Anthropogenic impact on nitrification dynamics in coastal waters of the Mediterranean Sea

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Abstract

The anthropogenic alteration of the nitrogen cycle results in the modification of the whole food web. And yet, the impact caused on nitrogen dynamics in marine systems is still very uncertain. We propose a workflow to evaluate changes to coastal nitrification by modelling nitrite dynamics, the intermediary compound. Nitrite concentrations were estimated with a simple steady state nitrification model, which was calibrated in 9 NW Mediterranean coastal sites with different anthropogenic pressures, located within 250km. The results obtained indicate that nitrite peaks are observed in winter and explained by nitrification response to temperature, but these dynamics are altered in impacted coastal waters. We found the second step of nitrification to be more sensitive to temperature, which entails a significant impact of climate change on the decoupling of the two steps of nitrification. The results could be extrapolated to numerous coastal regions of the Mediterranean Sea with similar characteristics.

Keywords

Nitrification dynamics, Coastal waters, Mediterranean Sea, Anthropogenic impact, Nitrogen cycle, Inorganic Nitrogen Speciation

1. Introduction

Rockström et al. (2009) established the alteration of the nitrogen (N) cycle as one of the three planetary processes, together with climate change and biodiversity loss, sufficiently
altered by human activity as to potentially have disastrous consequences for humans. Some authors are concerned about nitrogen being “the next carbon” (Battye et al., 2017). Due to their vulnerability to anthropogenically driven change, coastal zones have been highly impacted (Arhonditsis et al., 2000; De Vittor et al., 2016; Smith et al., 2014). Population growth and related nutrient sources such as agriculture, wastewater, urban runoff, and fossil fuels have increased nutrient inputs to coastal waters to many times their natural levels (Bricker et al., 2008). As such, many researchers have evidenced the impact that human activities have caused to food webs or biogeochemical processes in many coastal systems (Borja et al., 2004; Lundberg et al., 2005; Wang et al., 1999). In the Mediterranean Sea, direct wastewater discharges account for a large amount of the total nitrogen input (Powley et al., 2016; Stamou and Kamizoulis, 2008). Nearshore coastal waters (0-200 m) are particularly vulnerable and need a special attention, as the nutrient gradient from land to ocean is considerably large in the Mediterranean Sea (Flo et al., 2011).

Nitrification plays a crucial role in marine primary production (Yool et al., 2007) and in the N cycle of coastal zones (Damashek et al., 2016; McLaughlin et al., 2017). This process alone does not change the total amount of nitrogen in an ecosystem, but it affects its speciation and fate: nitrate (the product of nitrification) serves as substrate for denitrification, which removes N from the system via N₂ gas (Carini et al., 2010). Nitrification is generally described as a two-step process occurring under aerobic conditions: oxidation of ammonium to nitrite and oxidation of nitrite to nitrate (Kim, 2016). It links reduced and oxidized forms of nitrogen. Although ammonium oxidation is considered the limiting step, both steps are expected to be tightly coupled. However, evidence of decoupling in coastal waters was observed especially at high temperatures (Beman et al., 2013; Heiss and Fulweiler, 2016), which leads to the accumulation of nitrite. As an intermediary compound in many key biological processes, nitrite dynamics have historically been used as an indicator of the balance between oxidative and reductive pathways in marine systems (Lomas and Lipschultz, 2006). Nitrite production processes in aerobic waters include the oxidation of ammonia and assimilatory nitrate reduction by phytoplankton and heterotrophic bacteria, while removal pathways for nitrite include oxidation by nitrite-oxidizing bacteria and phytoplankton uptake (Schaefer and Hollibaugh, 2017).

Increasing evidence indicates that many environmental factors such as pH, temperature or oxygen concentration affect nitrification processes (Damashek et al., 2016; Schaefer and Hollibaugh, 2017). However, when it comes to how humans alter inorganic nitrogen transformations in marine environments research is still very scarce. Kim (2016) summarized
how climate change will alter marine N cycle and indicated the need for further research on
marine inorganic N transformations, while McLaughlin et al. (2017) and Bartl et al. (2018)
studied the alteration of nitrification caused by wastewater discharges and pointed out the
need for further research on anthropogenic nutrient effect on coastal biogeochemistry. Ocean
acidification results in reduced nitrification rates (Beman et al., 2010; Huesemann et al., 2002;
Kitidis et al., 2011) while other anthropogenic pressures such as N deposition (Kim, 2016) or
wastewater effluents might increase nitrification (McLaughlin et al., 2017). Clearly, the overall
anthropogenic effect on nitrification in coastal systems needs to be evaluated further. In the
Mediterranean Sea, the urbanization on the littoral zone has severely impacted the natural
balance of ecosystems (Lejeusne et al., 2010). Nitrification dynamics are altered in those areas
with high anthropogenic pressure, leading to a change in nitrogen cycling along the year
(Ordines et al., 2015). The modification of such an important process in the N cycle may have
consequences on phytoplankton abundance and diversity, with cascading effects on other
organisms. The importance of nitrification in the N biogeochemistry in coastal waters has
already been proved by many authors (Damashek et al., 2016; Heiss and Fulweiler, 2016;
Huesemann et al., 2002), but the mechanisms by which anthropogenic activity alters
nitrification in coastal waters need to be studied in more detail.

The aim of this study was to propose a simple workflow for the evaluation of nitrification
alteration in coastal waters due to anthropogenic activity. Nitrite, as the intermediary
compound in the two steps of nitrification, was used to study nitrification. We modelled nitrite
dynamics in several coastal sites located within approximately 250 km of coast. All modelled
water bodies have similar characteristics but different anthropogenic pressures, so that
nitrification parameters could be related to anthropogenic pressure. This methodology is
applicable to other coastal areas of the Mediterranean Sea, where nitrification is the main
driver of nitrite dynamics (Bianchi et al., 1994).

2. Materials and methods

2.1. Study area and analytical methods

The Jucar River Basin District (JRBD) lies in the Mediterranean coast of Spain, covering
42,735 km² with 574 km of coastline. The management plan defines 16 natural coastal water
bodies. In this study we focused on 9 of them, which belong to the typology II-A (moderately
influenced by freshwater inputs with salinity between 34.5 and 37.5 g Kg⁻¹). These water
bodies (presented in Figure 1) have similar geomorphology, littoral transport, dominant winds,
rainfall, area of fluvial basins, continental inputs and wet zones. C002 is the reference site for
typology II-A with no relevant anthropogenic influence, as determined by Romero et al. (2013) and Pachés et al. (2012) who evaluated pressures and impacts according to annex V of the Water Framework Directive (WFD). Some coastal waters in the JRBD are considered under the WFD as heavily modified due to the presence of a harbor (Figure 1); these water bodies were not included in our study.

Figure 1. Jucar River Basin District: natural coastal water bodies of typology II-A, heavily modified coastal water bodies (“which as a result of physical alterations by human activity is substantially changes in character” (European Commission, 2000)) and monitoring sites.

Hermosilla Gómez (2009) studied the influence of the sampling locations in the evaluation of the anthropogenic pressures and the ecological status of the coasts of Valencia. By means of statistical analysis, she determined that sampling sites should be located inshore (over the coast) and at the surface so that samples are taken from the area affected by anthropogenic eutrophication. Besides, the data and methods developed in Spain for the intercalibration exercise of the Mediterranean intercalibration group (MedGIG) within the WFD are most based on inshore sampling stations (MedGIG, 2009). Thus, in order to compare the results obtained in this study with previous ecological evaluations in the water bodies considered,
inshore sampling was more convenient. 46 monitoring sites were distributed all along the coast, with 4 to 7 stations in each water body. Each month from August 2008 until January 2011, water samples were taken from beyond the wave breakpoint at a 10 cm depth. Temperature was measured in situ with a multiparametric probe YSI (6600 V2).

Water samples were collected in plastic bottles, refrigerated, and carried to the laboratory within 12 hours. A Portasal 8410A salinometer was calibrated to determine salinity (I.A.P.S.O. Standard Seawater, Ocean Scientific International Ltd., K15 = 0.99986, S = 34.995‰). Samples were divided into several sets following the conservation procedures suggested by APHA (2005) and filtered through 0.45 µm cellulose acetate membrane filters (Millipore HAWP). These membranes are stored at -20 °C in order to break the cells for chlorophyll-a analysis.

For the determination of chlorophyll-a, the trichromatic method was used, based on visible spectroscopy (APHA, 2005). The filters are introduced in 6 ml of 90% acetone in water with 1% calcium carbonate. The optical density of the extract was determined at different wavelengths (630, 647 and 664 nm) to determine the pigment content, and at 750 nm to determine the optical density not due to chlorophyll-a. The equations proposed by Jeffrey and Humphrey (1975) were used for concentration calculations. The detection limit was 0.2 mgC.m-3 of chlorophyll-a.

Nutrients (ammonium, nitrite and nitrate) were analyzed with an Alliance Instruments Integral Futura air-segmented continuous-flow autoanalyzer, following the procedure described by Treguer and Le Corre (1975) and taking into account the remarks made by Kirkwood et al. (1991) and Parsons et al. (1984). The equipment optimization is carried out following Coakley (1981) theories. Ammonium and nitrite were analyzed with the filtered samples, right after filtration, while the samples kept for nitrate determination were frozen for a later analysis. Ammonium was measured based on Berthelot’s reaction. Under alkaline conditions, ammonium reacts with the hypochlorite forming a monochloramine. This compound, in the presence of phenol and an excess of hypochlorite, forms indophenol blue. The nitroprusside ion catalyzes the reaction and trisodium citrate eliminates the interference of Ca and Mg (Solórzano, 1969). Nitrite concentrations were determined with Shinn (1941) water analysis method, adapted for seawater by Bendschneider and Robinson (1952). This method is based on the reaction of nitrite ion with sulfanilamide in acidic conditions, producing a diazo compound that forms a pink complex with N-naphthylethylenediamine. For the determination of nitrate concentrations, this compound is reduced to nitrite by means of a Cu/Cd reducing column in basic conditions (pH = 8.5), following the method described by Grasshoff, (1976). Subsequently, nitrite is analyzed by the procedure described above. High
purity Merck reagents for analysis and ultra-pure water (Milli-Q 185) were used. The detection limits were 1.4x10^-3 mgN.L^-1 for ammonium and nitrate and 1.4x10^-4 mgN.L^-1 for nitrite.

2.2. Workflow

The proposed workflow is schematized in Figure 2 and explained in detail in next sections.

2.2.1. Model formulation

The equation solved is based on the principle of the conservation of mass. The main processes driving nitrite dynamics were studied from the literature and used for the development of the model, neglecting the less relevant processes. We applied a mass balance in zero dimensions in each water body which was considered as a control volume. As samples
were taken at the surface, we considered appropriate to neglect the effect of the sediment. Simple and well-known principles can be used to build simple models which can give an overall understanding of nitrogen processes. When a basic understanding of the system is aimed and the requirement to the precision of estimated values is low, simple models perform better (Højberg et al., 2007). Complex models require large amounts of data and such a model would not add value to our research purpose.

Nitrite production pathways in coastal waters include ammonia oxidation and assimilatory nitrate reduction by phytoplankton and heterotrophic bacteria, while removal pathways for nitrite include nitrite oxidation and phytoplankton uptake (Schaefer and Hollibaugh, 2017). Ammonium is the preferred form of nitrogen for phytoplankton uptake (Chau and Jin, 1998; Zouiten et al., 2013), so we considered direct nitrite uptake to be negligible. Additionally, the study area is characterized by low phytoplankton concentrations in natural conditions (Pachés et al., 2012). Thus, nitrite release by phytoplankton was also neglected. An analysis of the relationship between the model error and phytoplankton concentrations was carried out to confirm this assumption (see section 2.2.2). Bianchi et al. (1994) also determined that nitrite concentrations in the NW Mediterranean Sea are regulated by the two steps of nitrification. Therefore, we considered only nitrification processes to estimate nitrite: formed with ammonium oxidation (nitrification first step) and eliminated by nitrite oxidation (nitrification second step). Studies carried out in other coastal areas also pointed out the significant role of nitrification (Damashek et al., 2016; Schaefer and Hollibaugh, 2017).

At low nitrogen concentrations as those found in natural systems, the process of nitrification is generally represented by a first order kinetic reaction (Bowie et al., 1985). Previous studies typically considered only the temperature effect on nitrification. Some authors also introduced the effect of dissolved oxygen as a limiting factor (Chau and Jin, 1998; Umgiesser et al., 2003; Zouiten et al., 2013). Nonetheless, the samples used in this study, which were taken at the sea surface, had all high oxygen concentrations (averaged 8.3 ± 1.4 mg.O.L⁻¹ measured during campaigns). Consequently, we did not add oxygen limitation to our model. pH, which also affects nitrification rates (Park et al., 2007), was measured during the campaigns and variations were not relevant (averaged 8.14 ± 0.13).

Flo et al. (2011) showed that continental influence is the main driver of nutrient variability within 200m of coast in the NW Mediterranean Sea. The water bodies considered in this study have a length of >13km along the coast, and samples were taken at less than 50m from the coastline. As such, the continental influence on nutrient concentrations is much larger than the
effect of the dispersion along the coast. Additionally, as the two steps of nitrification are tightly
coupled (Schaefer and Hollibaugh, 2017), nitrite is oxidized to nitrate almost as fast as it is
created (see Table 1), whereas longitudinal mixing along >13 km is expected to be a much
slower process (Stamou and Kamizoulis, 2008). Therefore, we decided not to consider
dispersion with adjacent water bodies to simplify our model.

The main nitrogen sources to the JRBD coastal waters are agriculture and urban population
(Romero et al., 2013), which means that most of the nitrogen inputs are in the form of
ammonium or nitrate. Hence, we considered no relevant direct nitrite inputs. An analysis of
the relationship between the model error and salinity was carried out to confirm this
assumption (see section 2.2.2).

Equation 1 represents nitrite mass balance under these assumptions.

$$\frac{\partial [NO_2^-]}{\partial t} = k_1 \theta_1 T^{-20} [NH_4^+] - k_2 \theta_2 T^{-20} [NO_2^-]$$

Equation 1

Where, \([NO_2^-]\) is nitrite concentration (mg N L\(^{-1}\)), \([NH_4^+]\) is ammonium concentration (mg N L\(^{-1}\)), \(t\) is time (day), \(k_1\) is ammonium oxidation rate at 20°C (day\(^{-1}\)), \(\theta_1\) is temperature coefficient
for ammonium oxidation, \(k_2\) is nitrite oxidation rate at 20°C (day\(^{-1}\)), \(\theta_2\) is temperature
coefficient for nitrite oxidation, \(T\) is temperature (°C).

The steady state approach is very frequently used in water quality modelling (Chapra, 1997;
Wang et al., 2013). This approach enables the calculation of the nitrite concentration each
month, if the conditions found at the time of the sampling were maintained. Under this
assumption the accumulation term was set to zero. Nitrite concentrations can be estimated
with the following equation, derived from Equation 1:

$$[NO_2^-] = \left( \frac{k_1}{k_2} \right) \theta_1 \theta_2 T^{-20} [NH_4^+]$$

Equation 2

\(K_1\) and \(K_2\) depend mathematically on each other (see \(K_1\) and \(K_2\) depend mathematically on each
other (see Equation 2), making the separate calibration of both parameters unfeasible. The
same applied to \(\theta_1\) and \(\theta_2\). We defined \(K\) and \(\Theta\) as new parameters equivalent to the ratios
\(k_1/k_2\) and \(\theta_1/\theta_2\) respectively:

$$[NO_2^-] = K \cdot \theta T^{-20} [NH_4^+]$$

Equation 3

Thus, \(K\) represents the ratio of ammonium oxidation to nitrite oxidation, while \(\Theta\) represents
the ratio of ammonium to nitrite oxidation sensitivity to temperature.
Output sensitivity to input variables can be estimates with Spearman rank correlation coefficient in nonlinear but monotonic relationships (Pianosi et al., 2016). As such, the Spearman correlation coefficient was calculated between selected input (temperature and ammonium) and output variables to determine the relative importance in nitrite estimation. The calculation of this correlation confirms whether the selected input variables are relevant for the estimation of nitrite concentrations, or else the simplified model needs to be re-evaluated.

### 2.2.2. Model calibration and validation

We carried out a literature review to set parameter limits. The values found are presented in Table 1.

#### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>0.05 - 0.5</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$K_2$</td>
<td>0.5 - 10</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$\Theta_1$</td>
<td>1.02 - 1.12</td>
<td>-</td>
</tr>
<tr>
<td>$\Theta_2$</td>
<td>1.02 - 1.12</td>
<td>-</td>
</tr>
</tbody>
</table>

References: (Bowie et al., 1985; Chau and Jin, 1998; Myszograj, 2015; Zouiten et al., 2013)

Mean monthly values for all variables were calculated for each water body from August 2008 to January 2011. The dataset was divided in two sub-datasets; one was used as calibration data and the second as validation data. Odd monthly observations (1,3,4,...,29) of input and output variables were used for calibration whereas even observations (2,4,6,...,30) were left for validation (see Figure 2). The parameters $K$ and $\Theta$ were optimized to reproduce nitrite observed concentrations by minimizing the rooted mean squared error (RMSE) in calibration data. Then, the model was run with the validation dataset and nitrite estimations were compared to observations. The RMSE and the coefficient of determination ($R^2$) were calculated to estimate the goodness of fit.

Two of the neglected processes during the model formulation may be the main source of error to our model: phytoplankton release and continental inputs. To evaluate the source of error in the model, we calculated Spearman correlation between monthly error and phytoplankton and between monthly error and salinity in each water body.
Once the model was validated, the relationship between model parameter differences among water bodies and two physicochemical variables was evaluated to determine the source of spatial changes in nitrification dynamics. pH and dissolved oxygen were very similar in all water bodies as mentioned above and consequently not included in this evaluation. The two physical variables analyzed were temperature and salinity. The Spearman correlation coefficient between these two variables and calibrated model parameters was calculated to determine whether they may influence the studied nitrification parameter values.

Phytoplankton biomass is established as an indicator of the ecological status of coastal waters under the WFD. In the JRBD, Pachés et al. (2012) identified chlorophyll-a 50th percentile to be the most appropriate statistical parameter to measure anthropogenic pressure, and Romero et al. (2013) related phytoplankton to anthropogenic pressures such as population density, agriculture and industry. As such, we used chlorophyll-a 50th percentile as an indicator of the alteration provoked by human pressures. We calculated the Spearman correlation coefficient between model parameters and chlorophyll-a 50th percentile to determine whether anthropogenic pressures may have altered nitrification parameters.

3. Results

3.1. Variable values

Ammonium, nitrite and nitrate concentrations in each water body are presented in Figure 3, as well as temperature, salinity and chlorophyll-a. The highest N concentrations were found in C007, the water body located north of Valencia city. Chlorophyll-a was also high in C007; the most polluted site of the JRBD (Pachés et al., 2012; Temino-Boes et al., 2018). C002, the reference water body, had low N concentrations, together with C001 and C003. The lowest salinities were found in C005 and C007, with some low salinity events in C009. The latest water body corresponds to the discharge of the Jucar river. Temperature is slightly lower in sites C001 to C003.
Figure 3. Boxplot of ammonium, nitrite and nitrate concentrations of all water bodies from August 2008 to January 2011. Each data point corresponds to the mean concentration of all monitoring sites of a water body for a given time. Some outliers were also observed in C007 in N concentrations which do not appear in the figure due to the limits of the y-axis.

3.2. Model results

Spearman correlation between model forcings (water temperature and ammonium) and output variable (nitrite concentration) was calculated to determine which variable had the highest influence on nitrite concentrations in each water body. We found a significant rank correlation between nitrite and temperature in all water bodies except C005 and C007 (Table 2) which correspond to the sites with highest continental influence (see salinity in Figure 3). On the other hand, ammonium was significantly correlated to nitrite in all water bodies except C002 to C004. These sites correspond to the lowest observed inorganic nitrogen concentrations and chlorophyll-a (Figure 3).

Table 2

<table>
<thead>
<tr>
<th>Water Body</th>
<th>T (°C)</th>
<th>NH4 (mgN.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C001</td>
<td>-0.38*</td>
<td>0.49*</td>
</tr>
<tr>
<td>C002</td>
<td>-0.67*</td>
<td>0.28</td>
</tr>
<tr>
<td>C003</td>
<td>-0.55*</td>
<td>0.30</td>
</tr>
<tr>
<td>C004</td>
<td>-0.61*</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>Θ</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>C005</td>
<td>-0.36</td>
<td>0.56*</td>
</tr>
<tr>
<td>C007</td>
<td>0.08</td>
<td>0.79*</td>
</tr>
<tr>
<td>C008</td>
<td>-0.60*</td>
<td>0.85*</td>
</tr>
<tr>
<td>C009</td>
<td>-0.44*</td>
<td>0.57*</td>
</tr>
<tr>
<td>C010</td>
<td>-0.37*</td>
<td>0.52*</td>
</tr>
</tbody>
</table>

* Significant correlations at the 95% confidence level (p-value < 0.05)

We calibrated and validated the model for each water body, and the obtained results are presented in Figure 4. The parameters K and Θ obtained for each water body are shown in Table 3. We calculated the RMSE and R² in validation data (Table 3). Ammonium oxidation rate is often estimated as an order of magnitude lower than nitrite oxidation for surface water quality modelling as shown in Table 1. This observation agrees with our findings which established a K mean value of 0.17 (Table 3).

In most water bodies Θ was below 1, which indicates that nitrite oxidation is more sensitive to temperature changes than ammonium. Under this circumstance, nitrite peaks are observed in low temperature periods (December and January). The sites with the lowest anthropogenic pressures (C002 and C003) show clear nitrite peaks in the mentioned period. Only two water bodies (C007 and C008) presented a Θ higher than 1.
Figure 4. Mean nitrite measured (red line) and estimated (blue line) concentrations in all water bodies from August 2008 to January 2011.
Table 3
Calibrated parameters (K and θ) for each water body, root mean squared error (RMSE) and coefficient of determination (R²) between measured and estimated nitrite concentration in validation data

<table>
<thead>
<tr>
<th>Water body</th>
<th>K</th>
<th>θ</th>
<th>RMSE (mgN.L⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>C001</td>
<td>0.15</td>
<td>0.94</td>
<td>1.33E-03</td>
<td>0.55*</td>
</tr>
<tr>
<td>C002</td>
<td>0.08</td>
<td>0.80</td>
<td>7.10E-04</td>
<td>0.84*</td>
</tr>
<tr>
<td>C003</td>
<td>0.13</td>
<td>0.91</td>
<td>6.75E-04</td>
<td>0.71*</td>
</tr>
<tr>
<td>C004</td>
<td>0.20</td>
<td>0.98</td>
<td>1.49E-03</td>
<td>0.95*</td>
</tr>
<tr>
<td>C005</td>
<td>0.33</td>
<td>0.92</td>
<td>5.97E-03</td>
<td>0.70*</td>
</tr>
<tr>
<td>C007</td>
<td>0.09</td>
<td>1.27</td>
<td>2.46E-02</td>
<td>0.74*</td>
</tr>
<tr>
<td>C008</td>
<td>0.11</td>
<td>1.10</td>
<td>1.30E-02</td>
<td>0.72*</td>
</tr>
<tr>
<td>C009</td>
<td>0.25</td>
<td>0.97</td>
<td>8.37E-03</td>
<td>0.46*</td>
</tr>
<tr>
<td>C010</td>
<td>0.19</td>
<td>0.97</td>
<td>2.61E-03</td>
<td>0.31*</td>
</tr>
</tbody>
</table>

* Significant correlations at the 95% confidence level (p-value < 0.05)

No significant correlation was found in any water body between monthly error and chlorophyll-a (as a measure of phytoplankton), while the correlation with salinity was significant in water bodies C005 and C010 (Table 4). When salinity was low, the model error was higher due to continental nitrite inputs which were not considered in the model.

Table 4
Spearman rank correlation between error with chlorophyll-a (Chl-a) and salinity (S)

<table>
<thead>
<tr>
<th>Water Body</th>
<th>S</th>
<th>Chl-a</th>
</tr>
</thead>
<tbody>
<tr>
<td>C001</td>
<td>-0.27</td>
<td>-0.06</td>
</tr>
<tr>
<td>C002</td>
<td>-0.26</td>
<td>-0.11</td>
</tr>
<tr>
<td>C003</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>C004</td>
<td>0.34</td>
<td>0.29</td>
</tr>
<tr>
<td>C005</td>
<td>0.52*</td>
<td>-0.16</td>
</tr>
<tr>
<td>C007</td>
<td>0.34</td>
<td>0.26</td>
</tr>
<tr>
<td>C008</td>
<td>0.03</td>
<td>-0.09</td>
</tr>
<tr>
<td>C009</td>
<td>0.48*</td>
<td>-0.22</td>
</tr>
<tr>
<td>C010</td>
<td>0.33</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

* Significant correlations at the 95% confidence level (p-value < 0.05)

3.3. Analysis of spatial parameter variations
Spearman rank correlations between salinity, temperature and chlorophyll-a with model parameters and RMSE are calculated in Table 5. Both Θ and the RMSE have a high rank correlation with chlorophyll-a 50th percentile, an indicator of the ecological status of coastal waters. Additionally, salinity is related to the RMSE.

**Table 5**
Spearman Rank Correlation of model parameters and root mean squared error (RMSE) with mean salinity, mean temperature and chlorophyll-a 50th percentile.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salinity (g.kg(^{-1}))</th>
<th>Temperature (°C)</th>
<th>P50 Chlorophyll-a</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>-0.41</td>
<td>0.45</td>
<td>0.02</td>
</tr>
<tr>
<td>Θ</td>
<td>-0.42</td>
<td>0.50</td>
<td>0.80*</td>
</tr>
<tr>
<td>RMSE</td>
<td>-0.73*</td>
<td>0.42</td>
<td>0.97*</td>
</tr>
</tbody>
</table>

Note: Significant correlations at the 95% confidence level (p-value < 0.05) are marked with an asterisk.

Figure 5 shows how the parameter Θ, which represents the difference in temperature influence between ammonium and nitrite oxidation, is influenced by anthropogenic activity. Closer to big urban areas, like the city of Valencia in C007, Θ increases, while it decreases in C002 or C003 where the population is smaller. On the contrary, K does not have an anthropogenic influence (*Table 5*).
Figure 5. Model parameter Θ for each water body, 50th percentile of chlorophyll-a, population and Heavily modified water bodies (HMWB) due to the presence of a harbour.

4. Discussion

The natural annual cycle of nitrite showed peaks in cold months (December and January), as found in the reference water body C002 (Figure 4). However, this cycle was highly perturbed in anthropogenically altered coastal zones, with peaks occurring both in cold and warm seasons driven by ammonium concentrations. Our results demonstrate that the two steps of nitrification are decoupled in coastal waters, agreeing with late findings (Heiss and Fulweiler, 2016; Schaefer and Hollibaugh, 2017). Different speeds in the two steps of nitrification were also observed in the Jucar estuary (Romero et al., 2007). In our model, peaks occur due to the different response to temperature in ammonium and nitrite oxidation (parameter Θ). Many previous studies found high nitrite concentrations at warmer seasons (Bristow et al., 2015;
Heiss and Fulweiler, 2016; Schaefer and Hollibaugh, 2017) while others (such as this study) observed nitrite peaks at low temperatures (Pitcher et al., 2011).

The changes observed in nitrite dynamics among water bodies indicate a shift in nitrification temperature dependence parameter \( \Theta \) due to anthropogenic activity (Figure 5). Nitrification requires the mediation of a vast diversity of microorganisms, which makes it an essential process for marine life. Pachés et al. (2012) proved how anthropogenic activity is changing microorganism composition in coastal waters of the JRBD, which may explain the spatial differences found in ammonium and nitrite oxidation temperature parameters. Although no previous study considered this dependence in coastal waters, studies carried out in wastewater determined the dependence of nitrification temperature coefficient on microorganism composition and abundance (Myszograj, 2015). In addition, wastewater effluents alter the biogeochemical cycling and phytoplankton composition (Howard et al., 2017) which may have caused shifts in nitrification temperature dependence. Future research is required to describe the processes driving nitrification dependency on temperature. Previous studies identified a dependence of nitrification rates on salinity (Bernhard et al., 2007; Heiss and Fulweiler, 2016), and low salinity events with elevated concentrations of certain ammonium oxidizing archaea (Schaefer and Hollibaugh, 2017). Bernhard et al. (2005) indicated that the abundance and diversity of ammonium oxidizing bacteria is highly controlled by salinity, and Heiss and Fulweiler (2016) found lower nitrite oxidation rates with higher salinity events. Bianchi et al. (1999) linked high ammonium oxidizing rates with low salinity events in the NW Mediterranean Sea.

We found temperature to be an important driver of ammonium and nitrite oxidation decoupling under natural conditions, which entails climate change could have a great impact in this process. In the reference water body C002, which represents unaltered nutrient concentrations, the value of \( \Theta \) was lower than 1. This result indicates that under pristine conditions the second step of nitrification (nitrite oxidation) is more sensitive to temperature than the first step. Climate change will therefore have a greater impact in this second step. In addition, some studies forecast an important precipitation loss in the Jucar area (Chirivella et al., 2016; Miró et al., 2018) which would considerably reduce the riverine inputs of ammonium and other forms of nitrogen. The decrease in ammonium concentrations would have a direct effect on the rate of nitrification. Therefore, it may be expected to have lower nitrite concentrations due to reduced nitrification rates and a shift in nitrite peaks due to higher temperatures. Further research is needed to evaluate how the combined effect of nitrogen pollution and climate change will modify the nitrification process in coastal waters.
The model performed well in general. However, there was a wide variation among sites in the accuracy of the model. The coefficient of determination $R^2$ is lower in C001, C009 and C010 (Table 3). As the number of stressors increases, the functioning of the ecosystem is altered, and the estimation of nutrient concentrations is hindered (O’Meara et al., 2017). High nitrite events no longer occur due to natural conditions but rather to an unusual increase in human inputs. Continental inputs are the main source of error to sites C005 and C009 as shown by the Spearman correlations between error and salinity (see Table 4). Those water bodies have higher continental influence as indicated by the low salinities. The Jucar river discharges in C009, which is most probably the source of nitrite during the peaks not reproduced by the model. High nitrite concentrations in C010 also correspond to low salinity event indicating an external source of nitrite not simulated in our model. Although nitrite concentrations in C001 did not have a significant correlation with salinity, this water body is located close to the Ebro delta, which may influence nitrification dynamics in this water body. On the other hand, phytoplankton does not have a significant correlation with the error (Table 4) which indicates that neglecting phytoplankton uptake and release is not an important source of error to the model.

The proposed workflow is applicable to other coastal areas with samples taken at the surface to avoid the effect of dissolved oxygen limitation. pH variations along the year in the Mediterranean Sea are usually less than ± 0.1 pH units (Flecha et al., 2015), and therefore pH is not expected to be one of the main drivers of nitrification. The effect of phytoplankton and external inputs are the most important processes to be considered before applying the model to other coastal areas. In the Mediterranean Sea, marine waters are often oligotrophic (Vollenweider et al., 1996) and phytoplankton release of nitrite is presumably negligible in most areas. The nitrification kinetic model proposed could be extended to add other processes in the application to other study sites. However, the steps proposed in the workflow of Figure 2 can be followed for the study of any areas if the model is adapted. Future studies are needed to evaluate the mechanisms by which nitrification dynamics are altered by human activity and which are its most relevant consequences.

5. Conclusions

Our results show how nitrification dynamics are perturbed in highly populated coastal zones. Under natural conditions nitrite peaks are observed in winter due to low temperatures, but this tendency is completely altered in anthropogenically impacted water bodies. The change observed in the sensitivity to temperature of the two steps of nitrification was highly
correlated to chlorophyll-a 50th percentile, a measure of the ecological status (Spearman correlation r=0.80). Temperature was the main driver of monthly variation in natural conditions, which indicates a potential effect of climate change on nitrification dynamics. Nitrification is a fundamental process of nitrogen biogeochemistry. As a key nutrient, the alteration of the nitrogen cycle may result in the change of the whole food web in marine ecosystems. Further research concerning the human driven changes of the nitrogen cycle in marine environments is essential to enable experts to propose recovery measures and avoid reaching a point of no return.

Acknowledgements

Field data collection was supported by the Regional Ministry of the Environment, Water, Urban Planning and Housing. The work was partly supported by Erasmus Mundus - MAYANET Grant Agreement Number 2014-0872 / 001 - 001, funded with support from the European Commission.

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