI (11%) and overestimated in the SI (9%) this led to a global TPB overestimation of 2%. In the 2009–2012 period, simulation data are compatible with the growth dynamics of PPP that presents a typical biomass peak in late summer. Moreover, PPP simulated biomass from 2013–2014 showed a good concordance with observed data as happened for 2008–2009.

3.3. Mussels Grazing, Biomass and Condition Index

Figure 8 shows the simulated mussel grazing on the phytoplankton fractions in the FI and SI of Mar Piccolo. An increasing trend of values was observed from January to August–September periods, and then grazing was reduced from October to April to increase again the following months. This trend is common in the two Inlets, and slight higher grazing values were detected in the SI in comparison to the FI.

The most important phytoplankton fraction grazed by mussels was MPP while the contribution of the other two fractions was negligible.

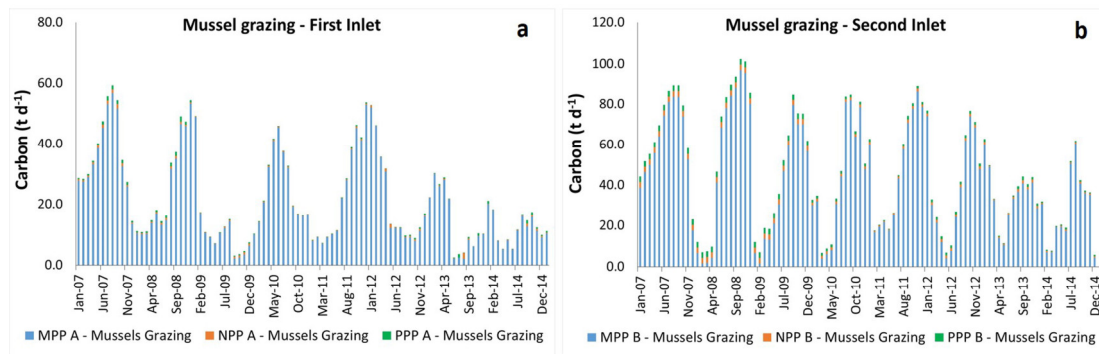


Figure 8. Simulation of mussel grazing on phytoplankton fractions (MPP, NPP and PPP) in terms of Carbon ($t d^{-1}$). (a)FI and (b) SI.

The simulation of mussel biomass dynamics due to mussel farming in MP is presented in Figure 9 together with the mussel condition index prediction and in situ data (see Table 1). The mussel biomass showed typically higher values in the period April–September when mussels reached their commercial size. Higher values were in the FI than in the SI, due to different seeding stock fractions equal to 1/3 in the FI and 2/3 SI.

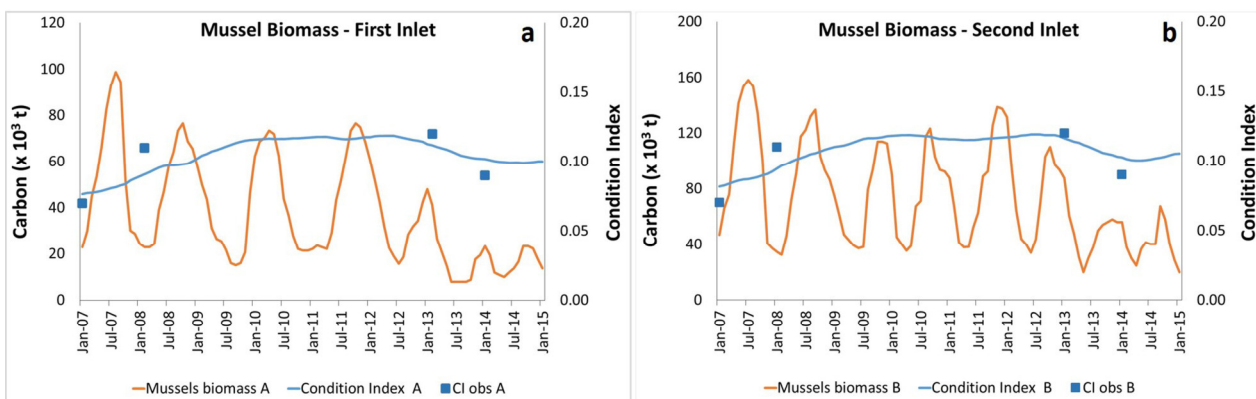


Figure 9. Simulation of the mussel biomass (wet weight of 10^3 t of Carbon), Condition Index prediction and in situ data in the FI (a) and SI (b) of Mar Piccolo.

The condition index simulation showed a rather stable trend from 2007 to April–May 2008, and then increased reaching its maximum value in September (0.12 and 0.10 in the SI), more pronounced in the SI than in the FI. In both Inlets, the CI showed a slight positive trend between 2010–2011, then stabilization between 2011–2012 and finally a negative trend that starts in 2013.

4. Discussion

Aiming to improve the simulation of the general dynamics of phytoplankton, mussel growth and farming cycle in MP, this work represents a refinement of the phytoplankton growth model previously developed for the Mar Piccolo case study [22].

Moreover, recent years upgrades included advanced simulation tools to better understand the complex mosaic of ecosystem functioning by providing additional information to support mussel farming [30].

Since phytoplankton modelling is the result of coupling physiological, physical and ecosystem processes, calibration through field data allows to overcome the limits imposed by functional relationship parametrization.

The MP ecosystem model [22] served to simulate boundary environmental conditions and bio-geochemical forcing factors for phytoplankton growth.

MP is an ecosystem subjected to wide temporal and spatial variations in hydro-chemical characteristics. In the 1990s, the presence of many urban sewers conditioned the quality of the waters and, over the years, eutrophication and summer blooms negatively affected the shellfish farming activities [20]. Between 2000 and 2005, the closure of the main sewerage outfalls improved the environmental quality of the ecosystem. Nutrient concentrations decreased and water transparency increased leading to the recovery of some marine plants, considered indicators of good environmental status [50]. However, in summer 2012 heatwaves worsened again the water quality and caused the widespread death of benthic invertebrates, mussels included [30]. Since then, the frequency of phenomenon is intensified and shellfish farming is going through another period of deep crisis [30].

As concerning phytoplankton, in situ observations evidenced a general decrease of the phytoplankton cell-size throughout the years. It is well known that the reduction of phytoplankton dimensions represents the universal ecological response to global climate warming [51,52]. Moreover, our results support the hypothesis that cell-size is influenced not only by temperature but also by nutrients [53]. In fact, previous our data indicated that the increase of temperature, the closure of the most urban sewage outfalls (2000–2005) and the following inorganic nutrient decrease [34] affected phytoplankton composition since nano-sized phytoflagellates (indicators of oligotrophic conditions) became dominant over the micro-sized diatoms [23]. In our study, the discrimination of phytoplankton biomass into different size fractions outlined the C partitioning towards the small-sized groups (PPP and NPP) typical of eutrophic environments where large phytoplankton (MPP) is favoured. In fact, PPP and NPP represented the most abundant available food source for filter feeders. PPP biomass values were similar to those previously observed out of the Mar Piccolo (Mar Grande, Ionian Sea) [54] and in other Mediterranean coastal and transitional systems [55]. In addition, NNP showed an increase of values throughout the years as opposed to the MPP biomass that instead decreased.

In addition to temperature raise and nutrient loading reduction, intensive mussel farming could also affect phytoplankton standing crop and cell-size composition. According to some nutrient budgeted models [56,57], nutrient release from shellfish cultures increased by 2.5- to 3-fold within a period of six years. However, in contrast with the theoretical models, the plant overloading of mussels seems to represent a control of eutrophication phenomena [22,30].

The updated version of the MP phytoplankton model showed a good performance after its calibration with in situ observations. In fact, this model is capable to reproduce the dynamics as well as the inter-annual variations of the phytoplankton size fractions.

From a global comparison between the simulated data, it emerged a major overestimation difference between the FI and the SI in 2013–2014 with respect to 2007–2008, probably due to a calculation delay, which prevents the model from responding immediately after a peak. Moreover, the calibration of the phytoplankton sub-model provided us with a better quantitative understanding of primary production in a stabilized period (i.e., no significant anthropogenic disturbance except for mussel farming) and raised information on mussel farming. In the previous phytoplankton sub-model of the FI of MP [22] calibrated with data collected in a meso-eutrophic condition, the feedback with nutrient regeneration seemed to be one of the most relevant aspects for phytoplankton groups (diatoms, dinoflagellates and phytoflagellates) succession. The updated version of sub-model allowed us to represent the importance of mussel grazing.

In winter, phytoplankton growth is favoured because grazing is low and new nitrogen is added from fall-winter runoff. During summer, the phytoplankton biomass minimum appears to be primarily caused by grazing, because mussels reached their commercial size and feed intensively on MMP, but also by low rates of phosphates [34].

The extension of simulations from 2007 to 2014 revealed the overall model capability in order to predict the phytoplankton and mussels' dynamics also during a not-typical climatic condition (i.e., 2012 heatwave). In order to recognize the model prediction

capability, simulated data and field data comparison were analysed in two close-up periods exploiting the top-down calculations for the mussel Carbon budget (see Table 1).

Furthermore, data analysis on mussel grazing simulation outputs suggested that the mussel grazing for the close-up period 2007–2008 is appreciably underestimated if related to mussel Carbon budget calculated from field data. Comparing the mussel Carbon budget estimation with the total simulated mussel grazing emerges that the calculated mean underestimation (2007–2008) for the MP is equal to 7%. A significant difference emerged from the estimated and simulated data comparison between the FI (3%) and the SI (9%).

The data analysis suggested that especially for 2013 the mussel grazing presents a mean underestimation of 10% if compared to mussel Carbon budget calculated from field data, conversely for 2014 the model predicts a mean mussel grazing overestimation equal to 6%. Particularly, in 2013 while the TPB prediction is significantly overestimated in the FI (12%) and in the SI (15%), the model recognizes a mussels' grazing underestimation both in the FI (8%) and in the SI (12%).

This data discrepancy is probably induced by changing environmental conditions (i.e., temperature increase, oxygen depletion) that affect much more mussels grazing efficiency than nutrient assimilation by phytoplankton.

In 2014, the model significantly underestimates both mussel grazing and phytoplankton biomass in the FI (respectively, 8% and 11%). Conversely, a significant overestimation was recorded for the SI (11%) and this is equally revealed by phytoplankton biomass prediction that was overestimated.

Globally, the results showed that at low mussel biomass, the average phytoplankton biomass displayed a positive response to increased nutrient loading (i.e., bottom-up control). Since mussel farming in MP can be considered intensive during some period of the year, the expected top-down control by bivalve grazing implies that bottom-up control of phytoplankton development due to nutrient dynamics becomes less important.

This is comparable with the hypothesis that in periods characterized by high mussel biomass, phytoplankton biomass was low and independent of external nutrient loading (i.e., top-down control) [58].

In conclusion, the simulation of phytoplankton dynamics presented in this work deals with the bottom-up control of phytoplankton biomass on mussels' growth and quality to tackle in the future with different environmental, socio-economic and political scenarios. Furthermore, this reflects the needs of mussel farming management as an ecosystem service considering phytoplankton biomass prediction as a key tool to improve ecosystem productivity. This newly calibrated phytoplankton model, in terms of utility, is very important in order to produce a performing instrument such as a real-time modelling. The simulation of the succession of the phytoplankton size-fractions provides a useful indicator of a system response to anthropogenic pressures; a calibration parameter for the size-fractions growth; an indicator of trophic changes; and management of the mussel production. This model will undoubtedly influence our approach to ecological issues to perform better monitoring programs for the future and to export this methodology in other geographical contexts.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/jmse10040462/s1. Table S1. Minimum, maximum, average values and standard deviations of the main water and biotic parameters measured in the two Inlets of Mar Piccolo during the period 2007–2014.

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