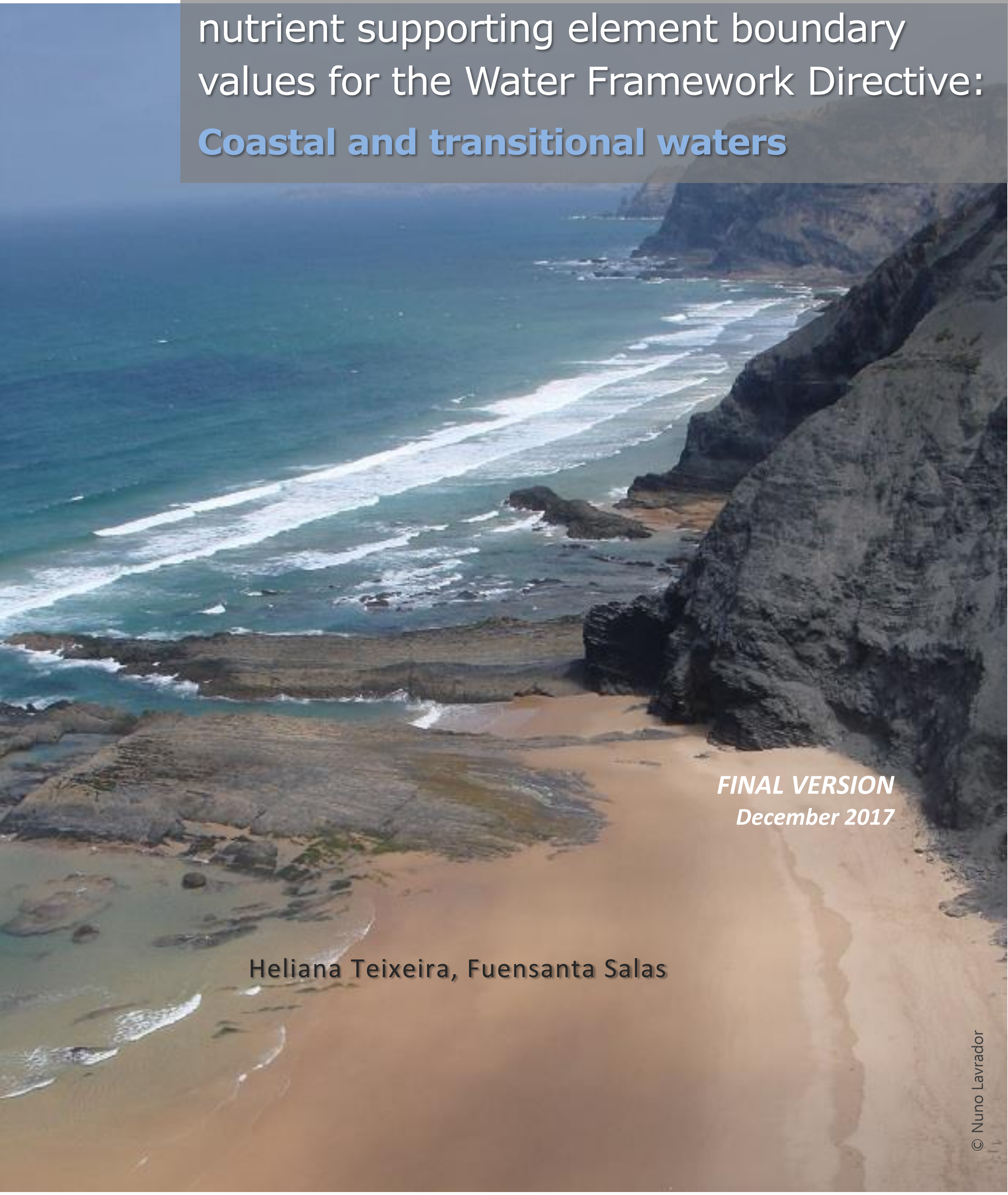
# The use of pressure response relationships between nutrients and biological quality elements as a method for establishing nutrient supporting element boundary values for the Water Framework Directive:

## Coastal and transitional waters

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# Content

# List of Figures

***Cover image*** Portuguese southwest coast, year 2010 © Nuno Lavrador

# List of Tables

## *List of abbreviations*

| | |
|---|---|
| ECOSTAT | Working Group on Ecological Status |
| WFD | Water Framework Directive |
| MSFD | Marine Strategy Framework Directive |
| CTRW | Coastal and Transitional waters |
| CW | Coastal waters |
| TRW | Transitional waters |
| FW | Freshwaters |
| BPG | Best Practice Guide |
| MS | Member States |
| BQE | Biological Quality Element |
| IC | Intercalibration |
| RSC | Regional Sea Convention |
| G/M | Good/Moderate |
| H/G | High/Good |
| TN | Total nitrogen |
| TP | Total phosphorus |
| DIN | Dissolved inorganic nitrogen |

## *MS abbreviations*

BE: Belgium
BG: Bulgaria
CY: Cyprus
DE: Germany
DK: Denmark
EE: Estonia
GR: Greece
SP: Spain
FR: France
FI: Finland
HR: Croatia
IE: Ireland
IT: Italy
LT: Lithuania
LV: Latvia
MT: Malta
NL: Netherlands
NO: Norway
PL: Poland
PT: Portugal
RO: Romania
SE: Sweden
SL: Slovenia
UK: United Kingdom

# 1. Summary

Intercalibrated data from the Water Framework Directive (WFD) for the biological quality element phytoplankton was used for deriving nutrient boundaries in coastal and transitional waters (CTRW). Overall the statistical approaches currently proposed in the Best Practice Guide, hereafter Guidance or BPG (Phillips *et al.* 2017), and accompanying toolkit revealed adequate for coastal lagoons (in TRW), but are not always robust to allow deriving nutrient boundaries in other transitional waters such as estuaries or for all CW types.

The datasets available for this report, that were analysed, provided good examples of the most common problems that may be encountered when following this approach in these water categories. Similar issues have been found in freshwater (FW) environments (Phillips *et al.* 2016), and the solutions presented in that report and proposed in the Best Practice Guide (BPG; Phillips *et al.* 2017) allow addressing partially the situations encountered also in CTRW.

Below we summarize a few of the most common issues encountered within the CTRW dataset available, which could impede establishing nutrient boundaries from the BQE Phytoplankton intercalibration (IC) data.

***Low number of observations*** In many CTRW the MS have adopted different EQR methods that have been intercalibrated and for which specific EQR boundaries have been established. This is very common within common types, and often within the same country. This leads to having several small subsets of data with EQRs that are not in the same scale and cannot be directly used in the statistical analyses. The low n per se is already compromising the representativeness and robustness of the results, and the type of analyses that can be performed. But this often leads to another problem, which is the ***insufficient coverage of the gradient of disturbance*** of interest.

Proposals on how to increase the n of the datasets available for analyses should be discussed, with recommendations on how that could be achieved without compromising the quality of the data and of the analyses. Recommendations could include, for example, when, how and which EQRs could be normalised, which type of factors (e.g. categorical factors or other continuous variables) should be added when merging datasets (factors that could help explain the variability once the data is pulled together), and which statistical analyses are more adequate.

***Interaction between explanatory variables*** There was often evidence of interaction between nutrient variables, in which cases the univariate linear regressions methods performed rather poorly. Bivariate regression models that could account for such relationships would provide better results for defining nutrient boundaries. The use of other available nutrient parameters (other than TN, TP and DIN tested at this stage) should also be tested. For example, some MS referred to and tested the N:P ratio in their own datasets with satisfactory results.

***Scattered and wedge shape data*** The datasets do not always contain all the variables needed to model what is actually controlling the EQR response to nutrients in the ecosystem. In many datasets the data are over-dispersed and do not allow for the use of the most simple linear regression approaches. There were particular cases where the presence of confounding factors (e.g. other pressures acting in the ecosystem) would compromise the use of such methods. Quantile regression, which is mentioned as an alternative for modelling BQE response to nutrient pressure in some river types, could also be a useful approach for testing in some of these CTRW datasets.

In this report we present already some preliminary nutrient boundaries in a few common types, whose results compared considerably well with the range of national of nutrient boundaries proposed by MS. Where data was available for comparison, in several cases the national boundaries were within the BPG proposed range or very near the proposed values. Finally, some of the issues mentioned require that further analyses are implemented, i.e. alternative approaches to those tested and currently included in the BPG toolkit (see BPG by Phillips *et al.* 2017). Results obtained with alternative approaches should be compared to understand whether differences and

improvements obtained justify the added effort of gathering extra information for the existing datasets, and implementing more complex statistical analyses.

# 2. Introduction

This work reports the pressure-response relationships found between nutrients and the biological quality element (BQE) phytoplankton in transitional and coastal waters. The phytoplankton was selected because it is deemed more responsive to the type of pressure under study, i.e. nutrients. Nevertheless other BQEs may also provide relevant insight for establishing nutrients standards in CTRW.

In this report we provide the context for the work developed (section 2.1), then describe the type of data available, the protocol followed for data exploration, and the statistical analyses tested (Chapter 3). Similarly to what was done for rivers and lakes in freshwaters (FW; Phillips *et al.*, 2016), the last sections of this report present and discuss the predicted nutrient concentrations (Chapter 4) to support good ecological status, resultant from the application of the regression and categorical analyses in transitional (Chapter 5) and coastal (Chapter 6) waters.

## 2.1. Background

The Working Group on Ecological Status (ECOSTAT), as part of the Common Implementation Strategy for the WFD and MSFD, agreed to address the topic of wide variations in the concentration of boundaries set by the MS. Inappropriate nutrient standards may hamper the ability of MS to establish adequate nutrient reduction targets where necessary and to achieve good ecological status, therefore it is important to set nutrient standards adequately supporting good ecological status.

The work presented in this report is part of the work proposed by the steering group for 2016-2017 focusing on the production of best practice guidelines for setting, checking and adjusting nutrient boundaries. The main goal of this report was to test the applicability, in CTRW, of the statistical approaches being proposed in the best practice guide (BPG) on nutrient standards for the Water Framework Directive (Phillips *et al.*, 2017); in support of the elaboration and further development of that guidance accounting for specificities of these water categories.

A previous report by Dworak *et al.* 2016 compared nutrient boundaries for transitional, coastal and marine waters across European MS. Results from questionnaires reported by Member States in relation to nutrient boundaries set for the WFD and the Marine Strategy Framework Directive (MSFD) revealed a huge variability in nutrient concentrations boundaries, but also in other relevant aspects such as the nutrient parameters and metrics used, the time of year assessed, the reference conditions established. It also revealed that often MS boundaries do not follow their Regional Sea Convention (RSC) nutrient standards.

# 3. Data analysis

## 3.1. Datasets available

The datasets available for this exercise represent common intercalibration (IC) types[1] across Geographic Intercalibration Groups (GIG) in the Water Framework Directive (WFD) (Table 1). For the Baltic, this report includes data for the single TRW common type defined and also for two of the nine CW common types in this GIG. For the Mediterranean, there is data for five of the six coastal waters common types defined for phytoplankton, and also for one TRW common type. For the North East Atlantic, five of 11 coastal water types (or subtypes) relevant for phytoplankton are considered in this report; as well as the estuarine single broad common type defined. For the

[1] The full list of common IC types across GIG can be accessed in Decision 2013/480/EU.

Black Sea, no data was available for this report, although MS in this GIG tested the BPG toolkit in their CW national datasets.

Data from 17 Member States (MS) was compiled (Table 2), which included essentially nutrient parameters and ecological quality ratio (EQR) values for the biological quality element (BQE) Phytoplankton. In most cases the parameter indicative of biomass (i.e. Chlorophyll *a*) was intercalibrated and not the full national classification system (Table 2; Decision 2013/480/EU). In a few cases, datasets reported also the raw Chl*a* data and supporting environmental data such as: turbidity, flushing regime, tidal range, distance to shore, salinity, pH, and dissolved oxygen.

The nutrient parameters available varied across water categories, i.e. coastal (CW) and transitional (TRW) waters, and also across regional GIG, but they were common within IC types. The parameters most commonly reported for nutrients were: dissolved inorganic Nitrogen (DIN), total Nitrogen (TN), and total Phosphorus (TP). Ammonia (NH4), Nitrates (NO3), Nitrites (NO2), soluble Phosphorus in the form of Orthophosphates (PO4), and Silica (Si) were reported only for common types in coastal Mediterranean waters. Nutrient data reported was taken either from specific seasons of the year, i.e. Summer (Su) or Winter (Wi), but in some cases all year round data was considered (Table 2). In a few cases, for the Mediterranean, the season information has not been reported.

To keep the examples comparable, in this report we have focused the analysis in the phytoplankton response to TN, TP and DIN parameters, since these are the most commonly applied across GIG and common types in both water categories, i.e. coastal and transitional (Table 2). For the analyses we kept the units used by the MS, in order to facilitate the comparison with their own established nutrient boundaries. However, for comparisons of boundaries across larger regions, a conversion of the nutrient concentrations into a common units scale was made as indicated in Table 2.

From the 27 datasets available for the present work, only 25 had initially associated EQR intercalibrated boundaries that allowed their use for deriving nutrient boundaries (Table 3). For the common types TW NEA11 and CW NEA3-4 the results of the WFD phytoplankton IC were only available at a later stage for this report. Where such information was available in due time, the data was analysed for selected EQR vs. nutrient combinations observed within common types (Table 4).

Table 1. Common intercalibration (IC) types defined within European coastal and transitional waters corresponding to the dataset used in the present report, See Decision 2013/480/EU for the complete list of common IC types.

| Common IC Type | Typology description | Countries sharing common type | Countries with data in this report |
|---|---|---|---|
| ***Transitional waters*** | | | |
| **BALTIC** | | | |
| BT1 | Very sheltered (coastal lagoons), oligohaline (0-8) | Lithuania (LT) and Poland (PL) | LT; PL |
| **MEDITERRANEAN** | | | |
| MEDPolyCL | Polyhaline coastal lagoons | | IT; FR; GR |
| **NORTH EAST ATLANTIC** | | | |
| NEA11 | Estuaries (very broadly defined type) | Belgium (BE), Germany (DE), Spain (SP), France (FR), Ireland (IE), Netherlands (NL), Portugal (PT), United Kingdom (UK) | NL; UK; IE; FR; SP; PT |
| **BLACK SEA** | | | |
| -- | *No common IC types defined for TRW in the Black Sea* | | |
| ***Coastal Waters*** | | | |
| **BALTIC** | | | |
| BC4 | Sheltered, lower mesohaline (5-8), <90 ice days | Estonia(EE) and Latvia (LV) | LV; EE |
| BC5 | Exposed, lower mesohaline (6-12), <90 ice days | Latvia (LV), Lithuania (LT) and Poland (PL) | LV; LT |
| **MEDITERRANEAN** | | | |
| MED I | Highly influenced by freshwater input | France (FR), Italy (IT) | IT |
| MEDIIA Adriatic | Moderately influenced by freshwater input (continent influence) | Italy (IT), Slovenia (SL) | IT Adriatic |
| MED IIA | | France (FR), Spain (SP), Italy (IT) | IT Tyrrhenian |
| MED IIIW | Continental coast, not influenced by freshwater input (Western Basin) | France (FR), Spain (SP), Italy (IT) | IT |
| MED IIIE | Not influenced by freshwater input (Eastern Basin) | Greece (GR), Cyprus (CY) | GR; CY |
| **NORTH EAST ATLANTIC** | | | |
| NEA 1-26A | Open oceanic, exposed or sheltered, euhaline, shallow | Spain (SP), France (FR), Ireland (IE), Norway (NO), United Kingdom (UK) | NO; FR; IE; SP; UK |
| NEA 1-26B | Enclosed seas, exposed or sheltered, euhaline, shallow | Belgium (BE), France (FR), Netherlands (NL), United Kingdom (UK) | BE; FR; NL; UK |
| NEA 1-26C | Enclosed seas, enclosed or sheltered, partly stratified | Germany (DE), Denmark (DK) | DE; DK |
| NEA 1-26E | Areas of upwelling, exposed or sheltered, euhaline, shallow | Portugal (PT), Spain (SP) | PT; SP |
| NEA 3-4 | Polyhaline, exposed or moderately exposed (Wadden Sea type) | Germany (DE), Netherlands (NL) | DE; NL |
| **BLACK SEA** | | | |
| BL1 | Coastal waters, mesohaline, microtidal (< 1 m), shallow (< 30 m), moderately exposed, mixed substratum | Bulgaria (BG), Romania (RO) | *No data available in this report; but RO tested toolkit* |

Table 2. Summary of datasets available for the current exercise for coastal (CW) and transitional (TRW) waters. Details provided regarding: existence of supporting environmental data, sampling period considered (season of the year: winter Wi, summer Su, spring Sp, autumn Au), parameters used (units used for comparison), EQR method used and existence of intercalibrated (IC) boundaries per country, and number of observations* (n) available per dataset.

| **Common Type** | **Water Category** | **Supporting Env data** | **Season** | **Nutrient parameters** (*units*) | | | | | | | | **Chl*a*** | **EQR** | **IC boundaries** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dataset *(MS original nutrient units)* | | *Yes/No; parameter* | | **DIN** | **NH4** | **NO3** | **NO2** | **TN** | **TP** | **OrtoP** | **Si** | | | Yes/No; Countries (n obs*) |
| **TRWBALBT1** *(mg L$^{-1}$)* | TRW | No | Su | | | | | µg L$^{-1}$ | µg L$^{-1}$ | | | µg L$^{-1}$ | EQR_Chla | Yes: LT (27); PL (29) |
| **TRWMEDpolyCL*a*** *(µg L$^{-1}$)* | TRW | No | -- | µg L$^{-1}$ | | | | µg L$^{-1}$ | µg L$^{-1}$ | | | | EQR (MPI) | Yes IT/GR (17) |
| **TRWMEDpolyCL*b*** *(µg L$^{-1}$)* | TRW | No | -- | µg L$^{-1}$ | | | | µg L$^{-1}$ | µg L$^{-1}$ | | | | EQR_Phyto | Yes: FR (15) |
| **TRWNEA11** *(µM)* | TRW | No | Wi | µmol L$^{-1}$ | | | | | | | | | EQR_Chla | Yes: 6 MS (232) |
| **CWBALBC4** *(µmol L$^{-1}$)* | CW | No | Su | | | | | µmol L$^{-1}$ | µmol L$^{-1}$ | | | µg L$^{-1}$ | EQR_Chla | Yes: LV (92); EE (44) |
| **CWBALBC5** *(mg L$^{-1}$)* | CW | No | Su | | | | | µmol L$^{-1}$ | µmol L$^{-1}$ | | | µg L$^{-1}$ | EQR_Chla | Yes: LT (65); LV (104) |
| **CWMEDI** *(µmol L$^{-1}$)* | CW | Y shore;T;Sal;pH;Turb;O2D | All | | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µg L$^{-1}$ | EQR_Chla | Yes: IT (88) |
| **CWMEDIIAdriatic** *(µmol L$^{-1}$)* | CW | Y shore;T;Sal;pH;Turb;O2D | All | | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µg L$^{-1}$ | EQR_Chla | Yes: IT (336) |
| **CWMEDIITyrrhenian** *(µmol L$^{-1}$)* | CW | Y shore;T;Sal;pH;Turb;O2D | All | | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | µg L$^{-1}$ | EQR_Chla | Yes: IT (245) |
| **CWMEDIIIE** *(µmol L$^{-1}$)* | CW | No | -- | | µmol L$^{-1}$ | µmol L$^{-1}$ | µmol L$^{-1}$ | | | µmol L$^{-1}$ | | µg L$^{-1}$ | EQR_Chla | Yes: GR/CY (99) |
| **CWNEA1-26A** *(µM)* | CW | Y Turb;flush | Wi | µmol L$^{-1}$ | | | | | | | | Log | EQR_Chla | Yes: FR (45); IE (45); UK(13); SPwc (8); NO/SPec/SPgc (23) |
| **CWNEA1-26B** *(µM)* | CW | Y TidalRangTurb;flush | Wi | µmol L$^{-1}$ | | | | | | | | | EQR_Chla | Yes: FR/NL (7); UKs (41); UKn (16); BE (8) |
| **CWNEA1-26C** *(µM)* | CW | Y Turb;flush | Wi | µmol L$^{-1}$ | | | | | | | | | EQR_Chla | Yes: DK(5);DE(3) |
| **CWNEA1-26E** *(µM)* | CW | Y Turb;flush | Wi | µmol L$^{-1}$ | | | | | | | | | EQR_Chla | Yes: PTsUpW/SP (27); PTUpW (9) |
| **CWNEA3-4** *(µM)* | CW | Y Turb;flush | Wi | µmol L$^{-1}$ | | | | | | | | | EQR_Chla | Yes: (14) |

*This does not mean complete observations available per analysis, since it depends on parameters combination in each analysis.

The EQR intercalibrated boundaries for phytoplankton at High/Good and Good/Moderate status (Table 3) used for the regression and categorical analyses (see Chapters 5 and 6) were taken from the WFD IC exercise (see Decision 2013/480/EU) and from unpublished recent IC results[2,3],.

In most cases, boundaries at High/Good and Good/Moderate status are the only ones available, since these have been the target of the WFD IC exercise. This means that for some datasets boundaries at the Moderate/Poor and Poor/Bad categories were not reported. These boundaries at lower quality classes are required to normalise data within common types, where MS have different IC EQR boundaries, but also to run some of the categorical analyses included in the BPG protocol and accompanying toolkit proposed by Phillips *et al.* (2017).

[2] The WFD intercalibration exercise has in the meantime finished but the Commission Decision with the IC results is not yet published.

[3] At the time of this report the WFD intercalibration exercise was still ongoing for some TRW and CW common types, thus intercalibrated EQR boundaries were not available for some MS for deriving nutrient boundaries. Despite the EQR boundaries are now updated in the present report, some calculations have not been performed.

Table 3. WFD intercalibrated (IC) EQR boundaries for BQE phytoplankton per common type (or country if they differ within type) and equivalent Ecological Quality Status (EQS) classes: High (H), Good (G), Moderate (M), Poor (P), Bad (B), (adopted from latest WFD IC results in 2017). (*EQR boundaries for datasets in italic blue were only available later in the process of this report and are not yet officially published*)

| Water Category | Common Type | Country | Datasets | EQR Boundaries | | | |
|---|---|---|---|---|---|---|---|
| | | | | H/G | G/M | M/P | P/B |
| TRW | BALBT1 | LT | ds1 | 0.83 | 0.57 | 0.39 | 0.29 |
| | | PL | ds2 | 0.77 | 0.61 | 0.5 | 0.4 |
| | MEDpolyCL | IT/GR | ds3 | 0.78 | 0.51 | -- | -- |
| | | FR | ds4 | 0.71 | 0.39 | -- | -- |
| | NEA11 | NL/UK/IE | ds25 | 0.8 | 0.6 | -- | -- |
| | | FR | ds26 | 0.67 | 0.393 | -- | -- |
| | | SP | ds27 | 0.67 | 0.37 | -- | -- |
| | | PT | ds28 | 0.667 | 0.467 | -- | -- |
| CW | BALBC4 | LV | ds5 | 0.82 | 0.67 | 0.33 | 0.23 |
| | | EE | ds6 | 0.83 | 0.67 | 0.33 | 0.23 |
| | BALBC5 | LV | ds7 | 0.65 | 0.39 | 0.33 | 0.2 |
| | | LT | ds8 | 0.87 | 0.6 | 0.28 | 0.21 |
| | MEDI | IT | ds9 | 0.85 | 0.62 | -- | -- |
| | MEDII Adriatic | IT | ds10 | 0.81 | 0.6 | -- | -- |
| | MEDII Tyrrhenian | IT | ds11 | 0.84 | 0.62 | -- | -- |
| | MEDIIIE | GR/CY | ds12 | 0.66 | 0.37 | -- | -- |
| | NEA1-26A | FR | ds13 | 0.76 | 0.33 | -- | -- |
| | | IE | ds14 | 0.82 | 0.6 | -- | -- |
| | | SP/NO | ds15 | 0.67 | 0.33 | -- | -- |
| | | UK | ds16 | 0.8 | 0.6 | -- | -- |
| | NEA1-26B | FR | ds17 | 0.67 | 0.44 | -- | -- |
| | | UK*south* | ds18 | 0.82 | 0.63 | -- | -- |
| | | NL/UK*north* | ds19 | 0.8 | 0.6 | -- | -- |
| | | BE | ds20 | 0.8 | 0.67 | -- | -- |
| | NEA1-26C | DK/DE | ds21 | 0.67 | 0.44 | -- | -- |
| | NEA1-26E | SP/PT*sUpW* | ds22 | 0.67 | 0.44 | -- | -- |
| | | PT*UpW* | ds23 | 0.88 | 0.49 | -- | -- |
| | NEA3-4 | DE/NL | ds24 | 0.8 | 0.6 | -- | -- |
| | Black Sea | *no data available* | | | | | |

## 3.2. Data check and exploration

For coastal and transitional waters we have largely followed the recommended steps in the Guidance protocol (2017). Thus, initially, we checked and explored the datasets in order to identify:

**(i) Outliers** (using box plots, Cleveland plots, and xy scatter plots), and, if justifiable, excluding these from subsequent analysis by marking the data set. See also the Guidance (2017) protocol on outliers' removal and reporting;

**(ii) Collinearity** among covariates (using Pearson correlation and/or VIF), in case bivariate or multivariate regressions are required for some of the case studies;

**(iii) Relationship** between **pressure and response** (using boxplots, xy scatter plots and coplots, GAM and segmented regression), also for checking presence of an adequate coverage of the gradient of disturbance, and to help identify linear parts of the relationship that should be used for fitting regressions. It is important to only use data within a linear range, when using the linear regression based methods proposed in the Guidance (2017), which assume a linear response between the variables: "*This can often be achieved by log10 transformation of nutrient; however, even after this, visual inspection may reveal non-linearity, often with sigmoid responses (i.e. with regions at the* *extremes of the distribution where there is little response of the biology to changed concentrations of nutrients. In the statistical toolkit, segmented regression methods are provided to test for linearity within the dataset; users need to be sure that the thresholds of interest are captured within the linear portion of the graph*". Linear range of the data was therefore checked by visual graphical analysis and/or by fitting a GAM and a segmented regression using the toolkit of the Guidance protocol. For non-linear relationships some alternatives were included in the Guidance, which were tested when potentially useful in some CTRW data examples.

**Homogeneity** and **Normality** were checked after performing the regression analysis, using the residuals of the regression models. These results are presented in Chapters 5 and 6, together with the regression models' results. The verification of **(iv) Homogeneity** was done using the residuals of the model; i.e. by plotting the residuals vs. fitted values. Checking for **(v) Normality** was done using the residuals of the model, i.e. by checking for normality in residuals distribution. Histograms of residuals were used to get some impression of normality, although this is not fully testing the assumption of normality.

In Chapters 5 and 6, and for each dataset, we present the results of the data exploration[4].

## 3.3. Statistical analysis for pressure-response relationships (BPG Toolkit)

The suitability of different statistical approaches proposed in the Guidance was tested in CTRW, using case study examples from the WFD intercalibration (IC) exercise. The ultimate goal was to test and support the adjustment of the protocol proposed in the Guidance to these water categories. In this sense some of the analyses here presented were performed with earlier versions of the toolkit and may not represent exactly the results obtained when using the fully developed toolkit now published. During this work, where significant pressure-response relationships have been observed, nutrient boundaries were derived. In any case we advise that MS run the analyses with the latest version of the toolkit and compare the results obtained.

Nutrient boundaries for CTRW were established based on different approaches, as described in the Guidance (Phillips *et al.* 2017):

***Regression analysis***, based on a continuous relationship between EQR and nutrients; here using univariate linear regression analysis (OLS linear regression of EQR and nutrients and Nutrients vs. EQR, and Orthogonal regression (RMA), i.e. geometric average of slope models from previous OLS models); and,

***Categorical methods***, by which nutrient thresholds are derived from the distribution of nutrient concentration across ecological quality status (EQS) classes: 1) average of upper and lower quartiles of adjacent classes; 2) average of median of adjacent classes, 3) upper 75th percentile, and

Additional categorical approaches were used, based on the 4) minimisation of mis-match of classifications for biology and nutrients and 5) Binomial Logistic Regression.

Where the above approaches proved unsuitable, bivariate and quantile regression approaches were tested in a few demonstrative examples. These and other alternative approaches are mentioned in the Guidance (Phillips *et al.* 2017) as potentially useful approaches in need of further testing and development.

In Chapters 5 and 6, and for each dataset, we present the detailed results of the statistical analyses[5].

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[4] *Full details of the exploratory analyses can be assessed in the excel files and R scripts provided as Annex.*
[5] *Full details of the statistical analyses can be assessed in the files (excel Toolkit templates and R scripts) provided as Annex.*

# 4. Nutrient boundaries

In this section we present a summary overview of the predicted nutrients boundaries obtained for each common type, based on different approaches outlined in the Guidance (2017):

a) univariate linear regression analysis (OLS linear regression of EQR vs. nutrients and nutrients vs. EQR, and standard major axis (SMA) regression, i.e. geometric average of slope models from previous OLS models); and
b) categorical analysis;
c) bivariate linear regression analysis;
d) additive quantile regression analysis.

The predicted boundaries were compared with national nutrient boundaries established by MS (Dworak *et al.* 2016), where available.

## 4.1. Overview of predicted nutrient boundaries

The datasets for which nutrient boundaries have already been derived from the regression and categorical analyses are indicated in Table 4 and Table 5, where some of the most common issues encountered are signalled (see ***Notes*** in tables).

Some Member States (MS) use a full intercalibrated phytoplankton EQR method (e.g. Italy, Greece, and France in TRW); however, the majority of the MS reported a partially intercalibrated EQR method based only on the Chl*a* parameter, because during the third IC phase many phytoplankton coastal experts provided accepted justifications for the use of only Chlorophyll-*a* for the establishment of the ecological status using the phytoplankton "Biological Quality element". Where different methods are used within a common type, and with different IC boundaries, in such cases, the datasets were split for the analysis; as for example in TRW in the Mediterranean, where Italy and Greece use a different method than that from France. Often, even using the common metric Chl*a*, the IC EQR boundaries also differed within type (Table 3), and those datasets had also to be split for analysis, despite that this reduces the already low number of observations available for the majority of types (Table 4). One solution to overcome the low number of observations would be to pull data together, by normalising EQRs to allow comparison in a same scale, as done for the TRW common type in the NEA (Table 5). To achieve this MS are required to report (or use) the full set of quality classes' boundaries. In addition, if normalisation takes place across broad or different types, additional explanatory variables factors might need to be considered to explain variation when predicting nutrient boundaries from such broad EQR datasets. Specific recommendations will be provided on this issue, in the Guidance by Phillips *et al.* (2017).

In many datasets the EQR values presented a significant percentage of the data (nearly 25%) beyond the expected EQR range [0-1], and often with very pronounced deviations. This occurred mainly where Chl*a* based EQR was being used for IC. In such cases, only extreme EQR values were removed from the analysis (e.g. EQR=32), since removing all EQR>1 would decrease the amount of data available for analysis but could also influence the statistical properties of the relationship between the phytoplankton BQE (i.e. EQR_Chl*a*) and nutrient pressure. However, this EQR ranges may indicate a problem in the established Reference Conditions (RC), in certain types. If the natural ranges of Chl*a* in the new datasets differ considerably from the ones used for establishing reference conditions and those used in the IC exercise, then the intercalibrated phytoplankton (Chl*a*) boundaries, defined within a 0-1 range, may compromise the prediction of robust nutrient boundaries. We suggest that these cases should be further scrutinized, in order to check the influence of this aspect in the predicted nutrient boundaries.

Some datasets have relatively few observations (low n; Table 4 and Table 5) which may compromise their use to apply some of the regression analyses proposed and also some of the categorical ones, since results might not be robust and representative enough. Many do not have a proper coverage of the full gradient of disturbance, and in particular of the range of interest to derive nutrient boundaries, i.e. from High to Moderate status. Both situations might be partially overcome if datasets within common types are normalised and analysed together. This would allow increasing the number of observations and the coverage of the gradient of disturbance. This is particularly relevant for MS lacking either good or bad quality samples/sites/conditions, since the full gradient of disturbance could still be captured at the common type scale.

Finally, evidence of potential interaction between nutrient parameters, of factors masking the pressure-response relationship (either positively, e.g. when the pressure is mitigated by other factor(s); or negatively, e.g. when multiple stressors occur simultaneously), and of over-dispersion in the data, indicates that alternative statistical approaches might be necessary to predict nutrient boundaries for some datasets.

Table 4. List of TRW datasets available for analysis, reflecting specific combination of countries with unique IC EQR boundaries and nutrient data available. Summary of the results for the analysis performed in each dataset correspond to Univariate linear regression type II, if not mentioned otherwise: BvR bivariate linear regression; AdQR additive quantile regression.

| GIG | Common Type | Country | dataset | Nutrient(s) tested | complete obs *n* | $R^2$ | *r* | *p*-value | Notes | Tool kit |
|---|---|---|---|---|---|---|---|---|---|---|
| **Transitional waters** | | | | | | | | | | |
| Baltic | BT1 | Lithuania | ds1 | TN | 25 | **0.41** | 0.641 | <0.001 | | vs3 |
| | | | | TP | 24 | **0.432** | 0.657 | <0.001 | | vs3 |
| | | | | N+P BvR | 23 | **0.556** | 0.745 | <0.001 | | vs3 |
| | | Poland | ds2 | TN | 13 | **0.86** | 0.927 | <0.001 | Check interaction TN-TP | vs3 |
| | | | | TP | 25 | **0.209** | 0.457 | 0.022 | Check interaction TN-TP | vs3 |
| | | | | N+P BvR | 23 | **0.088** | 0.296 | 0.154 | | vs3 |
| Mediterranean | polyhaline CL | Italy/Greece | ds3 | TN | 12 | **0.778** | 0.882 | <0.001 | H/G/M range not covered; predictions outside data range | vs3 |
| | | | | TP | 15 | **0.603** | 0.777 | <0.001 | | vs3 |
| | | *Analysis for:* | | DIN | | | | | *Not performed* | |
| | | France | ds4 | TN | 13 | **0.642** | 0.801 | <0.001 | Collinearity TN-TP | vs3 |
| | | | | TP | 14 | **0.868** | 0.932 | <0.001 | Collinearity TN-TP | vs3 |
| | | *Analysis for:* | | DIN | | | | | *Not performed* | |
| North East Atlantic | NEA11 | Netherlands/ UK/ Ireland | ds25 | DIN | 98 | **--** | -- | -- | EQS G/M class overlap | vs6c |
| | | France | ds26 | DIN | 15 | *excluded* | | | *insufficient gradient coverage; invert trend* | vs6c |
| | | Spain | ds27 | DIN | 55 | **--** | -- | -- | EQS G/M class overlap | vs6c |
| | | Portugal | ds28 | DIN | 7 | **--** | -- | -- | very low n; insufficient gradient coverage | vs6c |
| | | merged ds *normalised EQRs* | ds: 25, 27, 28 | DIN | 160 | **0.210** | -0.458 | <0.001 | Wedge-shape; EQS G/M class overlap | vs6c |
| | | | | DIN AdQR | 160 | **0.2049** | | <0.001 | | vs6c |

Table 5. List of CW datasets available for analysis, reflecting specific combination of countries with unique IC EQR boundaries and nutrient data available. Summary of the results for the analysis performed in each dataset correspond to Univariate linear regression type II, if not mentioned otherwise: BvR bivariate linear regression; LQR linear quantile regression; AdQR additive quantile regression.

| GIG | Common Type | Country | dataset | Nutrient(s) tested | complete obs *n* | $R^2$ | *R* | *p*-value | Notes | Tool Kit |
|---|---|---|---|---|---|---|---|---|---|---|
| **Coastal waters** | | | | | | | | | | |
| Baltic | BC4 | Latvia | ds5 | TN | 79 | **0.284** | 0.533 | <0.001 | Wedge-shape; predictions outside data range | vs3 |
| | | | | TP | 81 | **0.308** | 0.555 | <0.001 | Wedge-shape | vs3 |
| | | Estonia | ds6 | TN | 22 | **0.756** | 0.87 | <0.001 | EQS G/M class overlap | vs3 |
| | | | | TP | 40 | **0.26** | 0.501 | <0.001 | Wedge-shape | vs3 |
| | BC5 | Latvia | ds7 | TN | 98 | **0.48** | 0.693 | <0.001 | Wedge-shape; EQS G/M class overlap | vs3 |
| | | | | TP | 98 | **0.257** | 0.507 | <0.001 | Wedge-shape; EQS H/G class overlap | vs3 |
| | | Lithuania | ds8 | TN | 61 | **0** | 0.013 | 0.919 | No trend in data | vs3 |
| | | | | TP | 61 | **0.214** | 0.462 | <0.001 | Wedge-shape | vs3 |
| Mediterranean | MEDI | Italy | ds9 | TN | 82 | **0.098** | 0.314 | 0.004 | Wedge-shape | vs3 |
| | | | | TP | 83 | **0.043** | 0.208 | 0.059 | Wedge-shape | vs3 |
| | | *Analysis for:* | *NO3* | *NO2* | *NH4* | *PO4* | *Si* | | *Not performed* | |
| | MEDII Adriatic | Italy | ds10 | TN | 316 | **0.217** | 0.466 | <0.001 | Wedge-shape | vs3 |
| | | | | TN (LQR) | 332 (80th) | **0.615** | 0.785 | | EQR range >>1 | R |
| | | | | N+P (BvR) | 294 | **0.228** | 0.477 | <0.001 | H/G boundaries inversion | vs3 |
| | | | | TP | 309 | **0.066** | 0.258 | 0.6 | No trend in data | vs3 |
| | | *Analysis for:* | *NO3* | *NO2* | *NH4* | *PO4* | *Si* | | *Not performed* | |
| | MEDII Tyrrhenian | Italy | ds11 | TN | 228 | **0.088** | 0.297 | <0.001 | Wedge-shape | vs3 |
| | | | | TP | 228 | **0.005** | 0.068 | 0.307 | Wedge-shape | vs3 |
| | | *Analysis for:* | *NO3* | *NO2* | *NH4* | *PO4* | *Si* | | *Not performed* | |
| | MEDIIIE | Greece/ Cyprus | ds12 | NO3 | | **0.132** | 0.363 | <0.001 | | vs3 |
| | | *Analysis for:* | *NH4* | *NO2* | *PO4* | | | | *Not performed* | |
| North East Atlantic | NEA1-26A | France | ds13 | DIN | 45 | | | | *Not performed* | |
| | | Ireland | ds14 | DIN | 41 | | | | *Not performed* | |
| | | Spain/ Norway | ds15 | DIN | 30 | | | | *Not performed* | |
| | | UK | ds16 | DIN | 11 | | | | *Not performed* | |
| | NEA1-26B | France | ds17 | DIN | 4 | -- | -- | -- | very low n | |
| | | UKsouth | ds18 | DIN | 40 | | | | *Not performed* | |
| | | Netherlands/UKnorth | ds19 | DIN | 18 | | | | *Not performed* | |
| | | Belgium | ds20 | DIN | 3 | -- | -- | -- | very low n | |
| | NEA1-26C | Denmark/Germany | ds21 | DIN | 8 | -- | -- | -- | H/G/M range not covered; very low n | |
| | NEA1-26E | Portugal/ Spain | ds22 | DIN | 25 | | | | *Not performed* | |
| | | Portugal | ds23 | DIN | 6 | -- | -- | -- | very low n | |
| | NEA3-4 | *Germany/Netherlands* | ds24 | DIN | 14 | | | | *Not performed* | |

A summary of selected boundaries derived from the most adequate results (i.e. significant and/or meaningful) obtained for each common type, using the toolkit, are presented in Table 6 and Table 7. The nutrients analysed for this exercise were Total Nitrogen (TN), Total Phosphorus (TP) and Dissolved Inorganic Nitrogen (DIN), depending on the water systems. For these nutrients, two ranges of values are presented: the "most likely" taken from the minimum and maximum value predicted from the different regression and categorical approaches and a "possible" range taken from the maximum and minimum of the upper and lower quartiles of the regression residuals. Where other approaches have been tested (e.g. Bivariate Linear regression, Quantile regression, Binomial Logistic regression or other categorical approaches), the results are also presented along with derived nutrient boundaries.

Table 6. Predicted nutrient boundaries for several Transitional Waters common types, from the significant or most adequate approaches for each dataset (Toolkit excel vs3 or vs6c and/or R scripts).

| Transitional waters common type and methods | Nutrient boundaries | | Nutrient boundaries | |
|---|---|---|---|---|
| **BAL BT1** | | | | |
| **Lithuania** | **TN** | µg $L^{-1}$ | **TP** | µg $L^{-1}$ |
| **Regression methods (OLS & Type II):** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| Most likely boundary predicted | 1020 | 1224 | 73 | 89 |
| *range* | 928-1084 | 1218-1298 | 66-78 | 88-90 |
| *possible range* | 845-1187 | 1122-1333 | 61-86 | 82-99 |
| **Regression methods (bivariate TN+TP):** | | | | |
| Most likely boundary predicted | 1014 | 1218 | 71 | 89 |
| *range* | 939-1073 | 1128-1298 | 65-77 | 81-96 |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | | | 74 | 84 |
| average adjacent class median | 1101 | 1206 | 74 | 85 |
| 75th quartile of class | 1168 | 1235 | 72 | 85 |
| mis-match of biological v nutrient class (*Excel*) | 960 | 1240 | 67 | 83 |
| **Poland** | **TN** | µg $L^{-1}$ | **TP** | µg $L^{-1}$ |
| **Regression methods (OLS & Type II):** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| Most likely boundary predicted | 948 | 1072 | | |
| *range* | 940-956 | 1071-1073 | | |
| *possible range* | | | | |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | 662 | 1022 | | |
| average adjacent class median | 662 | 1022 | | |
| 75th quartile of class | 400 | 923 | | |
| mis-match of biological v nutrient class (*Excel*) | 600 | 900 | 88 | 101 |
| **MED Polyhaline Coastal Lagoons** | | | | |
| **Italy/Greece** | **TN** | µg $L^{-1}$ | **TP** | µg $L^{-1}$ |
| **Regression methods (OLS & Type II):** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| Most likely boundary predicted | 1039 | | 27 | 47 |
| *range* | 1031-1049 | | 25-28 | 44-53 |
| *possible range* | 840-1176 | | 17-38 | 25-97 |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | 1103 | | 23 | 63 |
| average adjacent class median | 1077 | | 23 | 66 |
| 75th quartile of class | 840 | 1463 | 28 | 25 |
| mis-match of biological v nutrient class (*Excel*) | 870 | | 21 | 97 |
| **France** | **TN** | µg $L^{-1}$ | **TP** | µg $L^{-1}$ |
| **Regression methods (OLS & Type II):** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| Most likely boundary predicted | 261 | 587 | 18 | 42 |
| *range* | 216-304 | 582-594 | 17-19 | 42-42 |
| *possible range* | 132-432 | 362-929 | 14-23 | 23-55 |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | 364 | 565 | 20 | 35 |
| average adjacent class median | 374 | 559 | 21 | 39 |
| 75th quartile of class | 432 | 470 | 21 | 23 |
| mis-match of biological v nutrient class (*Excel*) | 225 | 570 | 19 | 28 |
| **NEA 11 (NL, UK, RoI, SP, PT)** | **DIN** | µM | | |
| **Regression methods (OLS & Type II):** | **High/G** | **Good/M** | | |
| Most likely boundary predicted | 36 | 62 | | |
| *range* | (14-43) | (61-72) | | |
| *possible range* | 5-79 | 23-278 | | |
| **Additive Quantile regression method (rqss):** | | | | |
| 70th percentile | 65-85 | 190-240 | | |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | 49 | 80 | | |
| average adjacent class median | 47 | 82 | | |
| 75th quartile of class | 62 | 107 | | |
| mis-match of biological v nutrient class (*Excel*) | 50 | 72 | | |
| mis-match of biological v nutrient class (*R scripts*) | 53 | 75 | | |
| *range* | (47-59) | (66-83) | | |
| Logistic Binomial regression (*prob=0.5*) | 45 | 80 | | |

Table 7. Predicted nutrient boundaries for several Coastal waters common types, from the significant or most adequate approaches for each dataset (Toolkit excel vs3 or vs6c and/or R scripts).

| **Coastal waters common type and methods** | **Nutrient boundaries** | | | |
|---|---|---|---|---|
| **BAL BC4** | | | | |
| **Latvia** | **TN** | µM | **TP** | µM |
| **Categorical methods:** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| average adjacent class upper & lower quartiles | 29 | 30.4 | 0.68 | 0.72 |
| average adjacent class median | 29.4 | 29.4 | 0.70 | 0.77 |
| 75th quartile of class | 28.5 | 28.5 | 0.65 | 0.78 |
| mis-match of biological v nutrient class (*excel*) | 25.5 | 25.5 | 0.53 | 0.62 |
| **Estonia** | **TN** | µM | **TP** | µM |
| **Regression methods (OLS & Type II):** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| Most likely boundary predicted | 22.7 | 24.8 | | |
| *range* | 22.4-22.9 | 24.8-24.8 | | |
| *possible range* | 20.4-25.2 | 22.6-28.5 | | |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | 25.2 | 27 | 0.69 | 0.76 |
| average adjacent class median | 24.8 | 27.8 | 0.64 | 0.67 |
| 75th quartile of class | 22.2 | 28.5 | | 0.82 |
| mis-match of biological v nutrient class (*excel*) | 22.5 | 23.5 | 0.48 | 0.55 |
| **BAL BC5** | | | | |
| **Latvia** | **TN** | µg $L^{-1}$ | **TP** | µg $L^{-1}$ |
| **Regression methods (OLS & Type II):** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| Most likely boundary predicted | 312 | 368 | | |
| *range* | 292-327 | 366-370 | | |
| *possible range* | 260-360 | 330-410 | | |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | 332 | 353 | 20 | 21 |
| average adjacent class median | 331 | 347 | 20 | 22 |
| 75th quartile of class | 339 | 375 | | |
| mis-match of biological v nutrient class (*excel*) | 320 | 340 | 18 | 21 |
| **BAL BC5** | | | | |
| **Lithuania** | **TN** | µg $L^{-1}$ | **TP** | µg $L^{-1}$ |
| **Categorical methods:** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| average adjacent class upper & lower quartiles | | | 21 | 25 |
| average adjacent class median | 388 | 409 | 20 | 25 |
| mis-match of biological v nutrient class (*excel*) | 190 | 285 | 13 | 23 |
| **MED I (Italy)** | **TN** | µM | **TP** | µM |
| **Categorical methods:** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| average adjacent class upper & lower quartiles | | | 0.58 | 0.72 |
| average adjacent class median | 26 | 43 | 0.62 | 0.72 |
| 75th quartile of class | | | 0.71 | 0.81 |
| mis-match of biological v nutrient class (*excel*) | 24 | 39 | 0.57 | 0.76 |
| **MED II Adriatic (Italy)** | **TN** | µM | **TP** | µM |
| **Linear Quantile regression:** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| 80th percentile | 97 | 197 | | |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | 23.7 | 33.2 | | |
| average adjacent class median | 24.8 | 33.2 | | |
| 75th quartile of class | 28.6 | 43.4 | | |
| mis-match of biological v nutrient class (*excel*) | 23 | 30 | 0.35 | 0.58 |
| **MED II Tyrrhenian (Italy)** | **TN** | µM | **TP** | µM |
| **Categorical methods:** | **High/G** | **Good/M** | **High/G** | **Good/M** |
| average adjacent class upper & lower quartiles | 15.4 | 19.6 | 0.22 | 0.46 |
| average adjacent class median | 13.8 | 18 | 0.15 | 0.20 |
| 75th quartile of class | 16.8 | 28.3 | 0.40 | 0.87 |
| mis-match of biological v nutrient class (*excel*) | 15.3 | 20 | 0.34 | 0.42 |
| **MED III (Greece, Cyprus)** | **$NO_3$** | µM | | |
| **Categorical methods:** | **High/G** | **Good/M** | | |
| 75th quartile of class | 0.99 | 1.25 | | |
| mis-match of biological v nutrient class (excel) | 0.74 | 1.06 | | |

Below we provide an overview of the range of predicted nutrient concentrations for the High/Good and Good/Moderate boundaries for Total Nitrogen (TN; Figure 1 and Figure 3) and Total Phosphorus (TP; Figure 2 and Figure 4), across some common types in transitional and coastal waters. The range of values plotted for H/G and G/M was obtained from significant linear regression models (or other models as indicated in Table 4 and Table 5) and/or categorical approaches. The graphs allow for a quick comparison of the range of nutrient boundaries obtained across geographies and common types, when using these methods. The details of each analysis per type are provided in the next Chapters.

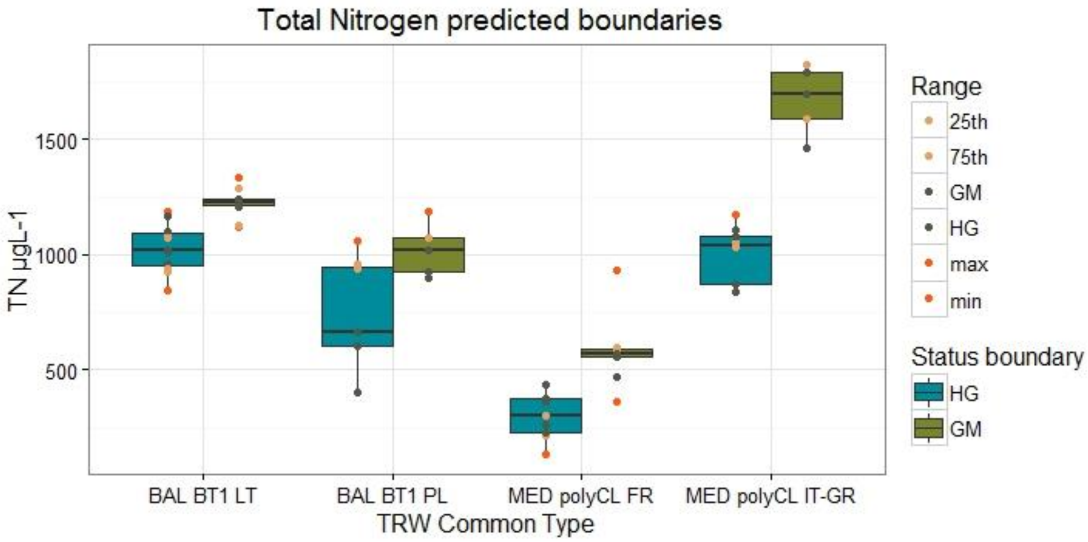



Figure 1. Summary of predicted values of Total Nitrogen concentration (TN µg $L^{-1}$) at the High/Good and Good/Moderate boundaries, for common intercalibration types within the Baltic (BAL BT1 for Lithuania and Poland) and the Mediterranean (MED polyhaline CL for France, Italy and Greece). Boxplots show the range of predictions by all tested methods: univariate linear regressions and categorical approaches (one grey dot for each predicted value), together with the upper (75$^{th}$) and lower (25$^{th}$) ranges for the boundary values as predicted by the RMA linear regression. The likely range of boundary values is also included, derived from the minimum and maximum values provided by the three linear models.

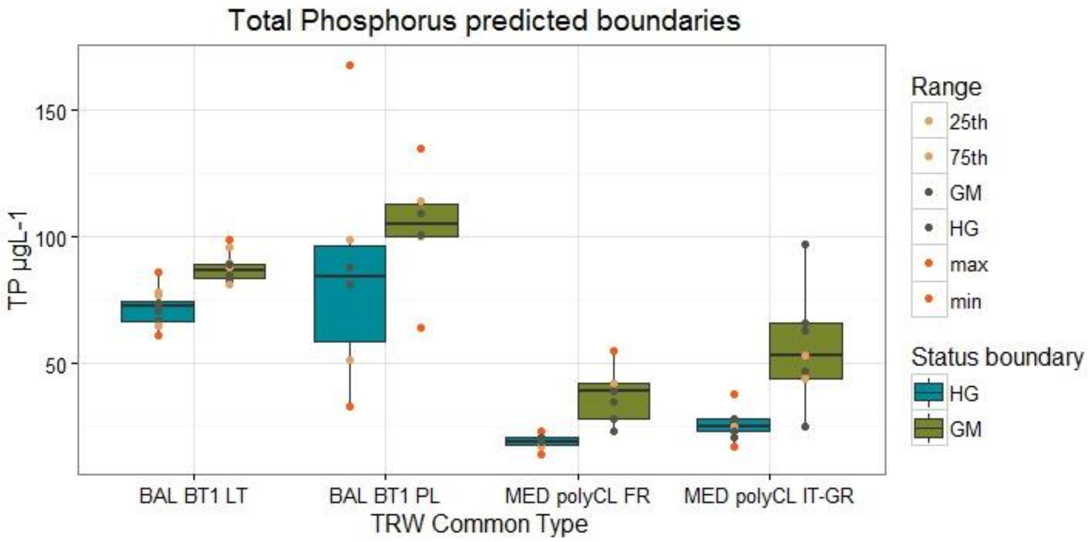



Figure 2. Summary of predicted values of Total Phosphorus concentration (TP µg $L^{-1}$) at the High/Good and Good/Moderate boundaries, for common intercalibration types within the Baltic (BAL BT1 for Lithuania and Poland) and the Mediterranean (MED polyhaline CL for France, Italy and Greece). Boxplots show the range of predictions by all tested methods: univariate linear regressions and categorical approaches (one grey dot for each predicted value), together with the upper (75$^{th}$) and lower (25$^{th}$) ranges for the boundary values as predicted by the RMA linear regression. The likely range of boundary values is also included, derived from the minimum and maximum values provided by the three linear models.

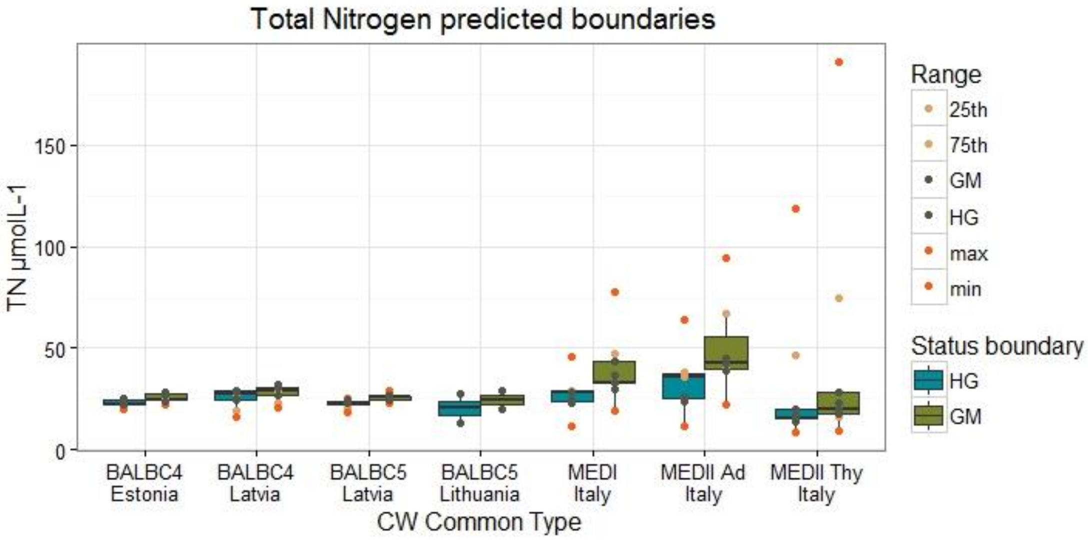



Figure 3. Summary of predicted values of Total Nitrogen concentration (TP µmol $L^{-1}$) at the High/Good and Good/Moderate boundaries, for common intercalibration types in coastal waters. Boxplots show the range of predictions by all tested methods: univariate linear regressions and categorical approaches (one grey dot for each predicted value), together with the upper ($75^{th}$) and lower ($25^{th}$) ranges for the boundary values as predicted by the RMA linear regression. The likely range of boundary values is also included, derived from the minimum and maximum values provided by the three linear models.

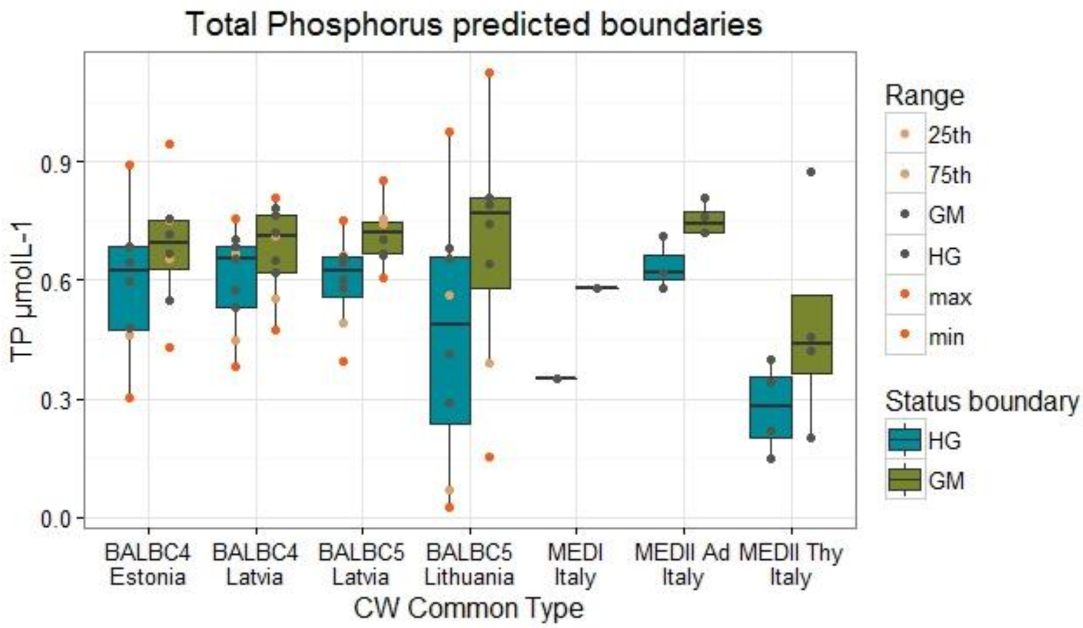



Figure 4. Summary of predicted values of Total Phosphorus concentration (TP µmol $L^{-1}$) at the High/Good and Good/Moderate boundaries, for common intercalibration types in coastal waters. Boxplots show the range of predictions by all tested methods: univariate linear regressions and categorical approaches (one grey dot for each predicted value), together with the upper ($75^{th}$) and lower ($25^{th}$) ranges for the boundary values as predicted by the RMA linear regression. The likely range of boundary values is also included, derived from the minimum and maximum values provided by the three linear models.

## 4.2. Comparison with national boundaries in TRW and CW

The nutrient boundaries estimated for CTRW GIG datasets using the approaches recommended in the Guidance should be compared with the nutrient boundaries reported by Member States (Table 8 and Table 9) within similar types (Dworak et al., 2016), and/or with other existing boundary values, for example adopted by the regional sea conventions (RSC). Where a mismatch is found between the boundary values predicted using methods in the toolkit and those reported by Member States and/or adopted within the RSC, then further consideration of the validity of the boundaries may be required (see Validation section in Phillips *et al.* 2017).

Table 8. Member States nutrient boundaries in TRW for comparison with BPG results. Confirmation is needed from MS experts for identifying national types included in IC dataset and also for selecting adequate boundaries for comparison, particularly for datasets marked*.

| Water Category | Common Type | Country | Dataset *in 2017* | Nutrient | Units | notes | MS Nutrient boundaries H/G (*Ref Cond*) | MS Nutrient boundaries G/M |
|---|---|---|---|---|---|---|---|---|
| **TRW** | **BAL BT1** | LT | ds1* | TN | ($\mu g l^{-1}$) | a) N CL salinity 0.5-5<br>b) N CL salinity <0.5<br>c) Plume CL salinity <2<br>d) Plume CL salinity 2-4<br>e) Plume CL salinity >4 | 940-1080 a,c (RC <750)<br>950-1070 b (RC <760)<br>430-670 d (RC <330)<br>130-250 e (RC <100) | 1090-1230 a,c<br>1080-1170 b<br>680-810 d<br>260-400 e |
| | | | | TP | ($\mu g l^{-1}$) | a) N CL salinity 0.5-5<br>b) N CL salinity <0.5<br>c) Plume CL salinity <2<br>d) Plume CL salinity 2-4<br>e) Plume CL salinity >4 | 60- 80 a,c (RC <47)<br>61-79 b (RC <48)<br>37-53 d (RC <29)<br>15-26 e (RC <11) | 81-136 a,c<br>80-130 b<br>54-84 d<br>27-33 e |
| | | PL | ds2 | TN | ($\mu g l^{-1}$) | salinity 0.5-5; winter mean | 650 | 980 |
| | | | | TP | ($\mu g l^{-1}$) | salinity 0.5-5; winter mean | *not available* | 120 |
| | **MED polyCL** | IT/GR | ds3 | TN | *not available* | | | |
| | | | | TP | *not available* | | | |
| | | | | DIN | ($\mu g l^{-1}$) | salinity <30 | *not available* | 420 (ca 30 µM) |
| | | FR | ds4 | TN | µM | | 50 | 75 |
| | | | | TP | µM | | 2 | 3 |
| | **NEA11** | NL | *ds25* | DIN | *not available* | | | |
| | | UK | *ds25** | DIN | µM | a) **mean** DIN at "Clear" waters: SPM >10, Salinity 25<br><br>**99th percentile:**<br>b) "Intermediate" waters: SPM 10-100, SPM midpoint:55<br>c) "medium turbidity" waters: SPM range:100-300, SPM mid-point 200<br>d) "very turbid" water SPM <300 | (RC <20) | 30 a<br>70 b<br>180 c<br>270 d |
| | | IE | *ds25* | DIN | *not available* | | | |
| | | FR | *ds26* | DIN | µM | normalized DIN salinity 33 | 20 | 29 (NEA26A)<br>33 (NEA26B) |
| | | SP | *ds27* | DIN | *not available* | | | |
| | | PT | *ds28* | DIN | *not available* | | | |

Table 9. Member States nutrient boundaries in CW for comparison with BPG results. Confirmation is needed from MS experts for identifying national types included in IC dataset and also for selecting adequate boundaries for comparison, particularly for datasets marked*.

| Water Category | Common Type | Country | Dataset *in 2017* | Nutrient | Units | notes | MS Nutrient boundaries H/G (*Ref Cond*) | G/M |
|---|---|---|---|---|---|---|---|---|
| CW | BAL BC4 | LV | ds5 | TN | *not available* | | | |
| | | | | TP | *not available* | | | |
| | | EE | ds6 | TN | µM | salinity 4-6; summer mean | 19.2 | 23.7 |
| | | | | TP | µM | salinity 4-6; summer mean | 0.4 | 0.5 |
| | BAL BC5 | LV | ds7 | TN | *not available* | | | |
| | | | | TP | *not available* | | | |
| | | LT | ds8* | TN | (µgl$^{-1}$) | salinity 5-18; summer mean | 130-250 (RC <100) | 260-400 |
| | | | | TP | (µgl$^{-1}$) | salinity 5-18; summer mean | 15-26 (RC <11) | 27-33 |
| | MEDI | IT | ds9* | TN | *not available* | | | |
| | | | | TP | µM | salinity 20-37; winter mean | (RC <0.24) | 0.6 |
| | MEDII Adriatic | IT | ds10 | TN | *not available* | | | |
| | | | | TP | µM | salinity 33-38; winter mean | (RC <0.23) | 0.37 |
| | MEDII Tyrrhenian | IT | ds11 | TN | *not available* | | | |
| | | | | TP | µM | salinity 33-38; winter mean | (RC <0.26) | 0.54 |
| | MEDIIIE | CY | ds12* | NO3 | µM | salinity >37.5; winter mean: (0.0091 mgl-1) | (RC <0.14) | 0.15 |
| | | GR | ds12 | NO3 | µM | annual mean; (0.023 mgl-1) | | 0.36 |
| | NEA1-26A | FR | *ds13* | DIN | µM | normalized DIN salinity 33 | 20 | 33 |
| | | IE | *ds14* | DIN | (mgl$^{-1}$) | salinity 34.5, winter and summer median | | 0.25 |
| | | SP | *ds15* | DIN | *not available* | | | |
| | | NO | *ds15* | DIN | *not available* | | | |
| | | UK | *ds16** | DIN | µM | winter<br>a) mean DIN at "Clear" waters: SPM >10, Salinity 32<br><br>99th percentile:<br>b) "Intermediate" waters: SPM 10-100, SPM midpoint:55<br>c) "medium turbidity" waters: SPM range:100-300, SPM mid-point 200<br>d) "very turbid" water SPM <300 | (RC <12 a) | 18 a<br><br>70 b<br><br>180 c<br><br>270 d |
| | NEA1-26B | FR | *ds17* | DIN | µM | normalized DIN salinity 33 | 20 | 29 |
| | | UK*south* | *ds18** | DIN | same as for ds16 UK | | | |
| | | UK*north* | *ds19** | DIN | same as for ds16 UK | | | |
| | | NL | *ds19* | DIN | *not available* | | | |
| | | BE | *ds20* | DIN | *not available* | | | |
| | NEA1-26C | DK | *ds21* | DIN | *not available* | | | |
| | | DE | *ds21* | DIN | *not available* | | | |
| | NEA1-26E | SP | *ds22* | DIN | *not available* | | | |
| | | PTsUpW | *ds22* | DIN | *not available* | | | |
| | | PT*UpW* | *ds23* | DIN | *not available* | | | |
| | NEA3-4 | DE | *ds23* | DIN | *not available* | | | |
| | | NL | *ds24* | DIN | *not available* | | | |

Where national Good/Moderate and High/Good nutrient boundaries (*Country*; Table 10) within common IC types were available (see Dworak *et al.*, 2016 for details on MS reported questionnaire), those values were compared with the range of nutrient boundary values resultant from the application of the BPG toolkit analyses. When linear regression results were not significant (see Table 4), then the results from the Categorical approaches (*Cat appr*) are used instead; in some cases only the predictions from the Minimise class difference approach (*Cat Mismatch*) were acceptable for use (Table 10). As explained by Phillips *et al.* (2017) this method appears to be the least sensitive to outliers and non-linear relationships, issues often encountered in the CTRW datasets. Further details on the analyses per type are provided in the next Chapters.

Table 10. Comparison of range of nutrient boundary values for TN, TP and DIN obtained with linear regression and/or categorical approaches, with the range of national good/moderate and high/good boundary values for several transitional (TRW) and coastal (CW) waters common IC types across Europe, where data was available for comparison.

| | **Nutrient boundaries** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Predicted** ***most likely range*** | | **National boundary** | | **Predicted** ***most likely range*** | | **National boundary** | |
| | **G/M** | **H/G** | **G/M** | **H/G** | **G/M** | **H/G** | **G/M** | **H/G** |
| | **Total Nitrogen (TN)** | | | | **Total Phosphorus (TP)** | | | |
| **Transitional Waters** | | | | | | | | |
| **BT1 LT** (μgl$^{-1}$) | 1206-1240 | 928-1146 | 1090-1230 *salinity<2* | 940-1080 *salinity<2* | 83-90 | -- | *81-136 salinity<2* | -- |
| **BT1 PL** (μgl$^{-1}$) | 900-1073 | -- | 980 | -- | *101 CatMismatch* | -- | 150 | -- |
| **MEDPolyCL IT/GR** | | | | | 25-97 | -- | 15 | -- |
| | **Dissolved Inorganic Nitrogen (DIN)** | | | | | | | |
| **NEA11** (μmoll$^{-1}$) | 66-83 *Cat appr* | 45-62 *Cat appr* | -- | 29-33 (range of UK, FR) | | | | |
| **Coastal Waters** | | | | | | | | |
| | **Total Nitrogen (TN)** | | | | **Total Phosphorus (TP)** | | | |
| **BC4 EE** (μmoll$^{-1}$) | 23.5-28.5 | -- | 23.7 | -- | *0.55-0.76 Cat app* | -- | 0.5 | -- |
| **BC4 LV** (μmoll$^{-1}$) | *26.9-31.9 Cat appr* | -- | 32.3 | -- | *0.62-0.78 Cat appr* | -- | 0.79 | -- |
| **BC5 LV** (mgl$^{-1}$) | 0.34-0.38 | -- | 0.36 | -- | 0.21-0.22 *Cat appr* | -- | 0.023 | -- |
| **BC5 LT** (mgl$^{-1}$) | *0.28-0.41 Cat appr* | -- | 0.26-0.40 | -- | *0.023-0.025 Cat appr* | -- | 0.027-0.033 | -- |
| **MED IIA Ad IT** Adriatic (μmoll$^{-1}$) | | | | | *0.58 CatMismatch* | -- | 0.37 | -- |
| **MED IIA IT** Tyrrhenian (μmoll$^{-1}$) | | | | | *0.42 CatMismatch* | -- | 0.54 | -- |
| | **Nitrates (NO3)** | | | | | | | |
| **MED III GR** (μmoll$^{-1}$) | 0.69-2.8 | -- | 0.36 | -- | | | | -- |

# 5. Transitional waters results

## 5.1. Baltic Sea

### 5.1.1. Common Type: TRWBALBT1

Phytoplankton intercalibration data from the common IC type BT1 in transitional waters was used. This type refers to sites in oligohaline (0-8) very sheltered coastal lagoons. This dataset contains data for two nutrient parameters, Total Nitrogen (TN) and Total Phosphorus (TP), for Chl*a* values, and EQR based on Chl*a,* from two countries: Lithuania (LT) and Poland (PL).

Exploratory analysis of the data and pressure response relationships between the phytoplankton EQR and nutrients are presented below per country: a) Lithuania and b) Poland.

Because the two countries have different IC EQR boundaries, the analyses for this type were performed separately in each country (Lithuania and Poland), using EQR IC boundaries adopted by each of them as indicated in Table 3. However this reduces the already low number of observations available for the analysis (n=56); so it would be useful to normalise the EQRs data and analyse the two countries together, comparing the results obtained. Since both MS reported all EQR quality boundaries (Table 3) normalisation could be attempted in future analyses.

#### 5.1.1.1. Lithuania (ds1_TRWBALBT1)

a) Data check

There are very few observations (n=27) in this dataset, however the EQR values available range at least three ecological quality status (EQS) classes as recommended for this exercise (Figure 5): High, Good and Moderate. The EQR boundaries used are the result of the WFD intercalibration exercise (Table 3). There is some overlap between some of the EQS classes, in particular for Good and Moderate in TN, and the Moderate class in TP, which covers almost all range of this nutrient distribution. There are two possible outliers in TP, at 104.75 µg $L^{-1}$ and 144.50 µg $L^{-1}$.

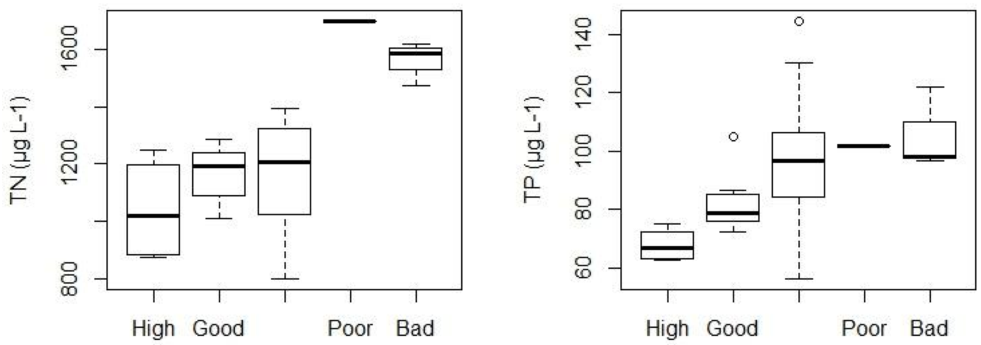



Figure 5. Range of Total Nitrogen (TN) and Total Phosphorus (TP) concentrations for Lithuanian sites grouped by ecological quality status (EQS) class: High, Good, Moderate, Poor and Bad.

Graphical interpretation of the data using histograms (Figure 6) revealed a slightly left-skewed distribution for Total Phosphorus (TP), which improved after a $\log_{10}$ transformation, performed before regression analyses (presented in the next section).

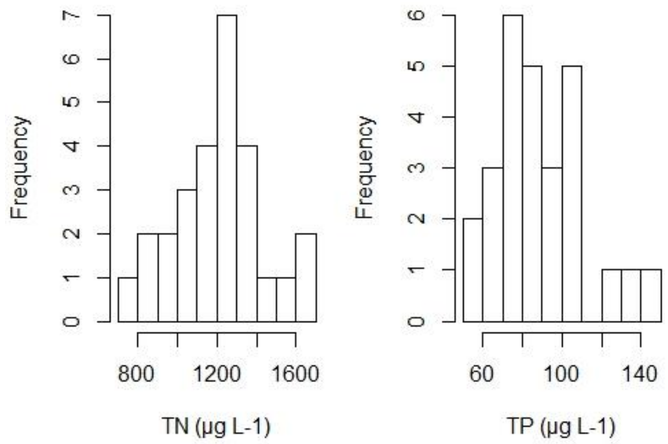



Figure 6. Range distribution of nutrient values for Total Nitrogen (TN) and Total Phosphorus (TP).

### i. Outliers

Eventual outliers for the variables of interest in this dataset are shown in Figure 7. There is an outlier in Total Phosphorus (144.50 µg $L^{-1}$), three outliers in Chl*a* (>100 µg $L^{-1}$) and two in the EQR variable. EQR outliers are beyond the EQR expected range of [0-1.0] (1.47; 1.65). The Cleveland plots distribution show however that these outliers may not be problematic and they will only be removed after analysing the xy relationships, following the nutrient Guidance protocol. The outliers finally removed from the analysis are reported in the next section.

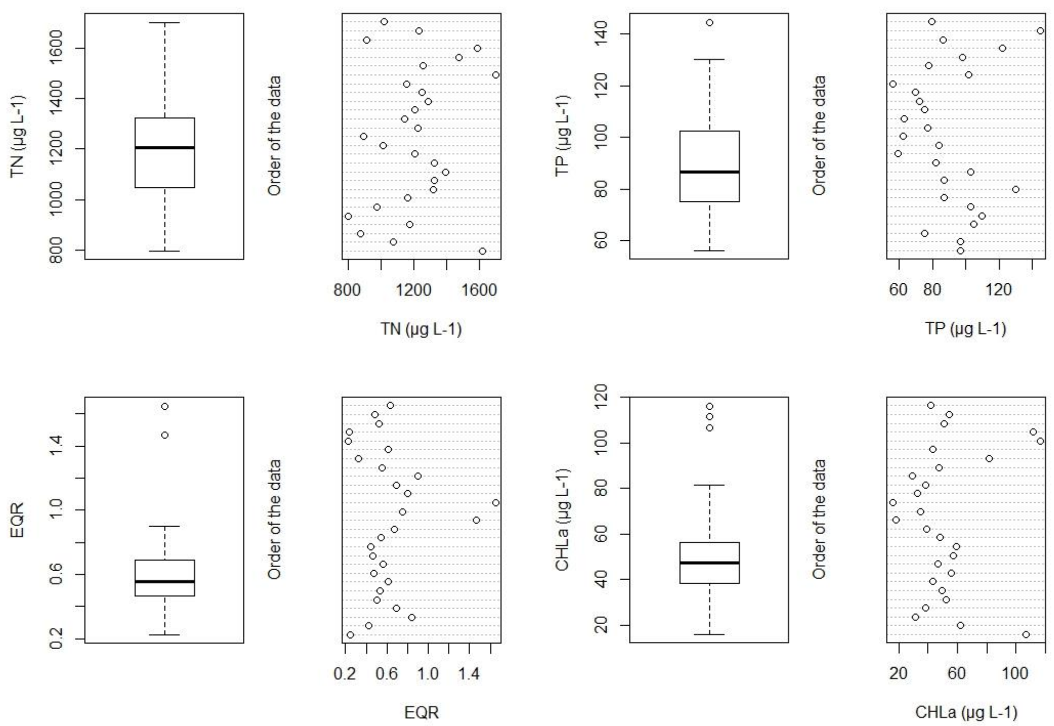



Figure 7. Outliers' verification in the predictors (TN, TP) and response (EQR, Chl*a*) variables, using Boxplot and Cleveland plots.

### ii. Collinearity among covariates

There is no collinearity (Pearson r = 0.28, n=27) among covariates Total Phosphorus (TP) and Total Nitrogen (TN) (Figure 8).

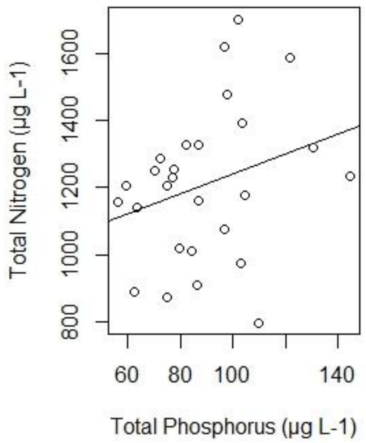



Figure 8. Scatterplot between nutrient explanatory variables: Total Nitrogen (TN) and Total Phosphorus (TP).

### iii. Relationship between X & Y

The correlation (Pearson r) between nutrients and EQR is near the acceptable threshold of 0.6 (EQR vs TN r= -0.478, n=27; EQR vs TP r= -0.554, n=27), but relationships do not follow clear linear patterns (Figure 9), presenting a slight wedge shape. The relationship does not change significantly with $\log_{10}$ transformed nutrient variables (EQR vs $\log_{10}$TN r= -0.451, n=27; EQR vs $\log_{10}$TP r=- -0.586, n=27). The linear regressions models to be applied use $\mathrm{Log}_{10}$ transformation of the nutrient concentration.

EQRs >1.0 were not removed, since they may bring relevant information for this analysis.

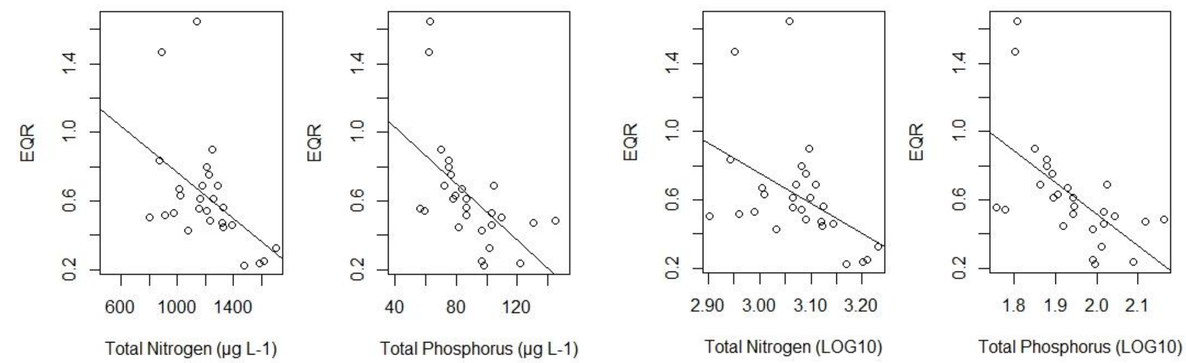



Figure 9. Scatterplot of the relationship of nutrient variables Total Nitrogen (TN) and Total Phosphorus (TP) with EQR, including all observations (left) and with $\log_{10}$ transformed explanatory variables (right).

A GAM model with segmented regression was fitted to the relationship of EQR vs TP to identify whether there are significant changes in the slope of the relationship at the end of the TP range, and help selecting linear regions of the data. However, linearity check with GAM and segmented regression failed using R script, since for one breakpoint (around the 100 – 120 µg $L^{-1}$ range) the error presented below kept occurring. We consider that we have very few data points, not allowing for a more robust analysis.

```
Error in segmented.lm(mod, seg.Z = ~x, psi = list(x =
c(Eb))) : only 1 datum in an interval: breakpoint(s) at
the boundary or too close each other
```

The fitted GAM showed almost a linear trend across the length of data used after three outliers' removal (Figure 10), and therefore no portion of the data has been dropped for the linear regression analyses in the next section.

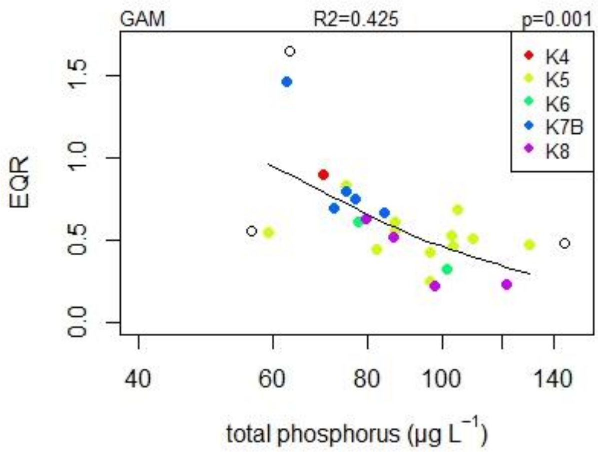



Figure 10. Scatter plot showing relationship between EQR and TP with fitted GAM model, points coloured by SITE, open circles outliers not used to fit model.

## b) Statistical analysis

For Lithuania, EQR relationships with Total Nitrogen (TN) and total Phosphorus (TP) were determined, using $log_{10}$ transformed nutrient data. The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Bivariate regressions EQR ~ TN + TP
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below (Table 11 and Table 12).

### *Univariate regression EQR ~ Total Nitrogen (TN)*

Two outliers were removed from the observations (Figure 11) and the analysis covered a nutrient range from 798 to 1700 µg$L^{-1}$. Regression of EQR with TN showed a significant acceptable correlation (r=0.641; Table 11).

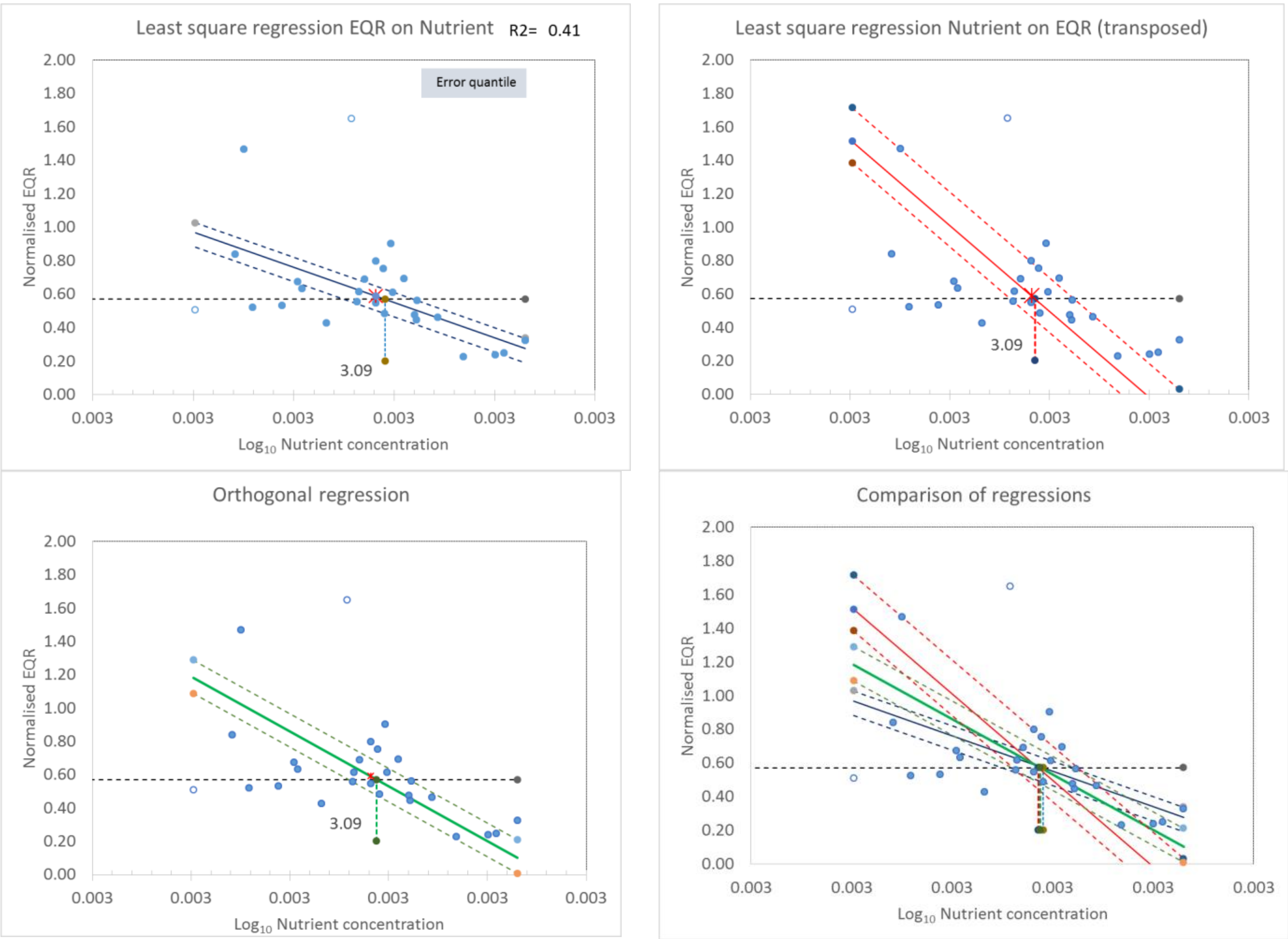



Figure 11. Regressions with total Nitrogen for Lithuania: (top left) OLS regression of EQR v log10 nutrient concentration; (top right) OLS regression of log10 nutrient concentration v EQR; (bottom left) Orthogonal type II regression of EQR v log10 nutrient concentration; (bottom right) comparison of above regressions. Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked

The TN model's validation may however indicate lack of suitability of these models (Figure 12). Despite that the residual frequency distribution has a normal distribution for LT, this is not the case for Poland which presents substantial skewness pattern. The residuals also show great variability along the fitted values for both nutrients (no homogeneity observed).

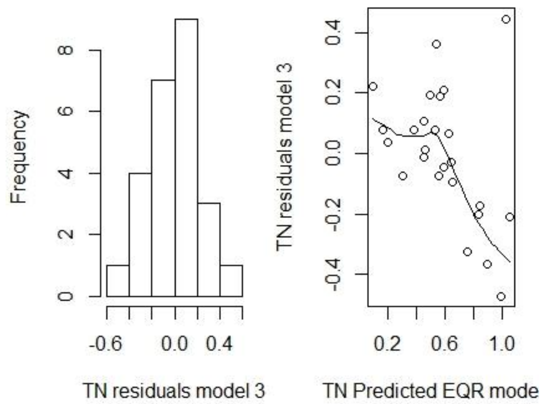



Figure 12. TN Model diagnosis of residual's normal distribution (histograms) and homoscedasticity (scatterplots), using ordinary residuals. Graphs are presented only for regression model 3 (RMA Orthogonal type II regression of EQR versus log10 of nutrient concentrations (TN) which is an average of model 1 – OLS EQR vs nutrient and model 2 OLS nutrient vs. EQR).

### *Univariate regression EQR ~ Total Phosphorus (TP)*

Three outliers were removed from the observations (Figure 13). The analysis covered a nutrient range from 59 to 130 µg $L^{-1}$ (Table 12).

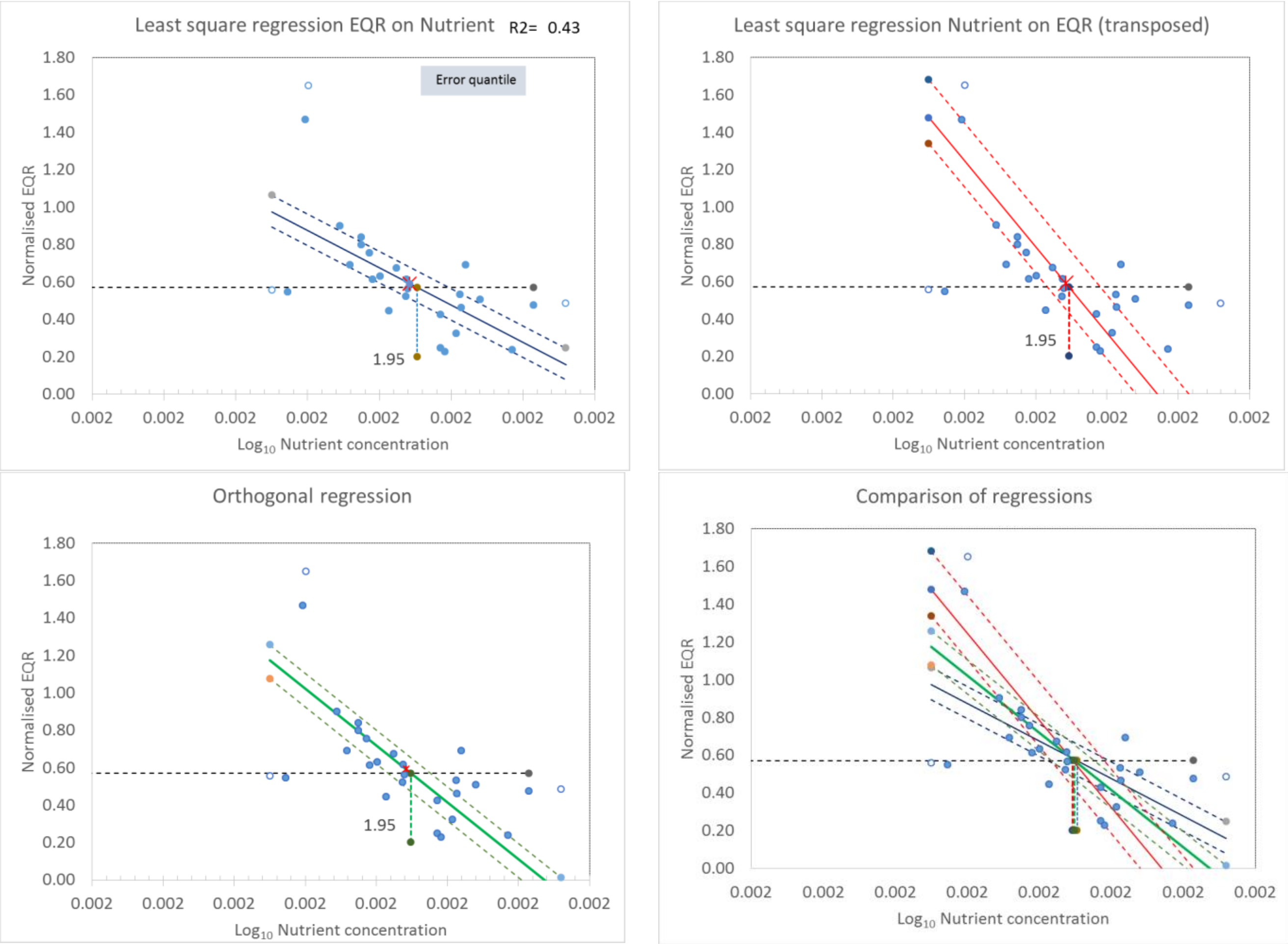



Figure 13. Regressions with total Phosphorus for Lithuania: (top left) OLS regression of EQR v log10 nutrient concentration; (top right) OLS regression of log10 nutrient concentration v EQR; (bottom left) Orthogonal type II regression of EQR v log10 nutrient concentration; (bottom right) comparison of above regressions. Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

Similarly to TN, also for TP the models' validation may indicate lack of suitability of these models (Figure 14). Although the residuals frequency distribution has a nearly normal distribution, the residuals show great variability along the fitted values for both nutrients (no homogeneity observed).

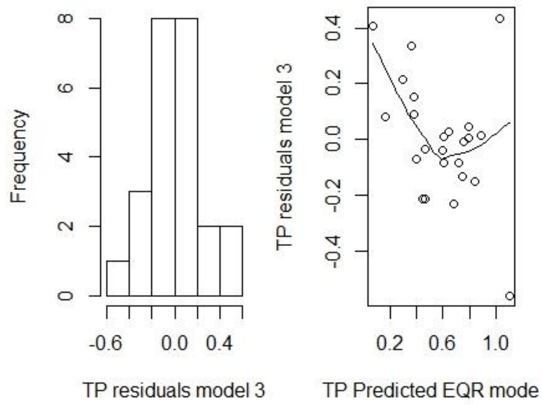



Figure 14. TP Model diagnosis of residual's normal distribution (histograms) and homoscedasticity (scatterplots), using ordinary residuals, for Lithuania. Graphs are presented only for regression model 3 (RMA Orthogonal type II regression of EQR versus log10 of nutrient concentrations (TN) which is an average of model 1 – OLS EQR vs nutrient and model 2 OLS nutrient vs. EQR).

### *Bivariate regressions EQR ~ TN + TP*

The bivariate model improves slightly from the univariate models for TN or TP previously presented (adjusted $R^2$ = 0.556; $p < 0.001$, n=23). The model validation plots (Figure 15) do not show particular problems with the model, the residuals show a normal distribution (Q-Q plot), although left panel graphs of residuals vs. fitted values and scale-location (Figure 15), used to assess homogeneity, indicate some spread towards higher values . Residuals vs. leverage panel does not show any observation highly influential (cook's distance >1) although observation 13 is near the limit.

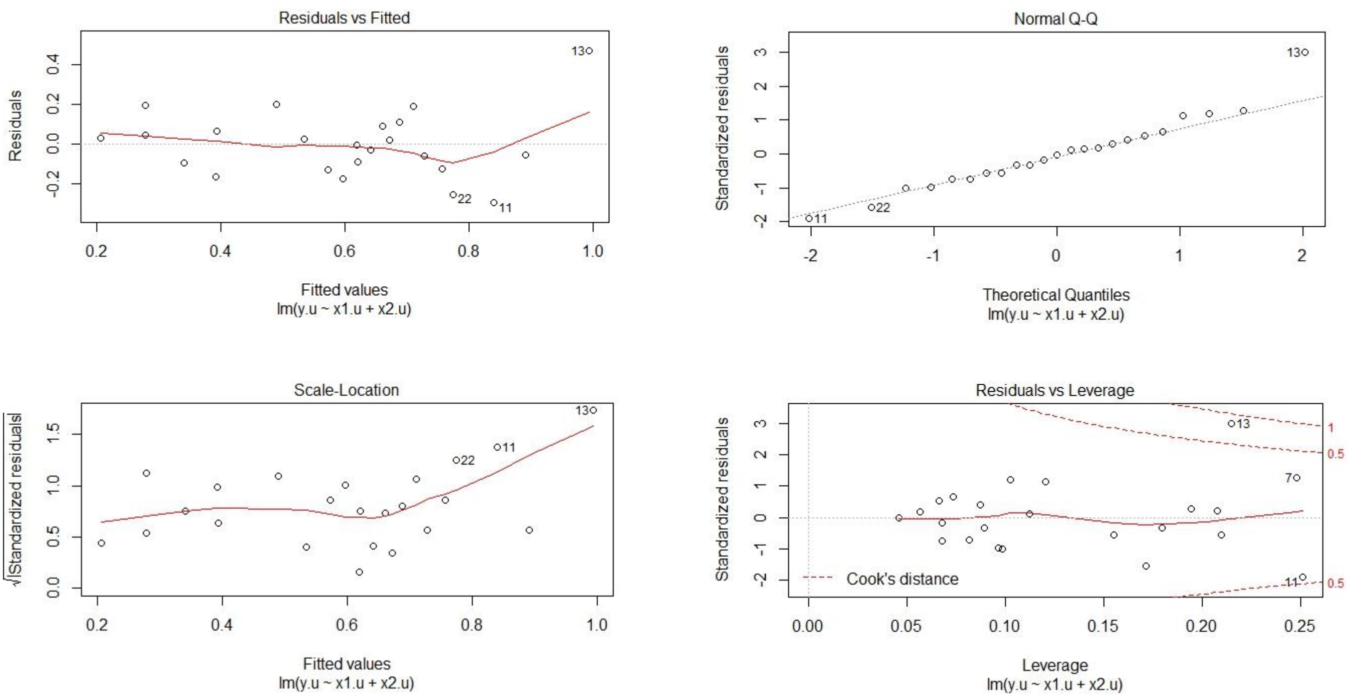



Figure 15. Model validation plots for multivariate model EQR ~ TN + TP, which presented the lowest AIC in comparison to the univariate models (AIC: NP mod = -9.499; N mod = -2.906; P mod = -3.885).

The model predicted nutrient boundaries for H/G and G/M for both nutrients are presented in Figure 16.

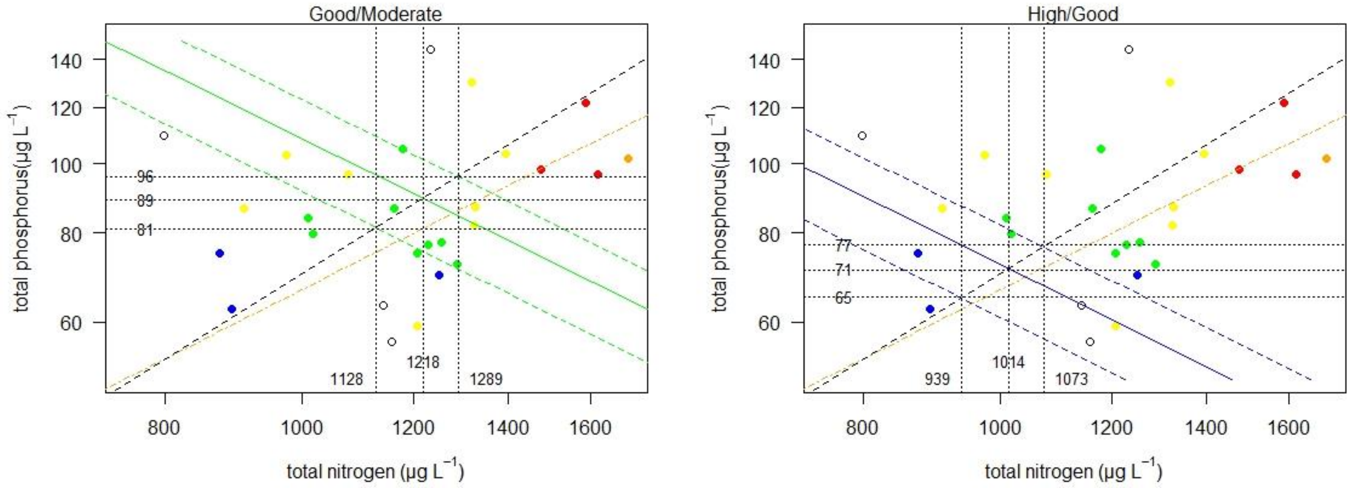
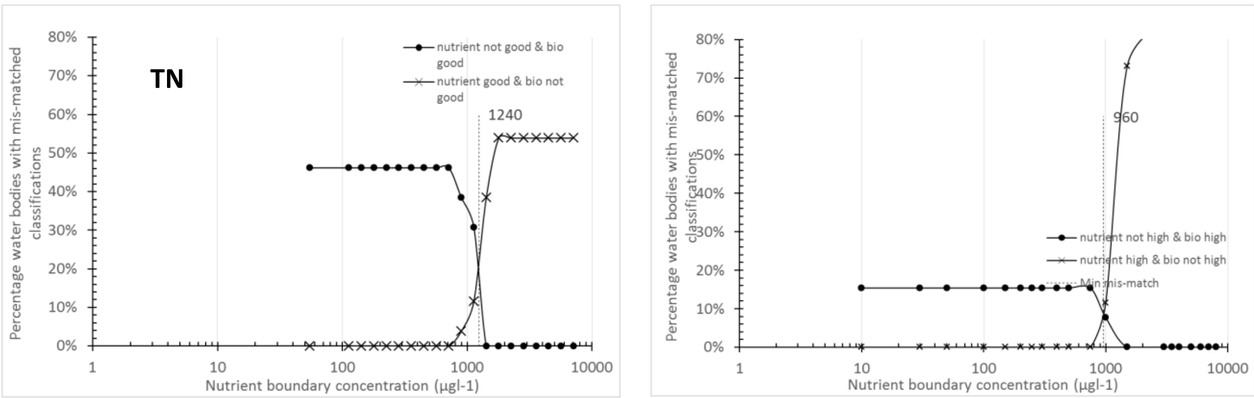



Figure 16. Relationship between TN and TP for coastal lagoons (within common type BT1) from the Baltic GIG. Points coloured by phytoplankton EQS class, dotted line marks the mean N:P ratio, broken orange line ratio of 15:1. Green and blue lines mark contours of the good/moderate and high/good boundaries predicted from the bivariate model.

### *Categorical Analyses*

Results of boundaries predicted by the categorical analyses for specific datasets within this TRWBALBT1 common type are presented in Table 11 and Table 12. The results obtained for H/G boundary for TN using the Average adjacent quartiles approach do not make sense, since they would indicate a higher nutrient concentration than that of the G/M boundary (G/M = 1126 < H/G = 1146 µg $L^{-1}$).

Below we show the graphs for the Minimise Mismatch Classification method for Lithuania, as example of outputs from this approach (Figure 17).



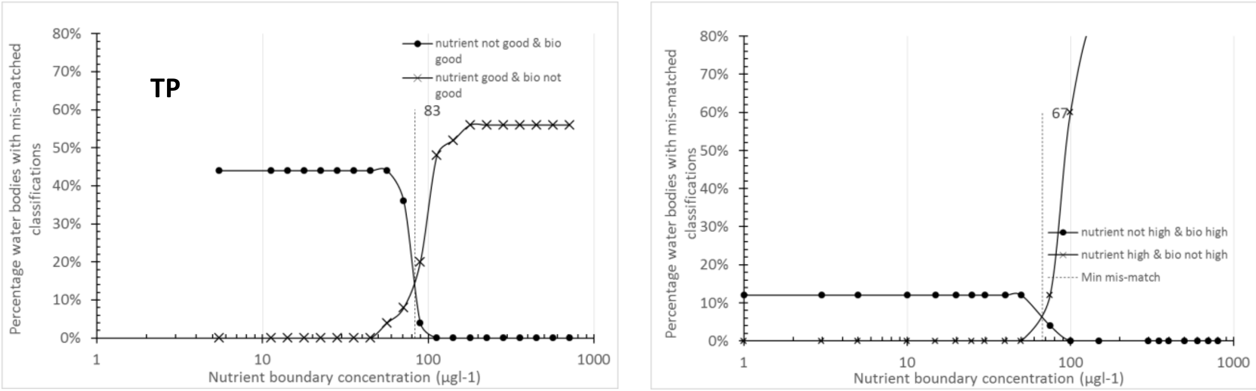


Figure 17. Relationship between percentage of mis-classified records comparing biological and nutrient classifications in comparison to value of nutrient boundary Good/Moderate (left) and High/Good (right). Broken line marks point of minimum mis-classification.

*Check the excel toolkit templates and R scripts for analyses' details (Annex files).*

### 5.1.1.2. Poland (ds2_TRWBALBT1)

#### a) Data check

There are very few observations (n=29) in this dataset. Although the EQR values range all five ecological quality classes (High to Bad), there is great overlap between some of the classes, both for TN and TP (Figure 18). The EQR boundaries used to establish quality classes are from the WFD IC exercise and are presented in Table 3. Both nutrients have two candidate outliers each (TN at 1121 and 2406 µg $L^{-1}$; TP at 160 and 297 µg $L^{-1}$).

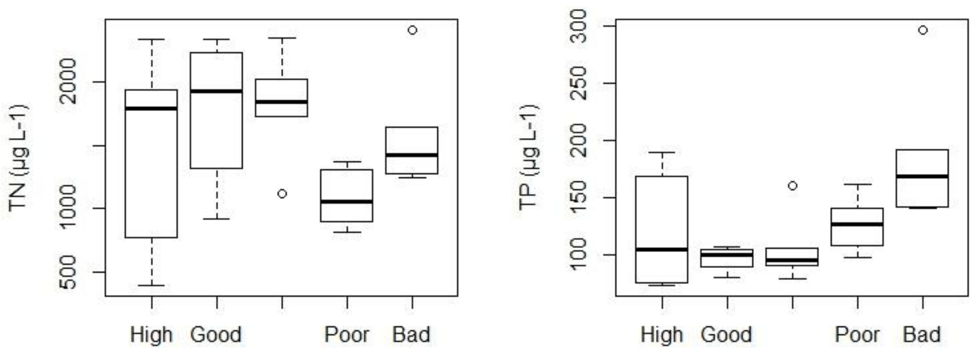


Figure 18. Range of Total Nitrogen (TN) and Total Phosphorus (TP) concentrations for Poland sites grouped by ecological quality status (EQS) class: High, Good, Moderate, Poor and Bad.

Total Phosphorus data presents a left-skewed distribution (Figure 19), which improved more towards a normal distribution after $log_{10}$ transformation. Both variables are transformed for linear regression analysis.

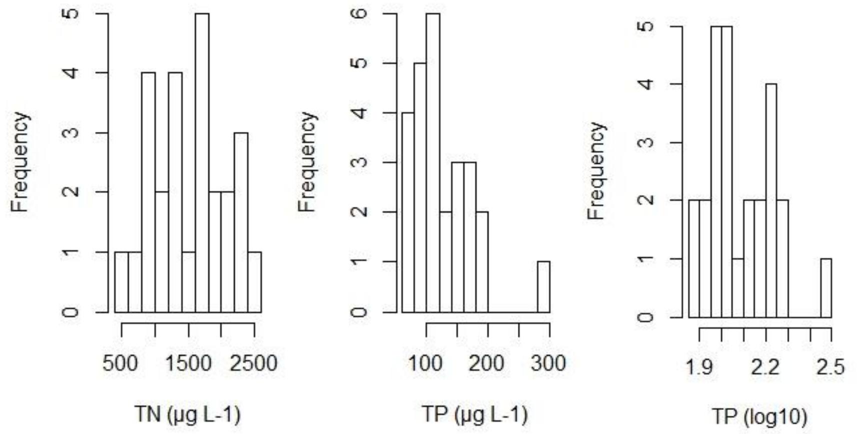



Figure 19. Range distribution of nutrient values for Total Nitrogen (TN) and Total Phosphorus (TP normal and log transformed).

### i. Outliers

Potential outliers in the variables of interest in this dataset are shown in Figure 20. The outlier value of TP (297 µg L$^{-1}$) had already came out in previous plots of distribution of this nutrient across the EQS classes (in Figure 18). The observation with EQR outlier > 1.0 will only be removed if justifiable by other evidence. The Cleveland plots distribution show however that these outliers may not be problematic outliers and will only be removed after analysing the *xy* relationships, following the nutrient Guidance protocol (2017). The outliers finally removed from the analysis are reported in the next section.

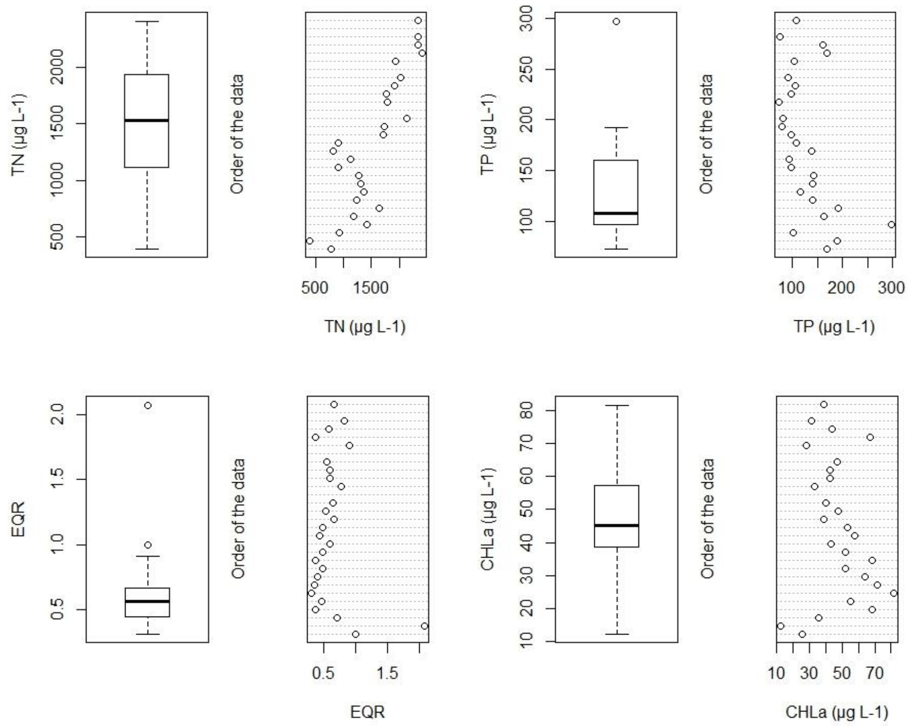



Figure 20. Outliers' verification in the predictors (TN, TP) and response (EQR, Chl*a*) variables, using box plots and Cleveland plots.

### i. Collinearity among covariates

There is no collinearity (Pearson r =-0.253, n=29) between covariates Total Phosphorus (TP) and Total Nitrogen (TN) (Figure 21).

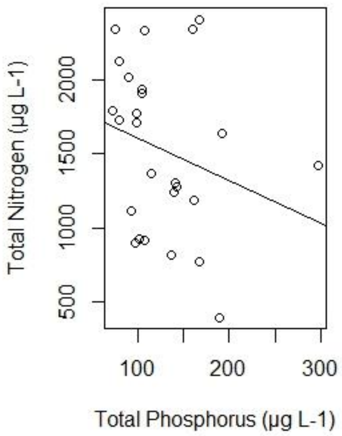



Figure 21. Scatterplot between nutrient explanatory variables Total Nitrogen (TN) and Total Phosphorus (TP).

### ii. Relationship between X & Y

The correlation between nutrients and EQR is very low (EQR vs TN r= -0.287, n=29; EQR vs TP r= 0.009, n=29) with no clear patterns (Figure 22). The EQR outlier previously mentioned (>1.0) appears to be interfering greatly with the relationships observed, particularly for TP whose relationship with EQR improves substantially after outlier removal (EQR vs TP r= -0.427, n=28). However for TN the relationship after EQR removal shows opposite trends to ecological expectations, with an increase of EQR with TN, even if evidences are still very weak (EQR vs TN r= 0.145, n=28). In the univariate regressions outliers will be dealt separately for each nutrient. The univariate regressions models applied in section 4 will use $Log_{10}$ transformation of the nutrient concentration.

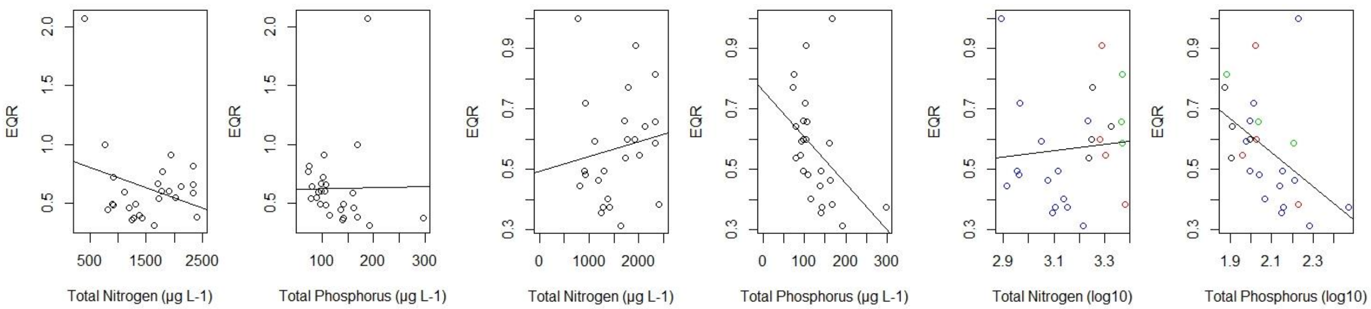



Figure 22. Scatterplot of the relationship of nutrient variables Total Nitrogen (TN) and Total Phosphorus (TP) with EQR, including all observations (left), removing EQR outlier >1 (centre), and after EQR>1 removed and nutrient variables log10 transformed (right, coloured by Site).

There is a sign of a possible interaction between explanatory variables TN and TP since most sites with high TN and relatively good ecological status seem to have a very low TP concentration (Figure 22 right). Observing the coplot below (Figure 23) we can see that the relationship of EQR with TN changes for high TP values (above 150 µg $L^{-1}$, upper right panel).

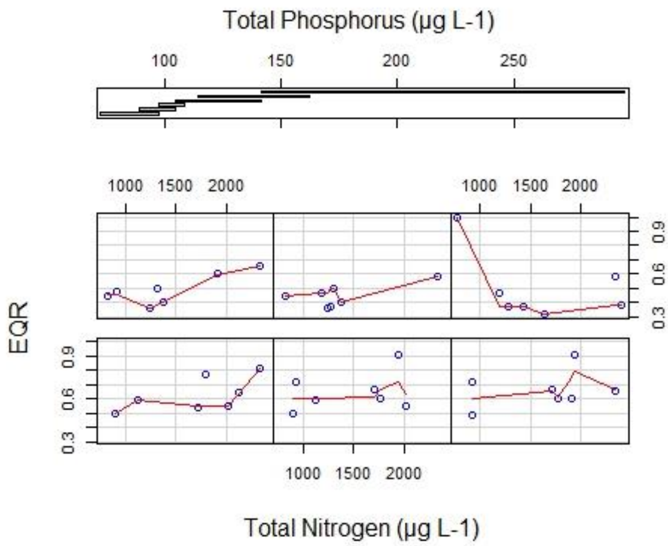
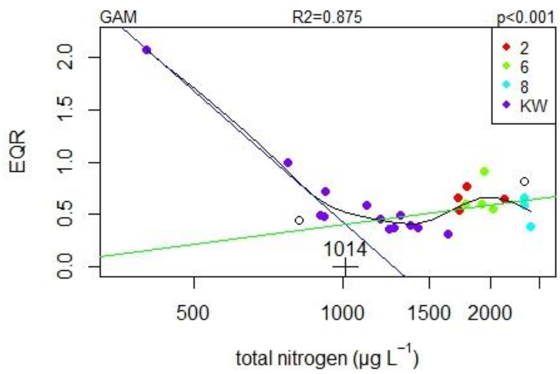



Figure 23. Coplot showing evidences of an interaction between explanatory variables TN and TP. EQR starts decreasing with increasing TN but only for higher TP values of after approx. 150 µg $L^{-1}$ (upper right panel).

To be able to use the linear regressions (toolkit) to predict TN boundaries we would need to remove the non-linear gradient of the data (with TN > 1600 µg $L^{-1}$). This break point is estimated more accurately with GAM and segmented regression (Figure 24) at TN = 1014 µg $L^{-1}$. However this breakpoint will leave very few observations for the regression analysis (n=5).

Ideally, for this dataset we should consider multiple regression analysis, including both explanatory variables (TN and TP), as suggested in the Guidance (2017): "… where there is good evidence of N and P co-limitation; threshold values may be interdependent and multiple regression allows values of one variable to be predicted for the full range of values of the second".




Estimated break point (1013.972) at higher end of TN gradient with its lower (957.9393) and upper (1073.282) confidence limits.

Figure 24. Scatter plot showing relationship between EQR and TN with fitted GAM model, points coloured by SITE, open circles outliers not used to fit model.

### b) Statistical analysis

For Poland, EQR relationships with Total Nitrogen (TN) and total Phosphorus (TP) were determined, using $log_{10}$ transformed nutrient data. The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Bivariate regressions EQR ~ TN + TP
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below (Table 11 and Table 12).

***Univariate regressions EQR ~ Total Nitrogen (TN)***

In the Polish dataset two outliers were removed from the observations. The linear range of the data covered a nutrient range approximately from 400 to 1640 $\mu gL^{-1}$ and observations out of the linear range were dropped from the analysis (Figure 25). Despite that this breakpoint differs from that proposed by the GAM and segmented regression (Figure 24) this option leaves us with a slightly more representative number of observations (n=13 instead of 5) and linear regression results obtained show a very strong relationship (r=0.927; Table 11).

Dropped observations belong all to a particular subset of the data, after which none observation from this subset has remained in the analysis (belonging to sites '2', '6', and '8'). When analysed separately it showed a low and non-significant correlation between EQR and TN (r=0.471). All observations in this subset present Total Nitrogen values >1710 $\mu gL^{-1}$, which is higher than any of the values observed in the remaining samples in this dataset (belonging all to site 'KW'). Previously we had seen that an interaction with TP (Figure 23) might explain the relatively high EQR values encountered in some samples with the highest TN levels.

In this case, the risk of using TN boundaries derived based solely on a subset of the data, is that it would place all samples in one of the subsets below the nutrient standards for TN, even if half of them present at least Good ecological status according to the Phytoplankton BQE. So nutrient classifications in support ecological status should be modelled together and results compared. The *Site* influence should also be further scrutinized and discussed with additional local data and MS expertise, possible testing the influence of this factor in a multivariate model.

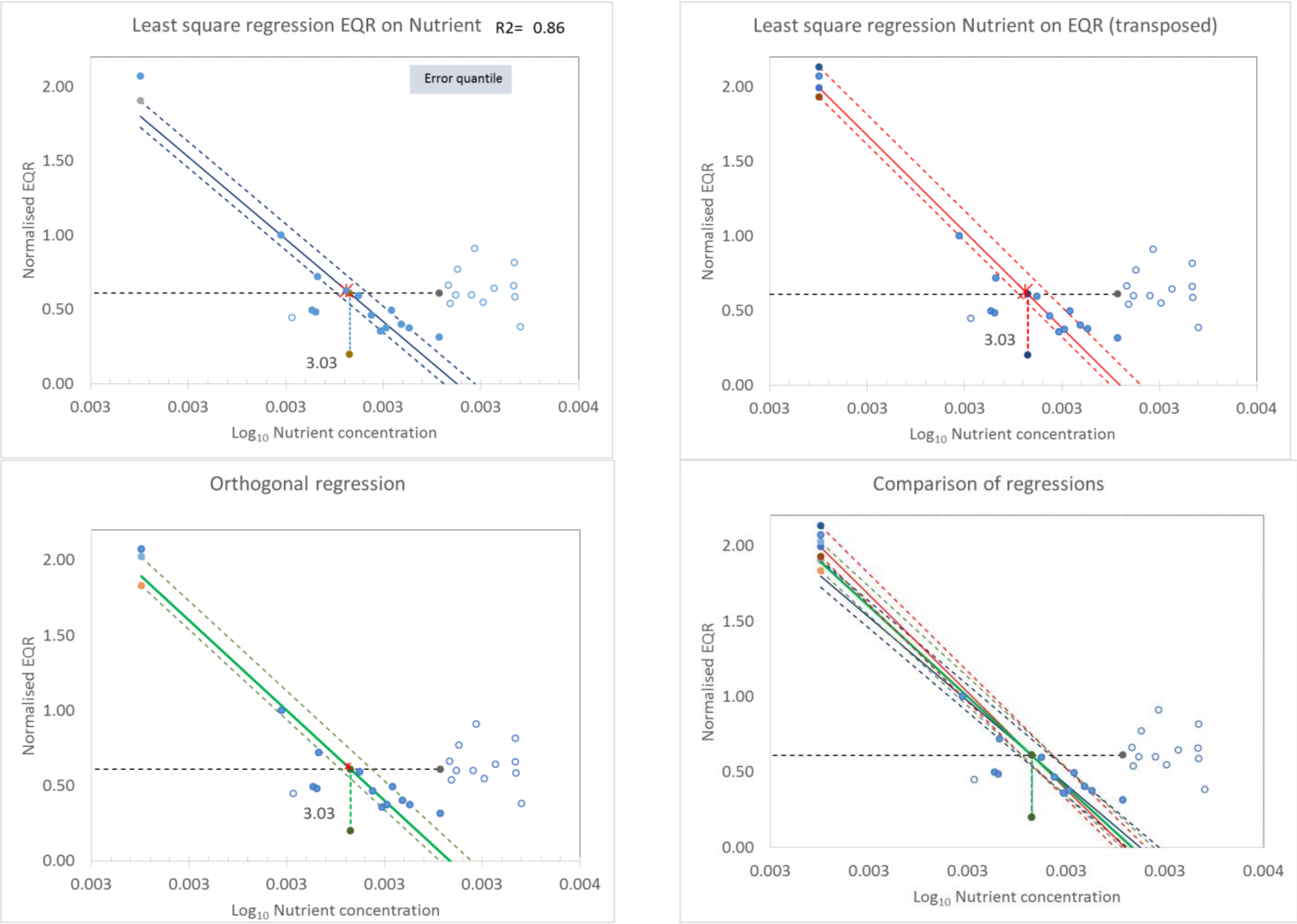



Figure 25. Regressions with Total Nitrogen for Poland: (top left) OLS regression of EQR v log10 nutrient concentration; (top right) OLS regression of log10 nutrient concentration v EQR; (bottom left) Orthogonal type II regression of EQR v log10 nutrient concentration; (bottom right) comparison of above regressions. Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

The TN model's validation may however indicate lack of suitability of these models (Figure 26). The residual frequency distribution does not have a normal distribution for Poland, presenting substantial skewness pattern. The residuals also show great variability along the fitted values for both nutrients (no homogeneity observed).

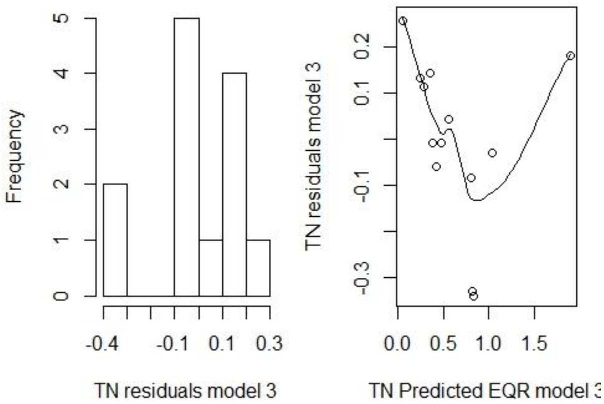



Figure 26. TN Model diagnosis of residual's normal distribution (histograms) and homoscedasticity (scatterplots), using ordinary residuals. Graphs are presented only for regression model 3 (RMA Orthogonal type II regression of EQR versus log10 of nutrient concentrations (TN) which is an average of model 1 – OLS EQR vs nutrient and model 2 OLS nutrient vs. EQR).

### *Univariate regressions EQR ~ Total Phosphorus (TP)*

Results of the linear regression analyses for TP are presented for Poland in Figure 27, but the model explains very poorly the variation in the data ($R^2$=0.209, Table 4). The graphs below show the different nutrient (TP) boundaries proposed by regressions approaches applied.

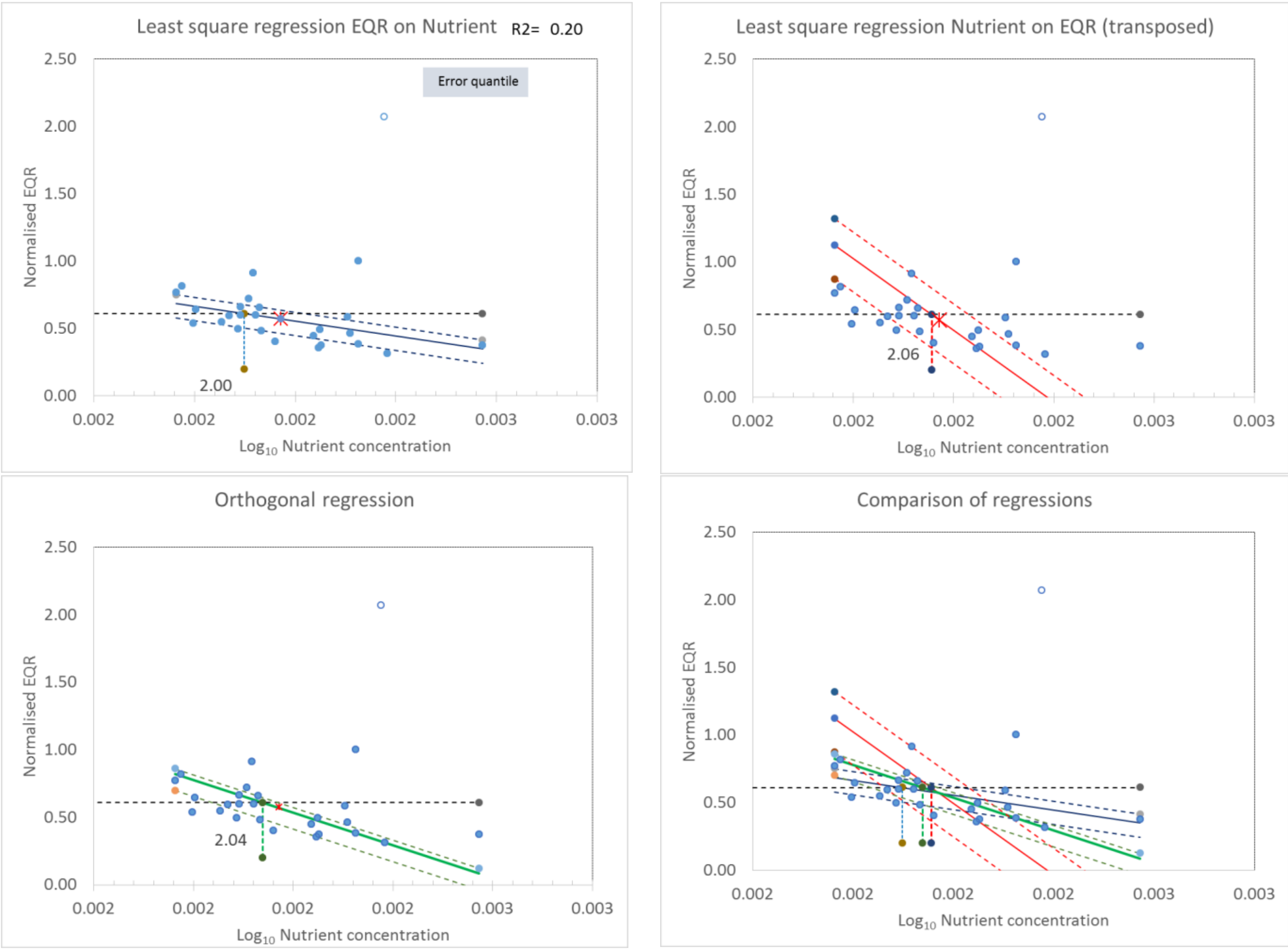



Figure 27. Regressions with Total Phosphorus for Poland: (top left) OLS regression of EQR v log10 nutrient concentration; (top right) OLS regression of log10 nutrient concentration v EQR; (bottom left) Orthogonal type II regression of EQR v log10 nutrient concentration; (bottom right) comparison of above regressions. Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

As for TN, models' validation for TP indicates lack of suitability of these models (Figure 28). The residual frequency distribution has a nearly normal distribution, but the residuals show great variability along the fitted values for both nutrients (no homogeneity observed).

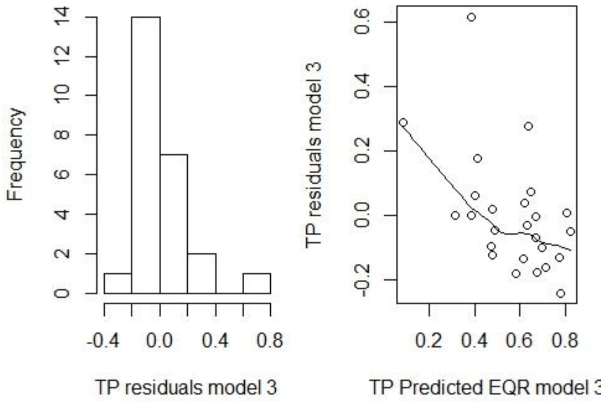



Figure 28. TP Model diagnosis of residual's normal distribution (histograms) and homoscedasticity (scatterplots), using ordinary residuals, for Poland. Graphs are presented only for regression model 3 (RMA Orthogonal type II regression of EQR versus log10 of nutrient concentrations (TN) which is an average of model 1 – OLS EQR vs nutrient and model 2 OLS nutrient vs. EQR).

***Bivariate regressions EQR ~ TN + TP***

The possibility of a significant interaction between nutrients was not observed, as the bivariate model considering both TN and TP is not significant (adjusted $R^2$ = 0.088; p-value= 0.1542, n=23). *Check and run R scripts for analyses' details (in Annex).*

***Categorical Analyses***

Results of boundaries predicted by the categorical analyses for specific datasets within this TRWBALBT1 common type are presented in Table 11 and Table 12. *Check the excel toolkit templates for analyses' details (in Annex).*

### 5.1.1.3. Nutrient boundaries

Here we present the predicted nutrient boundaries at the Good/Moderate and High/Good boundaries of TN and TP (µg $L^{-1}$) within common this type TRWBALBT1 per country: Lithuania (LT) and Poland (PL). The different sets of boundaries proposed were derived from univariate, and bivariate (LT), linear regression models and categorical analysis. Details for each approach are presented in the next sections.

Attention must be taken where H/G boundaries surpass nutrient concentrations values predicted for the G/M boundaries (highlighted red in the table). Also, the low $R^2$ (<0.36) obtained in TP model for Poland indicates that the univariate linear regression approach is not adequate for this data set, and thus boundaries proposed cannot be trusted. Results obtained are summarised in Table 11 and Table 12, presented per country and nutrient.

Table 11. Summary of predict values of nutrient concentration at the Good/Moderate and High/Good boundaries of TN ($\mu g\ L^{-1}$), per country, Lithuania (LT) and Poland (PL), within common type TRWBALBT1, results obtained by regression and categorical analyses.

| MS | Phytoplankton Models | $R^2$<br>*p-value*<br>n | Nutrient range TN*<br>$\mu g\ L^{-1}$ | GM TN $\mu g\ L^{-1}$ | | | HG TN $\mu g\ L^{-1}$ | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| **Lithuania**<br>EQR_Chl*a*<br>Boundaries:<br>HG 0.83<br>GM 0.57<br>MP 0.39 | EQR v TN (RMA) | **0.410**<br>*<0.001*<br>n=25 | 875 - 1700 | 1224 | 1218 | 1233 | 1020 | 928 | 1084 | GM<br>1122-<br>1333<br>HG<br>845-<br>1187 |
| | EQR v TN+TP | **0.556**<br>*<0.001*<br>n=23 | 798 1700 | 1218 | 1128 | 1298 | 1014 | 939 | 1073 | |
| | Average adjacent quartiles | | | 1126 | | | 1146 | | | |
| | Average adjacent classes | n=25 | | 1206 | | | 1101 | | | |
| | Average 75th quartile | | | 1235 | | | 1168 | | | |
| | Minimise class difference | | | 1240 | | | 960 | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| **Poland**<br>EQR_Chl*a*<br>Boundaries:<br>HG 0.77<br>GM 0.61<br>MP 0.5 | EQR v TN (RMA) | **0.860**<br>*p<0.001*<br>n=13 | 400 - 1640 | 1072 | 1071 | 1073 | 948 | 940 | 956 | GM<br>900-<br>1187<br>HG<br>400-<br>1056 |
| | EQR v TN+TP | **0.088**<br>*p=0.154*<br>n=23 | 798 1700 | -- | | | -- | | | |
| | Average adjacent quartiles | | | 1022(1823) | | | 662(1645) | | | |
| | Average adjacent classes | n= 13<br>(24) | (400 - 2406) | 1022(1777) | | | 662(1788) | | | |
| | Average 75th quartile | | | 923(1908) | | | 400(2032) | | | |
| | Minimise class difference | | | 900(1200) | | | 600(950) | | | |

*Despite that MS reported data in mg $L^{-1}$, to enable comparison with other TRW units presented are in $\mu g\ L^{-1}$.

Table 12. Summary of predict values of nutrient concentration at the Good/Moderate and High/Good boundaries of TP (µg $L^{-1}$), per country, Lithuania (LT) and Poland (PL), within common type TRWBALBT1, results obtained by regression and categorical analyses.

| MS | Phytoplankton Models | $R^2$ *p-value* n | Nutrient range TP µg $L^{-1}$ | Most likely boundary GM TP µg $L^{-1}$ | | | Most likely boundary HG TP µg $L^{-1}$ | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | |
| **Lithuania** EQR_Chl*a* Boundaries: HG 0.83 GM 0.57 MP 0.39 | EQR v TP (RMA) | **0.432** *p<0.001* n=24 | 59 - 130 | 89 | 88 | 90 | 73 | 66 | 78 | GM 82-99 HG 61-86 |
| | EQR v TN+TP | **0.556** *<0.001* n=23 | 56 145 | 89 | 81 | 96 | 71 | 65 | 77 | |
| | Average adjacent quartiles | n=24 | | 84 | | | 74 | | | |
| | Average adjacent classes | | | 85 | | | 74 | | | |
| | Average $75^{th}$ quartile | | | 85 | | | 72 | | | |
| | Minimise class difference | | | 83 | | | 67 | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | |
| **Poland** EQR_Chl*a* Boundaries: HG 0.77 GM 0.61 MP 0.5 | EQR v TP (RMA) | **0.209** *p=0.022* n=25 | 73 - 297 | 109 | 100 | 114 | 81 | 51 | 99 | GM 64-135 HG 33-168 |
| | EQR v TN+TP | **0.088** *p=0.154* n=23 | 798 1700 | | | | | | | |
| | Average adjacent quartiles | n=25 | | 97 | | | 131 | | | |
| | Average adjacent classes | | | 98 | | | 102 | | | |
| | Average $75^{th}$ quartile | | | 103 | | | 168 | | | |
| | Minimise class difference | | | 101 | | | 88 | | | |

## 5.2. Mediterranean Sea

### 5.2.1. Common Type: TRWMEDpolyCL

Phytoplankton intercalibration data from the type TRWMEDpolyCL in transitional waters was used. This dataset contains data for Total Nitrogen (TN), Total Phosphorus (TP) and Dissolved Inorganic Nitrogen (DIN). There are different methods being used across Member States (MS) sharing this typology. The MPI score is used by Italy and Greece, while France uses a different EQR Phytoplankton method. Analyses will be presented for both groups separately.

Since they had different EQR method and IC EQR boundaries, we split data to run analyses for this type; using the Italian and Greek shared EQR boundaries (taken from Table 3) and the French data and respective EQR boundaries separately, as shown below. However this generated very few observations available per analysis and it should be considered the possibility of pulling data together (i.e. normalising EQRs) if all EQS boundaries are made available by the MS.

### 5.2.1.1. Italy and Greece (ds3_TRWMEDpolyCLa)

a) Data check

The nutrient distribution (only showed for TN and TP, $\mu g L^{-1}$) along the available EQS classes (Figure 29) shows that data for TN does not cover the full range of disturbance required for deriving nutrient boundaries at the Good/Moderate range. Furthermore, only two EQR boundaries have been provided, at High/Good and Good/Moderate. One outlier for TN (716.4 $\mu g\ L^{-1}$) and two for TP (84.95 $\mu g\ L^{-1}$; 89.8 $\mu g\ L^{-1}$) might need to be excluded from the analysis, but this will be confirmed after checking for xy relationships.

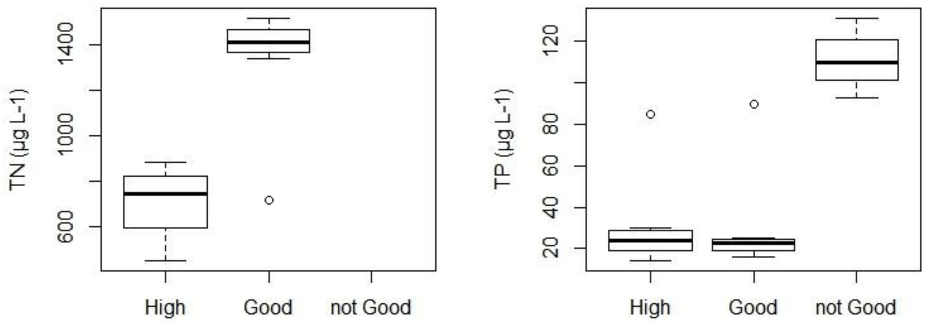



Figure 29. Range of TN and TP concentrations for sites grouped by ecological status classes (EQS: High, Good and below Good).

There are also very few observations in this dataset (n=17 for TP and DIN, and n=14 for TN) with nutrients presenting a non-normal distribution.

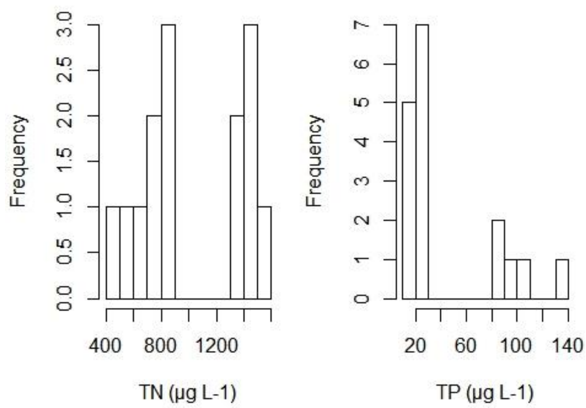



Figure 30. Range distribution of nutrient values (TN and TP).

**i. Outliers**

Checking for outliers in the predictors and response variables (X & Y), using Boxplot and Cleveland plots of DIN, TN, TP, and MPI score (the EQR equivalent method for IT and GR). No outliers observed while analysing distribution of each variable alone (Figure 31).

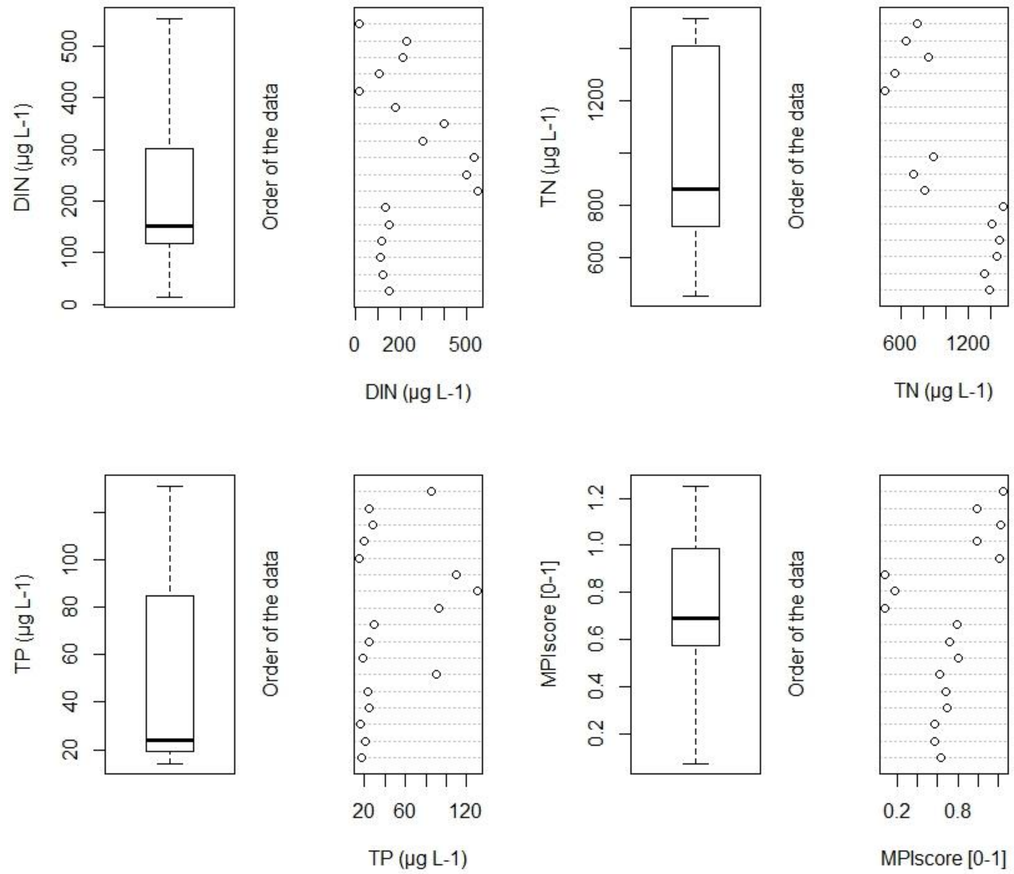



Figure 31. Outliers verification in the predictors (DIN, TN and TP) and in the response variable, EQR here represented by the MPI score method.

### ii. Collinearity among covariates

It was not observed collinearity (Figure 32) between any of the explanatory variables, using Pearson Correlation (r): TN vs TP r=0.128; TN vs DIN r=-0.210; and TP vs DIN r=0.05.

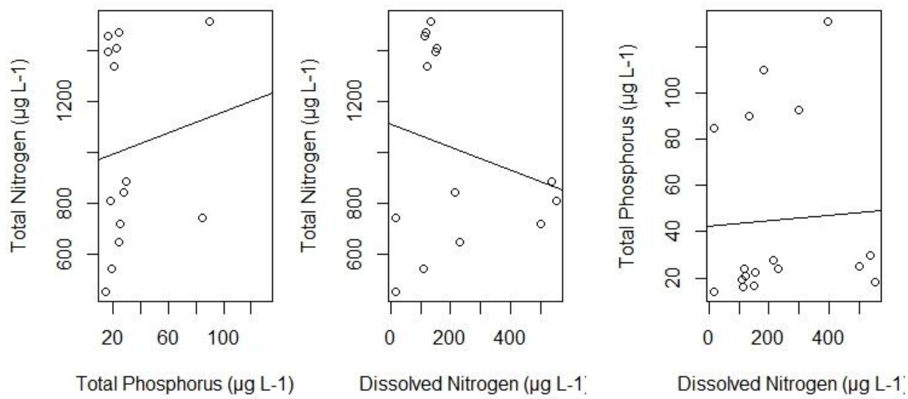



Figure 32. Scatter plots between explanatory variables showing no collinearity.

### iii. Relationship between X & Y

Total Phosphorus (TP) and Total Nitrogen (TN) show a relatively good negative correlation with the MPI score, presenting the highest Pearson correlations between pressure and response variables: TN (r= -0.78) and TP (r=-0.57). MPI score relationship with DIN was weaker (r= -0.260). There was also no evidence of interaction between the explanatory variables, as shown by the coplot (showed only for TN and TP, since these will be the ones used for the univariate regression analyses) (Figure 33).

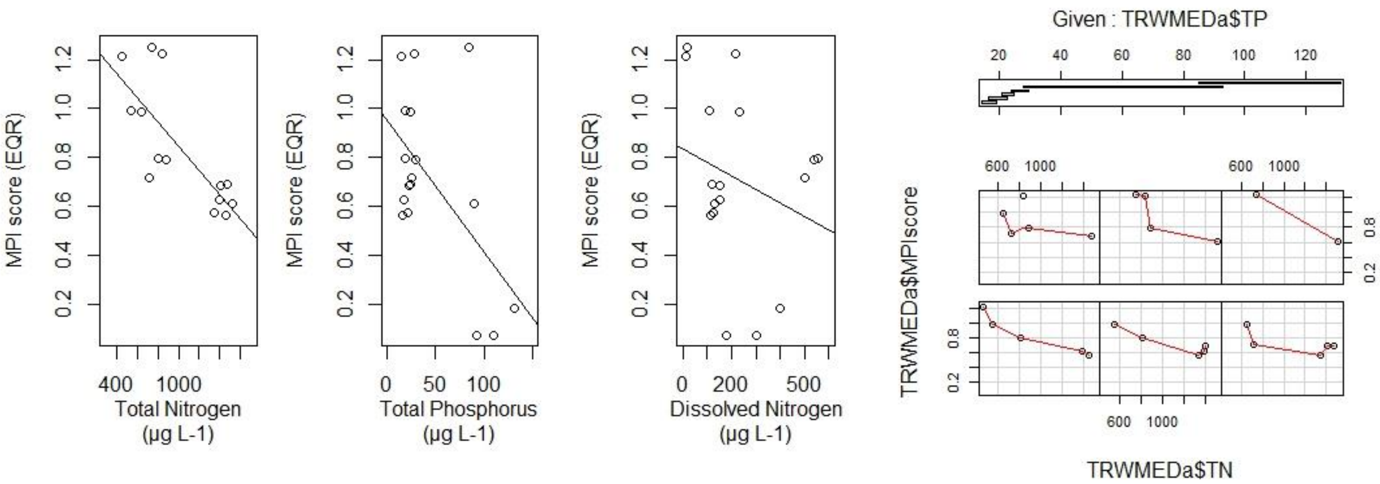



Figure 33. Scatter plots of the relationship between nutrient variables and the Phytoplankton EQR method (MPI score), and coplot for checking possible interaction between TN and TP.

The xy analyses revealed also that for TN (using the Guidance excel toolkit) there were two outliers beyond the 0.975 upper quantile of the data (in the *xy* distribution) used for outlier detection: at 716.4 µg $L^{-1}$ and 840.2 µg $L^{-1}$. For TP also two outliers were identified using similar procedure, at 15.9 µg $L^{-1}$ and 84.95 µg $L^{-1}$.

## b) Statistical analysis

Linear regression analyses included Italy (IT) and Greece (GR), and used the common EQR IC boundaries adopted by both of them as indicated in Table 3. EQR relationships with Total Nitrogen (TN) and total Phosphorus (TP) were determined using $\log_{10}$ transformed nutrient data. The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below (Table 13 and Table 14).

### *Univariate regressions EQR ~ Total Nitrogen (TN)*

For Italy and Greece, the regression analysis of MPI score (EQR method) with TN (r=0. 882, n=12) was good and above the advisable r (caution for r<0.6). Figure 34 shows that the EQR relationship with the nutrient ($log_{10}$ transformed data) presented a linear trend and besides the two outliers no data had to be dropped for this analysis.

For this dataset we need to be cautious since the model is predicting for outside the range of available data (Figure 34). As shown in section 3, there was no data below Good ecological status. Boundaries predicted should be further evaluated against data covering the full, or at least a wider, gradient of disturbance.

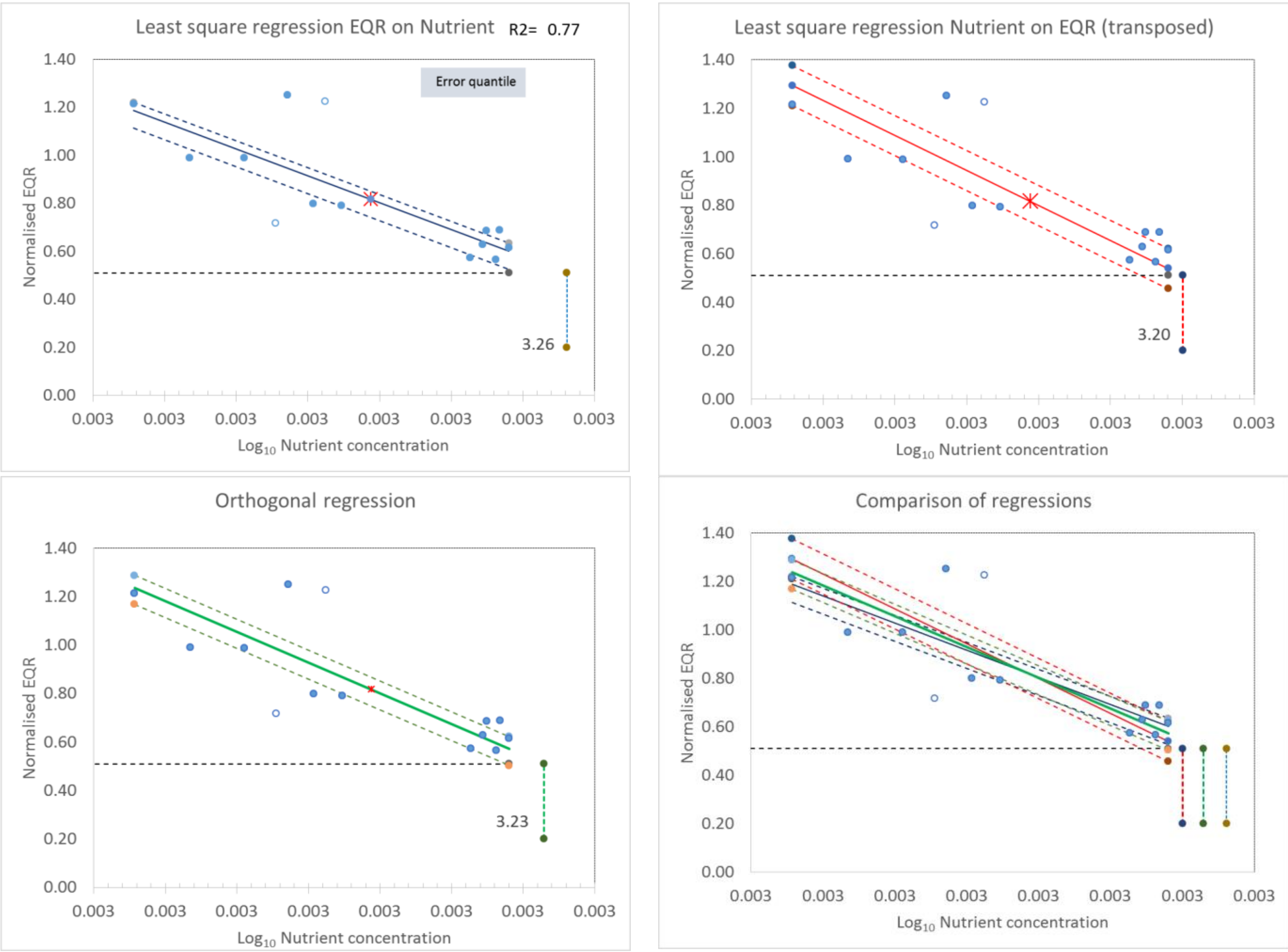



Figure 34. Regressions with Total Nitrogen for Italy & Greece: (top left) OLS regression of EQR v log10 nutrient concentration; (top right) OLS regression of log10 nutrient concentration v EQR; (bottom left) Orthogonal type II regression of EQR v log10 nutrient concentration; (bottom right) comparison of above regressions. Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

### *Univariate regressions EQR ~ Total Phosphorus (TP)*

Regressions' results of MPI score with TP was good (r=0.777, n=15) and above the advisable r (caution for r<0.6). Figure 35 shows that the EQR relationship with the nutrient (log10 transformed data) presented a linear trend and besides the two outliers no data had to be dropped for this analysis.

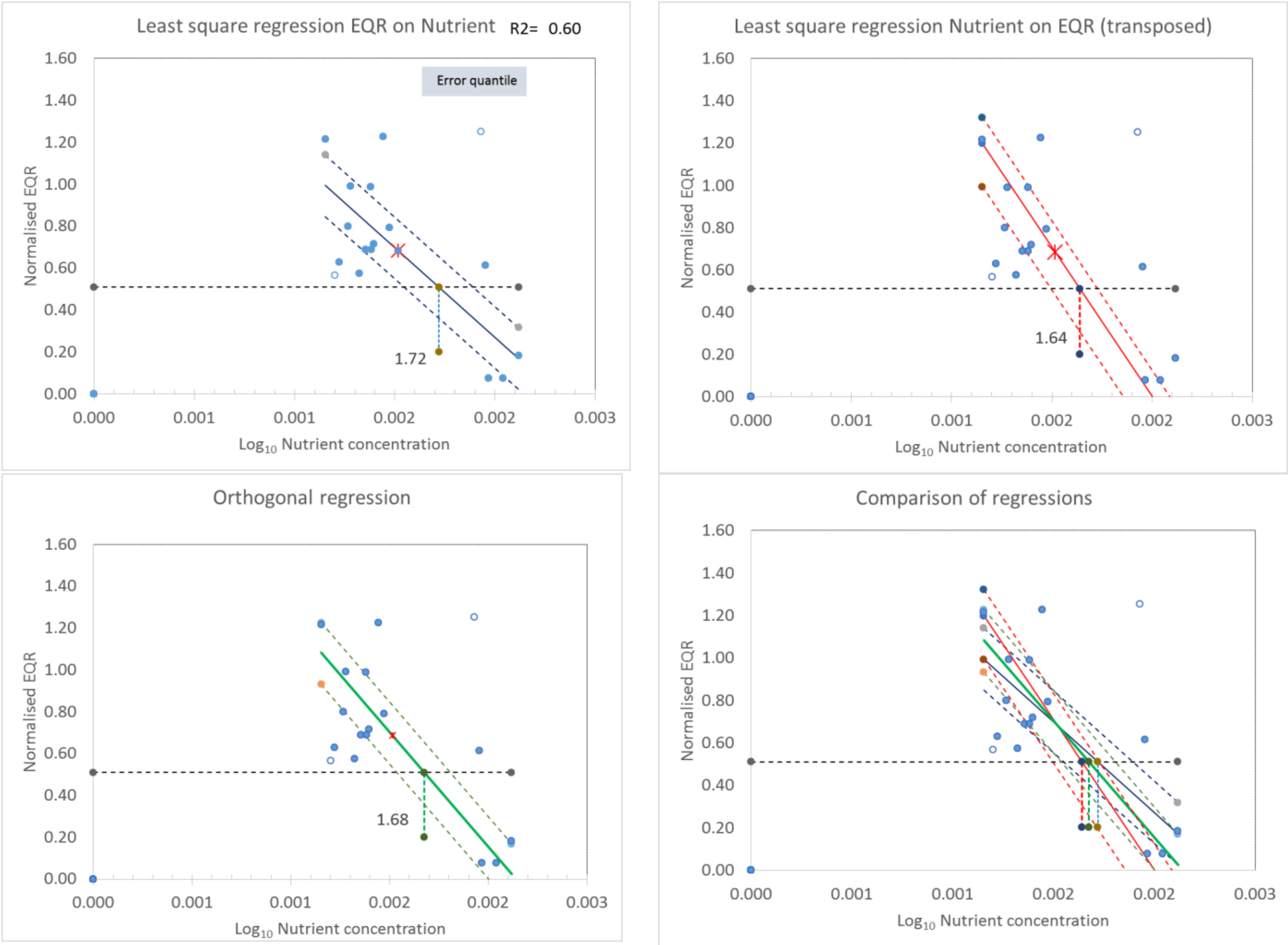



Figure 35. Regressions with Total Phosphorus for Italy & Greece: (top left) OLS regression of EQR v log10 nutrient concentration; (top right) OLS regression of log10 nutrient concentration v EQR; (bottom left) Orthogonal type II regression of EQR v log10 nutrient concentration; (bottom right) comparison of above regressions. Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

***Categorical Analyses***

There was not enough data covering the entire range of quality/disturbance, as the exploratory analyses had shown, and therefore some approaches could not determine an appropriate nutrient boundary for TN in the IT/GR data (Table 13). This stresses the importance of compiling a comprehensive dataset that covers a wider spectrum of conditions, containing as much as possible a balanced number of observations across several EQS classes (at least until the Moderate status).

Again, pulling data together could also be an option, but EQR methods' constrains must first be assessed (e.g. possible normalisation across all countries within a type).

#### 5.2.1.2. France (ds4_TRWMEDpolyCLb)

a) Data check

There are very few observations for France (n=15). The nutrient range available covers at least three ecological status classes (EQS, Figure 36) allowing to proceed with the analysis. There is however an unbalanced distribution across the good end classes with great overlap between High and Good. A slightly left-skewed distribution of the nutrient data can be observed (Figure 37), however regression analyses will use $log_{10}$ transformed data.

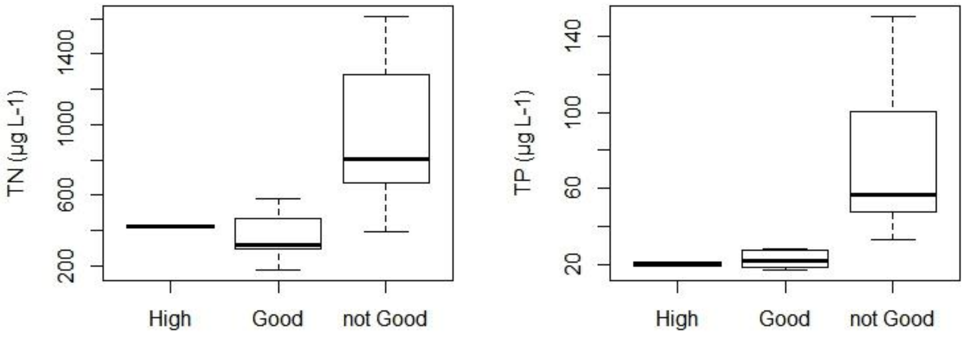



Figure 36. Range of TN and TP concentrations for France by ecological quality status (EQS) class.

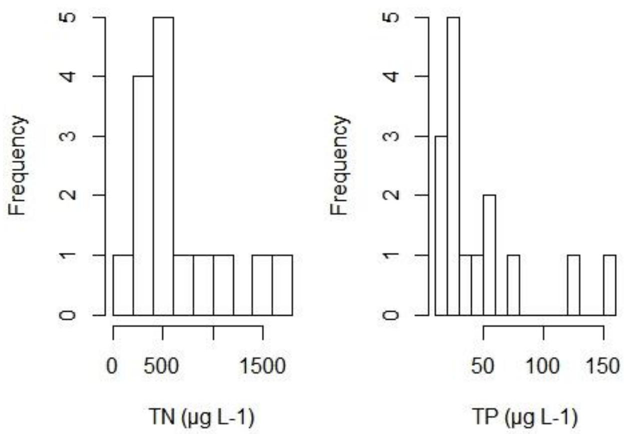



Figure 37. Range distribution of nutrient values TN and TP for the French dataset.

## i. Outliers

Checking for outliers in the predictors and response variables (X & Y), using Boxplot and Cleveland plots for TN (µg $L^{-1}$), TP (µg $L^{-1}$), and EQR (FR). The outliers that appear in the boxplots (for TN, TP and DIN) are not very spread apart from the remaining data if we observe the Cleveland plots (Figure 38). Outliers' removal will be decided after checking xy relationships.

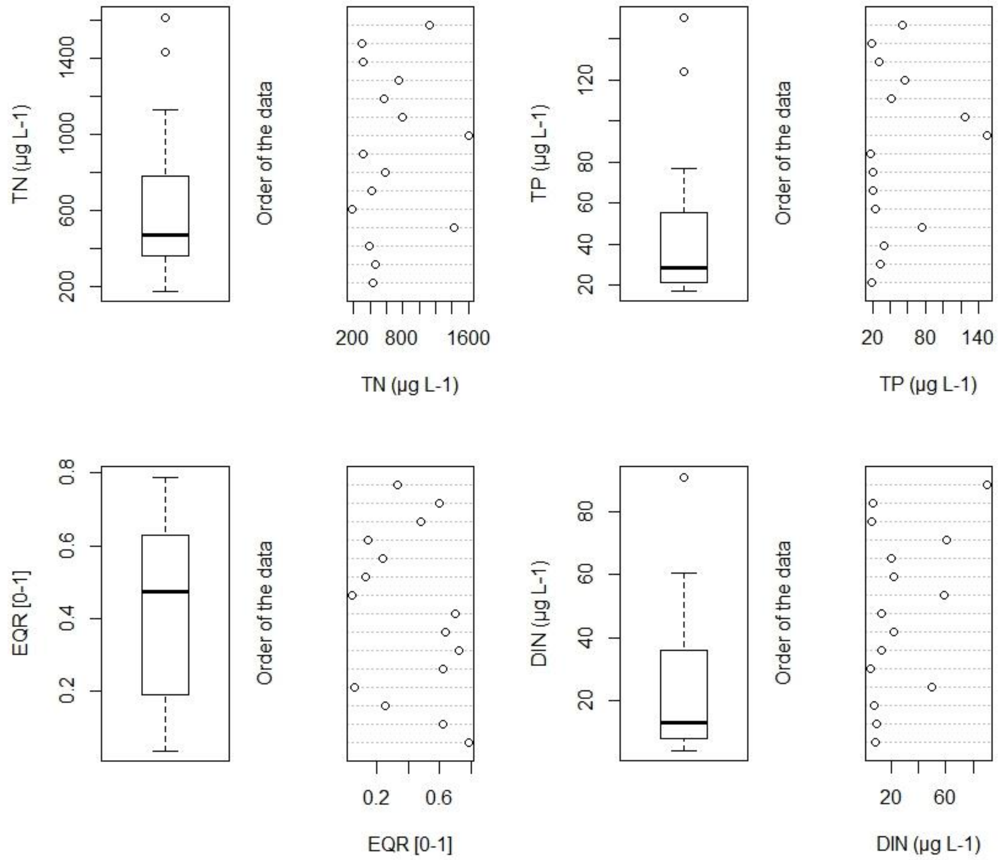



Figure 38. Outliers' verification in the predictors (TN, TP, DIN) and response variable EQR for the Phytoplankton, using boxplots and Cleveland plots.

### i. Collinearity among covariates

High correlation between covariates TP vs. TN (r= 0.811) and between TN vs. DIN (r= 0.807) (Figure 39). TP and DIN are not collinear (r= 0.529). If bivariate regressions are to be applied we must consider which variables to select, however VIF value < 8 indicate no collinearity between these covariates.

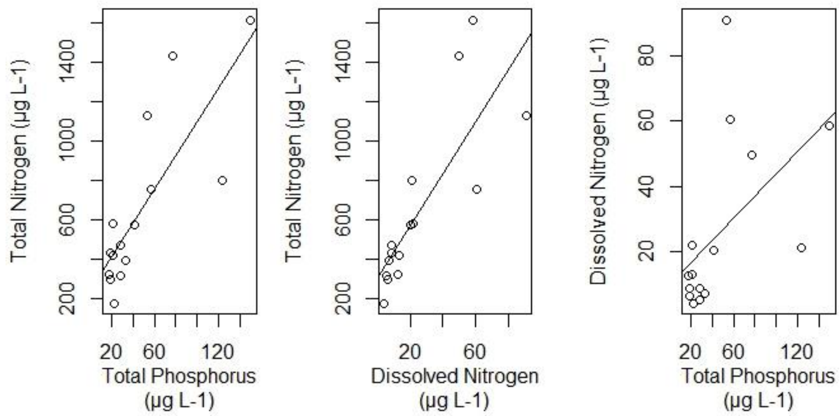



Figure 39. Scatterplots between explanatory variables TN, TP and DIN (µg $L^{-1}$).

### ii. Relationship between X & Y

All nutrient variables, Dissolved Nitrogen (DIN), Total Phosphorus (TP) and Total Nitrogen (TN), show a relatively good negative correlation (Pearson correlation, r) with the EQR of the Phytoplankton: EQR vs TN r = -0.745; EQR vs TP r = -0.798; EQR vs DIN: r = -0.600.

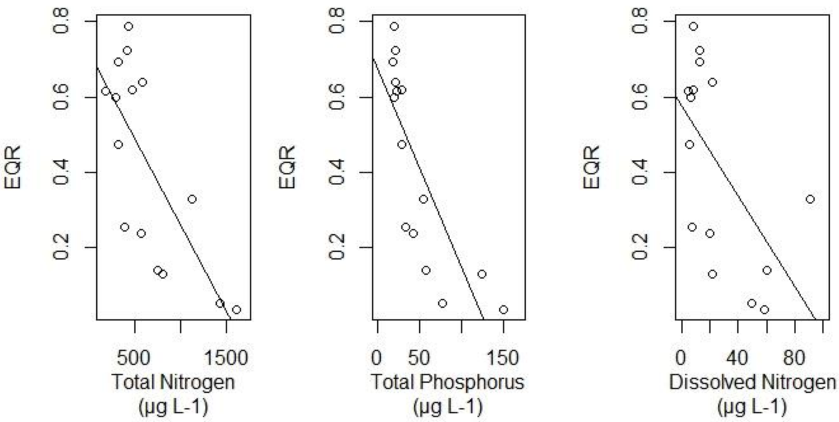



Figure 40. Scatterplots of the relationship between nutrient variables (TN, TP, and DIN, µg $L^{-1}$) and the Phytoplankton EQR method for France.

## b) Statistical analysis

Linear regression analyses for the French dataset (FR) used the EQR IC boundaries adopted by this MS as indicated in Table 3. EQR relationships with Total Nitrogen (TN) and total Phosphorus (TP) were determined using $\log_{10}$ transformed nutrient data. The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below (Table 13 and

Table 14).

### *Univariate regressions EQR ~ Total Nitrogen (TN)*

Regression results of EQR with TN are good (r=0.801; n=13).

To run regression analyses for this type we used the EQR boundaries (taken from Table 2) and the proposed nutrients boundaries are presented in Table 13, with indication of possible ranges.

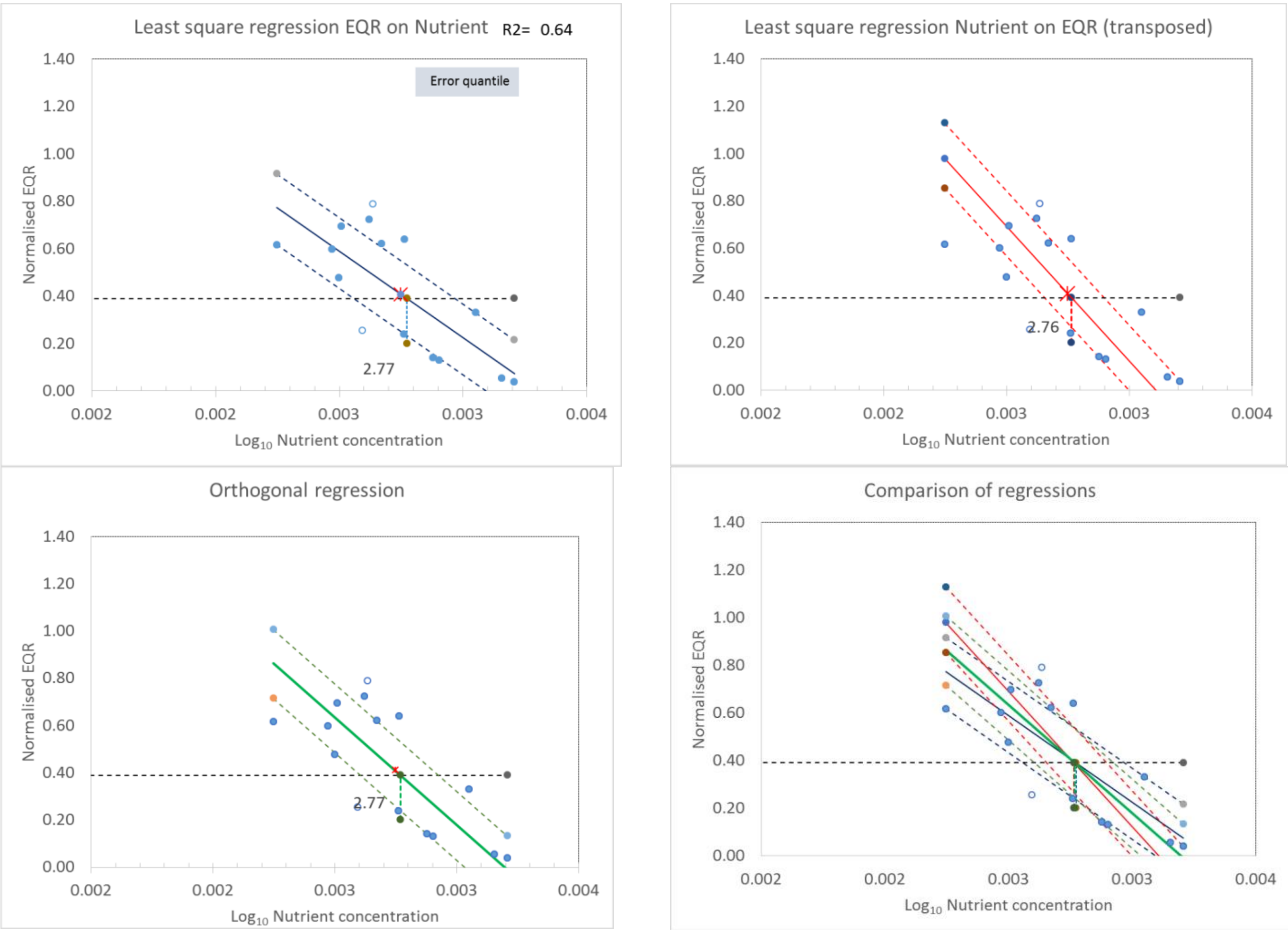



Figure 41. Regressions with Total Nitrogen for France: (top left) OLS regression of EQR v log10 nutrient concentration; (top right) OLS regression of log10 nutrient concentration v EQR; (bottom left) Orthogonal type II regression of EQR v log10 nutrient concentration; (bottom right) comparison of above regressions. Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

### *Univariate regressions EQR ~ Total Phosphorus (TP)*

Regressions' results of EQR Phytoplankton with TP were very good (r=0.932, n=14). The EQR relationship with the nutrient (log10 transformed data) presented a linear trend and, besides the two outliers, no data had to be dropped for this analysis (Figure 42).

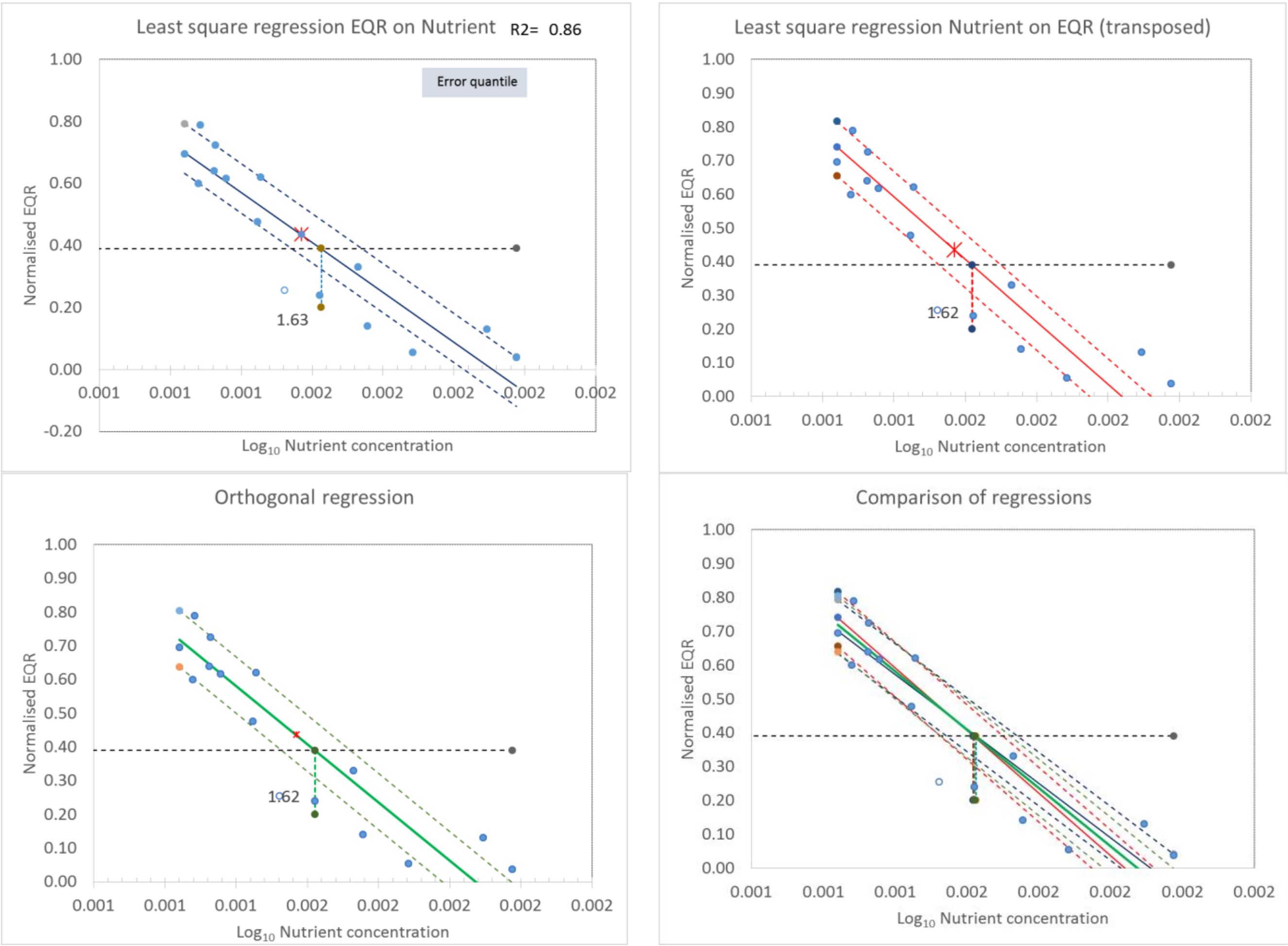



Figure 42. Regressions with Total Phosphorus for France: (top left) OLS regression of EQR v log10 nutrient concentration; (top right) OLS regression of log10 nutrient concentration v EQR; (bottom left) Orthogonal type II regression of EQR v log10 nutrient concentration; (bottom right) comparison of above regressions. Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

### *Categorical Analyses*

Results of boundaries predicted by the categorical analyses for France within this common type are presented in Table 13 and

Table 14. *Check the excel toolkit templates for analyses' details (in Annex).*

#### 5.2.1.3. Nutrient boundaries

Here we present the results obtained for nutrient boundaries at the Good/Moderate and High/Good boundaries of TN and TP (µg $L^{-1}$) within common this type. The different sets of boundaries proposed were derived from univariate linear regression models and categorical analysis. Details for each approach were presented in previous sections.

Boundaries proposed are summarised in Table 13 and

Table 14, presented per country and nutrient.

Table 13. Summary of predict values of nutrient concentration at the Good/Moderate and high/Good boundaries of TN (µg L$^{-1}$), per countries (IT/GR, and FR), within common type TRWMEDpolyCL, results obtained by regression and categorical analyses.

*prediction beyond the nutrient available range requires caution

| MS | Phytoplankton Models | $R^2$<br>*p-value*<br>n | Nutrient range<br>TN<br>µg L$^{-1}$ | Most likely boundary | | | | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | GM TN<br>µg L$^{-1}$ | | | HG TN<br>µg L$^{-1}$ | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| **Italy/ Greece**<br>EQR_Phyt<br>(MPIscore)<br>Boundaries: | EQR v TN (RMA) | **0.778**<br>*<0.001*<br>n=12 | 454 - 1515 | 1695* | 1588 | 1826 | 1039 | 1031 | 1049 | GM no data<br>HG 840-1176 |
| HG 0.78 | Average adjacent quartiles | | | no data | | | 1103 (1095) | | | |
| GM 0.51 | Average adjacent classes | n=12 | | no data | | | 1077 | | | |
| | Average 75th quartile | (14) | (454 - 1515) | 1463 | | | 840(824) | | | |
| | Minimise class difference | | | 1790* | | | 870 | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| **France**<br>EQR_Phyt<br>Boundaries: | EQR v TN (RMA) | **0.642**<br>*<0.001*<br>n=13 | (177 - 1612) | 587 | 582 | 594 | 261 | 216 | 304 | GM 362-929<br>HG 132-432 |
| HG 0.71 | Average adjacent quartiles | | | 565(544) | | | 364(365) | | | |
| GM 0.39 | Average adjacent classes | n=13 | | 559(560) | | | 374(373) | | | |
| | Average 75th quartile | (15) | | 470(428) | | | 432(429) | | | |
| | Minimise class difference | | | 570 | | | 225 | | | |

Table 14. Summary of predict values of nutrient concentration at the Good/Moderate and High/Good boundaries of TP (µg L$^{-1}$), per countries (IT/GR, and FR), within common type TRWMEDpolyCL, results obtained by regression and categorical analyses.

| MS | Phytoplankton Models | $R^2$ *p-value* n | Nutrient range TP µg L$^{-1}$ | Most likely boundary | | | | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | **GM TP µg L$^{-1}$** | | | **HG TP µg L$^{-1}$** | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| **Italy/ Greece** EQR_Phyt (MPIscore) | EQR v TP (RMA) | **0.603** *p<0.001* n=15 | 14 - 131 | 47 | 44 | 53 | 27 | 25 | 28 | GM 25-97 HG 17-38 |
| Boundaries: | Average adjacent quartiles | n=15 (17) | (14 - 131) | 63 | | | 23(24) | | | |
| HG 0.78 | Average adjacent classes | | | 66 | | | 23 | | | |
| GM 0.51 | Average 75$^{th}$ quartile | | | 25 | | | 28(29) | | | |
| | Minimise class difference | | | 97 | | | 21 | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| **France** EQR_Phyt | EQR v TP (RMA) | **0.868** *p<0.001* n=14 | 17 - 150 | 42 | 42 | 42 | 18 | 17 | 19 | GM 23-55 HG 14-23 |
| Boundaries: HG 0.71 | Average adjacent quartiles | n=15 (17) | (17 - 150) | 35(37) | | | 20 | | | |
| GM 0.39 | Average adjacent classes | | | 39(40) | | | 21 | | | |
| | Average 75$^{th}$ quartile | | | 23(27) | | | 21 | | | |
| | Minimise class difference | | | 28 | | | 19 | | | |

## 5.3. North East Atlantic

### 5.3.1. Common Type: TRWNEA 11

There is one single common type established for TRW in the NEA region, the NEA11 (Table 1). Phytoplankton intercalibration data from the common IC type NEA11 (n=175 obs) in transitional waters was used. This is a very broadly defined type containing estuaries in the North East Atlantic. The estuaries within this type are likely to differ on features that could influence the outcome of the Phytoplankton and nutrient relationships across this type. However this dataset contains limited parameters for accounting for such specificities, so this example will use available information on the best way possible to attempt deriving nutrient boundaries based on the information available, thus the values obtained will need to be validated with independent data. Despite possibly validation approaches are discussed in the guidance, the validation step is out of the scope of the present exercise.

Data included in this dataset: nutrient parameter Dissolved Inorganic Nitrogen (DIN), mean winter value; and EQR based on Chl*a* from six countries: Netherlands (NL); UK; Ireland (IE); France (FR); Spain (SP); and Portugal (PT).

#### a) Data check

It is important to compile a dataset that spans at least four ecological quality classes, and that shows a linear relationship for at least H, G, M and has $r^2 > 0.36$. The WFD intercalibration (IC) resulted in different EQR boundaries across MS within this type (Table 3), therefore EQRs have been normalized (nEQR) (using toolkit template

*TKit_Normalise.xlsx*) to be able to pull all datasets together and derived nutrient boundaries for this common water type (Table 4). Identity of each dataset is preserved to be able to check for the effect on the results obtained.

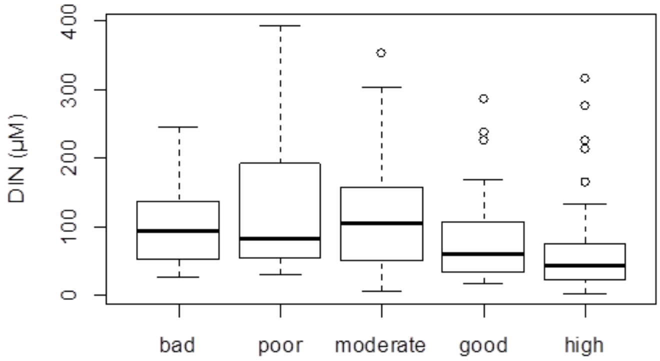



Figure 43. DIN concentrations (µM) across the EQS classes for all datasets pulled together. Outliers identified in the pulled dataset at DIN concentrations of: 353.3969; 288.3561; 227.2500; 239.8122; 316.6975; 227.1283; 276.4942; 166.3536; and 213.8841 µM.

Data covers all five EQS classes (Figure 43), with DIN concentrations slightly decreasing towards higher quality classes, nevertheless, some overlap is observed in particular for the classes below good status. Inspecting each dataset separately (Figure 44 and Figure 45) it can be seen that some datasets benefit from being pulled together and use information from the other datasets, in particular data from Portugal (ds28), as it does not cover a gradient of disturbance that allows establishing a relationship with nutrient concentration for deriving nutrient boundaries. The French data (ds26) shows an inverse trend of the distribution of DIN concentrations across the EQS classes to what would be expected (Figure 44b) and Figure 45), and should be excluded from the analysis.

a)

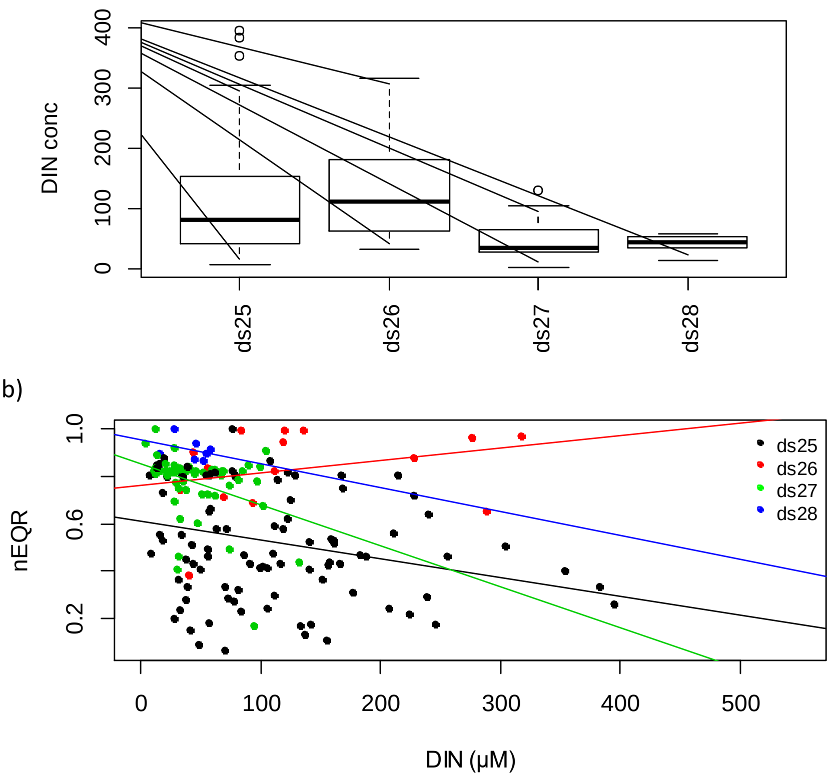


Figure 44. a) Dissolved Inorganic Nitrogen (DIN) concentrations (µM) range in each dataset within common type NEA11: ds25 (NL, UK, IE), ds26 (FR), ds27 (SP), and ds28 (PT); outliers identified at DIN concentrations of: 353.3969; 394.4761; 382.1658; and 131.2800 µM. b) Relationship of nutrient concentrations with normalised EQRs, trend lines showed for each dataset within NEA11.

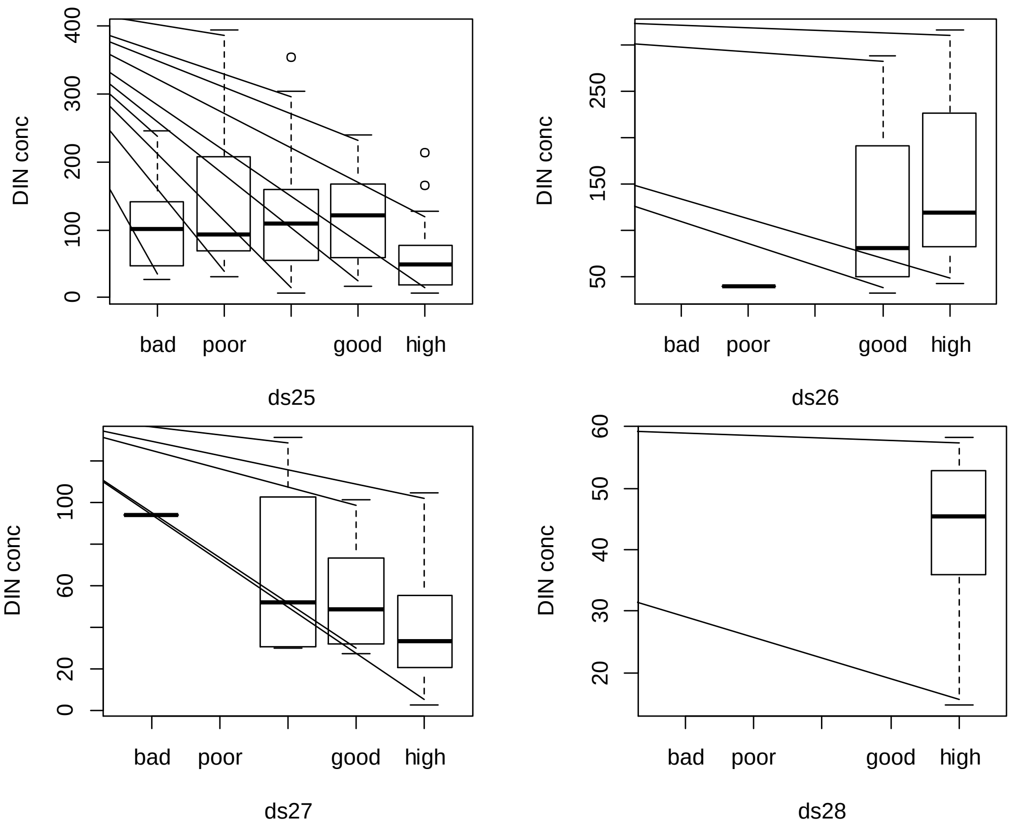


Figure 45. DIN concentrations (µM) across the EQS classes for each dataset separately: ds25 (NL, UK, IE), ds26 (FR), ds27 (SP), and ds28 (PT).

The regressions show a weak relationship of EQR with DIN, with r values below that recommended (>0.6): r =-0.308 and r=-0.310, respectively for EQRs and nEQRs. The plots (Figure 46 left) show that the regression is very scattered, suggesting that other pressures besides DIN are contributing to EQR decrease and influencing data distribution (i.e. wedge-shaped distribution). In this case other approaches such as quantile regression, or the use of an upper quantile categorical (e.g. 75th), or binomial logistic regression, will be considered. Removing the French dataset (ds26) the strength of the relationship increases slightly: r=-0.347 and r=-0.431, respectively for EQRs and nEQRs (Figure 46) but data is still very scattered. The overlap between EQS classes decreased slightly, in particular for the H/G/M range of interest (Figure 47).

*When removing ds26:*

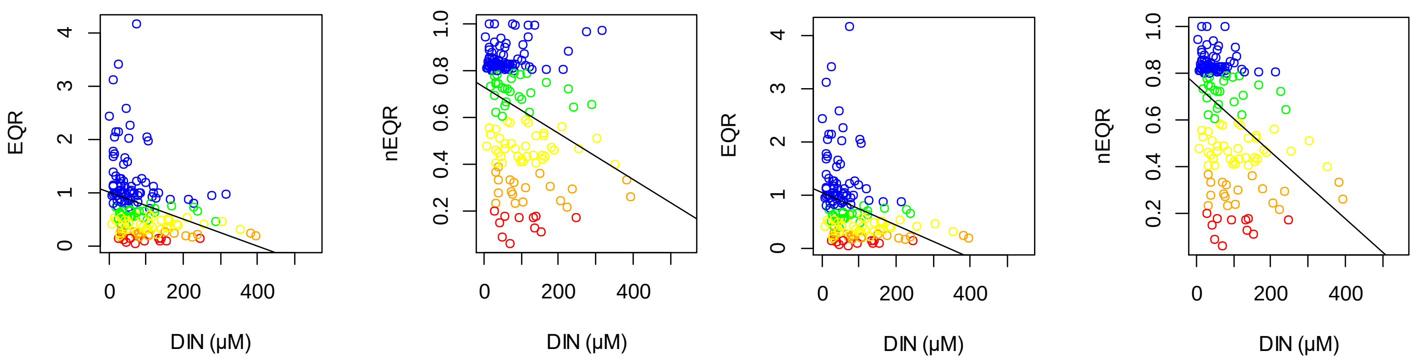


Figure 46. Scatterplot of the relationship of nutrient Dissolved Inorganic Nitrogen (DIN) concentrations (μM) with intercalibrated EQRs (left) and normalized nEQRs (right), including observations from all datasets in the NEA11 Type (top) and removing dataset from France (ds26) (bottom); color by EQS class.

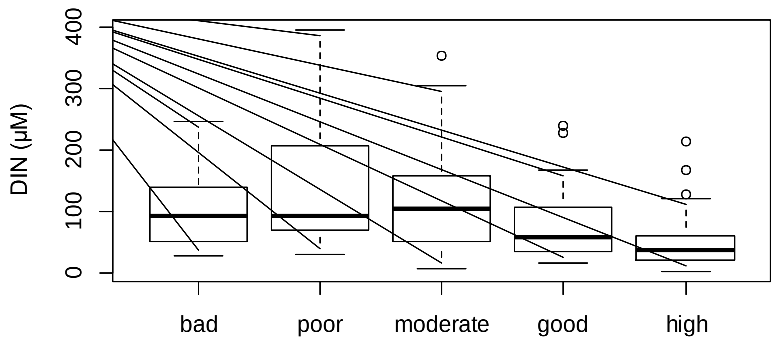



Figure 47. DIN concentrations (μM) across the EQS classes for NEA11 datasets after ds26 removal. Outliers identified at DIN concentrations of: 353.3969; 227.2500; 239.8122; 166.3536; 213.8841; and 128.2423 μM.

Conclusions from NEA11 data check:

***Data removal:*** ds26 will be removed from this analysis as it presents a pattern opposite to that expected, differing from all other datasets, and there are no additional explanatory variables available in this dataset that can help further explain that.

***EQS and EQR data use:*** Normalised EQRs will be used for regression based approaches and the EQS classifications will be used for categorical approaches.

***Outlier removal:*** Those outliers highlighted will be considered for their effect and eventual removal, but not removed *a priori*.

***Gradient cover:*** Full range of disturbance covered when datasets are combined within common type. The slight overlap of classes within the interval of interest (H/G/M) will be tested for significance of differences between nutrient concentrations across classes.

***Strength of the relationship:*** Weak relationship ($r^2$ <0.36) between biological and nutrient data.

***Data shape:*** Scattered wedge-shaped data distribution, indicating that other pressures besides DIN are affecting Phytoplankton and contributing to EQR decrease.

Data exploration indicates that linear regression methods (OLS and Type II regression) may not be adequate for deriving nutrient boundaries for this common type using the available dataset, thus quantile regression and categorical approaches will also be considered and compared. Most of the graphs presented for data exploration can be performed using the R scripts templates available with toolkit vs 6c (*TKit_check_data.R* and *TKit_CoPlot.R*).

### b) Statistical analysis

Excel toolkit analysis revealed that the correlation between Chl*a* based nEQR and DIN, though significant (p<0.001), is lower than r<0.6 (r=-0.458) and thus caution is needed when interpreting the predicted nutrient boundaries for DIN (μM) for this NEA11 common type, using the available dataset (Table 15). Some critical data points have been highlighted, essentially data points with the highest DIN concentrations observed (>238 μM), and their removal was tested and had no significant influence in the outputs of the regression previously presented. The outliers were kept in the dataset because: a) other approaches will be tested; and b) they may not be real outliers as they correspond to the tail of the distribution, eventually covering the full gradient of disturbance and potentially having an important influence on the regression outputs.

Significant differences between the nutrient concentrations in adjacent quality classes were only observed between the H/G and were not significant for the G/M classes (non-parametric Wilcoxon Rank Sum Test not significant $p$=0.052). The DIN concentration in Good is higher than in High but that was not the case between Moderate and Good classes, therefore the G/M boundary values provided by categorical methods based on the quantiles (Table 15) must be treated with extreme caution.

Quantile regression could be more adequate to the data pattern distribution observed in this dataset; however it is not fully developed in the current toolkit version. An alternative could be the minimisation of mis-match method (Table 15) as is the least sensitive to outliers and non-linear relationships, although it does require a significant correlation which, as mentioned, was not verified for the G/M boundary.

Because the excel toolkit outputs provide no uncertainty for the minimization of mis-match of class method, a boot-strap approach was included in the R scripts (*Tkit_mismatch3_HG.R* and *Tkit_mismatch3_GM.R*). Using this approach (Figure 48), the mean estimated high/good boundary for DIN is 52.5 µM, within a range of 47-59 µM, with a total mismatch classifications rate of 30%, ranging from 28-34%. For the good/moderate the mean estimated boundary is 74.5 µM, which is within the range of 66-83 µgl-1. At this point the total mis-match of classifications is 28% and lies within the range of 24-34%. Although not shown here, a sufficient number of iterations have been used to achieve convergence (read details in the Phillips *et al.* 2017 BPG Appendix 1).

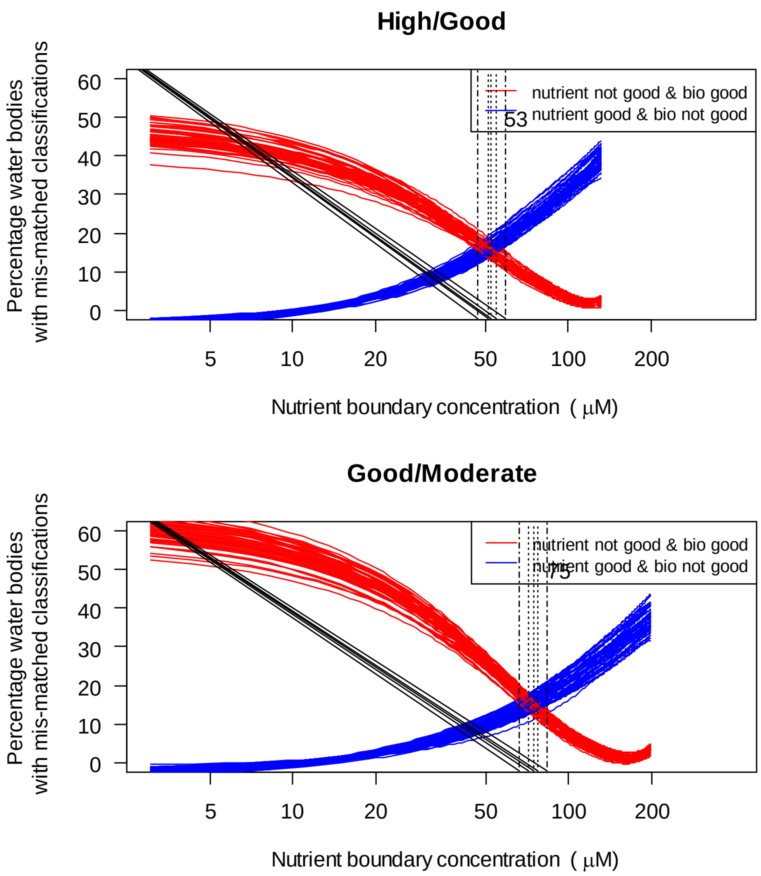
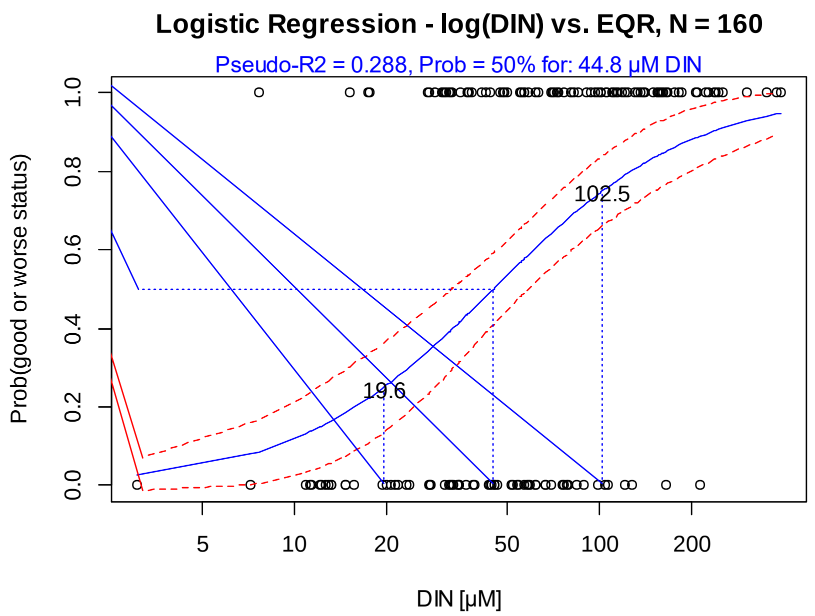



Figure 48. Relationship between percentage of mis-classified records comparing biological and nutrient classifications in comparison to value of nutrient boundary. Vertical lines mark the range of cross-over points where the mis-classification is minimized, together with the mean nutrient concentration. (each line shows a sub-sample of the data set selected at random).

Binomial Logistic regression is also included in the latest version of the toolkit (*TKit_LogisticRegHigh.R* and *TKit_LogisticRegGood.R*) and is the most reliable categorical method, and should be used when linear modelling is not appropriate. However, like other methods its boundary estimates will be influenced if other pressures are operating. As this is often the case in estuaries, where multiple pressures are occurring simultaneously, the results must be interpreted with caution. This seems also to be the case for this dataset from the NEA11 common type. Several criteria are being analysed for their usefulness for supporting threshold selection in function of e.g. type of data, ecological constraints, and regulatory use of the boundaries, possibly for future inclusion in the toolkit.

The binomial logistic regression of DIN on biology (nEQRs), for both the H/G and the G/M range are presented in (Figure 49). Nutrient boundary estimates are presented for a 50% probability of being in moderate or worse status for the G/M, or in good or worse for the H/G (Table 15), but nutrient values at lower and higher probability thresholds (25% and 75%) are also presented, which provide for precautionary and non-precautionary values.

a)




b)

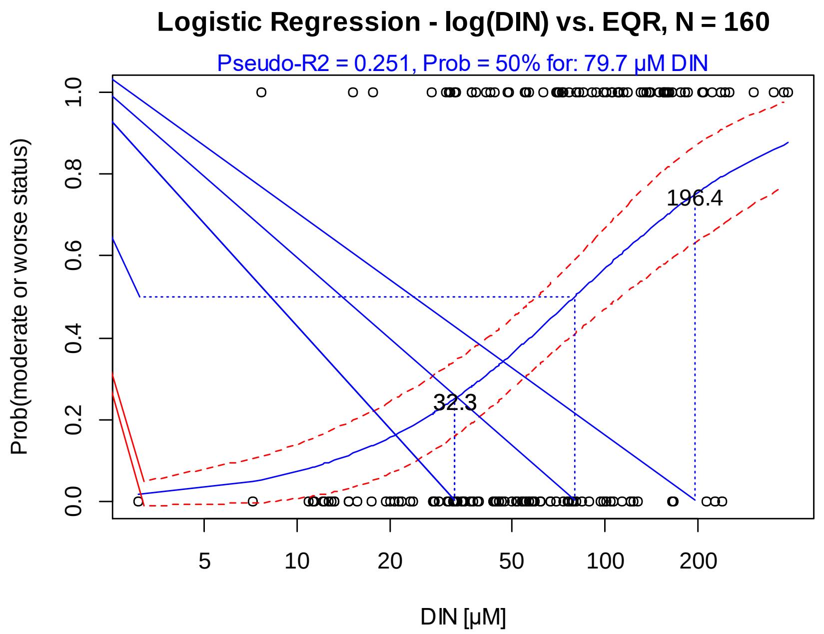



Figure 49. Binomial logistic regression of DIN on probability of being a) good or worse status and b) moderate or worse status (normalized EQRs used). Lines show potential boundary values at different probabilities of being a) good or worse status and b) moderate or worse.

Quantile regression is not included in the current toolkit version, but some testing has been done which we show below. The best Additive Quantile Regression model (adjusted-$R^2$=0.2049, p<0.001) obtained (lower AIC) assumed no significant effect of dataset, i.e. country of origin of the data (NL, UK, IE; SP; and PT). The possible range of nutrient boundaries for H/G and G/M, considering the quantile 0.7, are indicated by the vertical lines in the plot (Figure 50; Table 15). Boundaries derived for a highest quantile may be the solution when other pressures, other that nutrients, are downgrading the biological status. These boundaries are not precautionary and indicate a clear risk of negative effect on biota when such nutrient values are reached. The boundaries here indicated should however be taken with caution until further discussion and guidance on quantile selection for the purpose of this work is discussed.

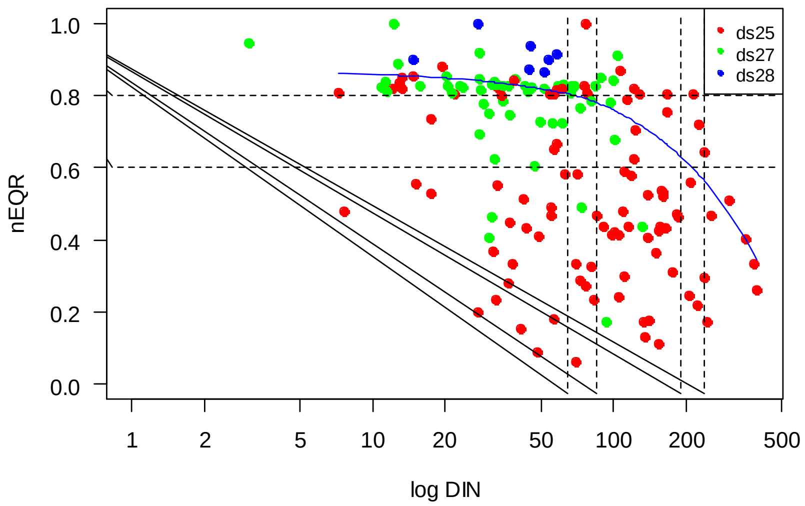



Figure 50. Quantile regression fit at the 70th quantile (Additive Quantile Regression Smoothing rqss using quantreg R package by Koenker) for nEQR v DIN (µM) in the NEA11 common type. Horizontal lines indicated EQR boundaries at H/G and G/M, and vertical lines possible nutrient boundaries range for H/G and G/M, at this quantile.

### 5.3.1.1. Nutrient boundaries

An overview of the boundaries suggested for DIN by the different approaches tested is provided in Table 15. Where the regressions correlation obtained is lower than 0.36 the results need to be taken with caution. Also, where the nutrient concentrations in adjacent classes Good and Moderate are not significantly different (Wilcoxon Rank Sum Test), the categorical methods based on quantiles need also to be taken with extreme caution.

Table 15. Summary of predicted boundary values at the Good/Moderate and High/Good for DIN (µM) in common type NEA11 (n=160), derived from the most adequate approaches for this dataset (using excel toolkit vs 6c and R scripts). Results from regression and categorical methods are presented, those in red need to be taken with caution.

| Methods tested for nutrient boundaries NEA11 | | DIN µM | | *notes* |
|---|---|---|---|---|
| **Regression methods (OLS & Type II):** | | **High/G** | **Good/M** | |
| Most likely boundary | predicted | 36 | 62 | $R^2$ = 0.21; p-value <0.001; n =160 |
| | *range* | (14-43) | (61-72) | |
| | *possible range* | 5-79 | 23-278 | |
| **Additive Quantile regression method (rqss):** | | | | |
| 70th percentile | | 65-85 | 190-240 | $R^2$ = 0.209; p-value <0.001; n =160 |
| **Categorical methods:** | | | | |
| average adjacent class upper & lower quartiles | | 49 | 80 | Wilcoxon Rank Sum Test: |
| average adjacent class median | | 47 | 82 | H/G p-value = 0.002 |
| 75th quartile of class | | 62 | 107 | G/M p-value= 0.052 |
| mis-match of biological v nutrient class (*excel toolkit*) | | 50 | 72 | |
| mis-match of biological v nutrient class (*R scripts*) | | 53 | 75 | |
| | *range* | (47-59) | (66-83) | |
| Binomial Logistic regression (*prob=0.5*) | | 45 | 80 | |

## 5.4. Black Sea

Does not apply since no common types for Transitional Waters (TRW) have been defined in the Black Sea.

# 6. Coastal waters results

## 6.1. Baltic Sea

For the Baltic Sea coastal waters we have gathered data from two common types: CWBALBC4 and CWBALBC5 (Table 2). In both common types nutrient data is available for two parameters, Total Nitrogen (TN) and Total Phosphorus (TP). Within each of the types the Member States have adopted different EQR methods/IC EQR boundaries (Table 3), and therefore the data will be split for the pressure-response relationship analysis.

### 6.1.1. Common Type: CWBALBC4

Within this common type, Latvia and Estonia have defined an EQR based on the Chl*a* metric, and each MS has different intercalibrated EQR boundaries (Table 3). The datasets for Latvia and Estonia, each with 92 and 44 samples respectively, will be analysed separately. The predicted nutrient boundaries obtained for Total Nitrogen and Total Phosphorus within this common type are presented in section 6.1.1.3.

### 6.1.1.1. Latvia (ds5_CWBALBC4)

a) Data check

The EQR values range the full spectrum of ecological quality from High to Bad, and in general there is a good distinction between quality classes along the gradient of concentration of the stressors (TN and TP, Figure 51). The exception is for the Good/Moderate classes that present an overlap, for both nutrients. This range of concentrations is one for which equivalent nutrient boundaries would have to be derived.

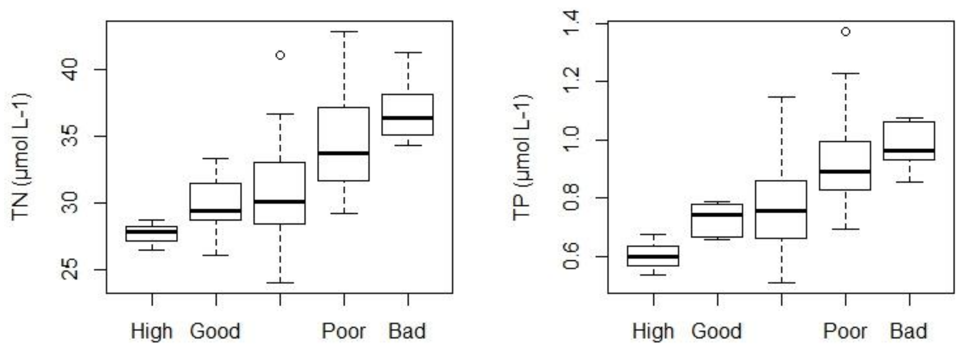



Figure 51. Range of Total Nitrogen (TN) and Total Phosphorus (TP) concentrations for Latvia sites grouped by ecological quality status classes (EQS): High, Good, Moderate, Poor, Bad.

Nutrient data are slightly left skewed, but distribution approximates normal after $\log_{10}$ transformation (Figure 52).

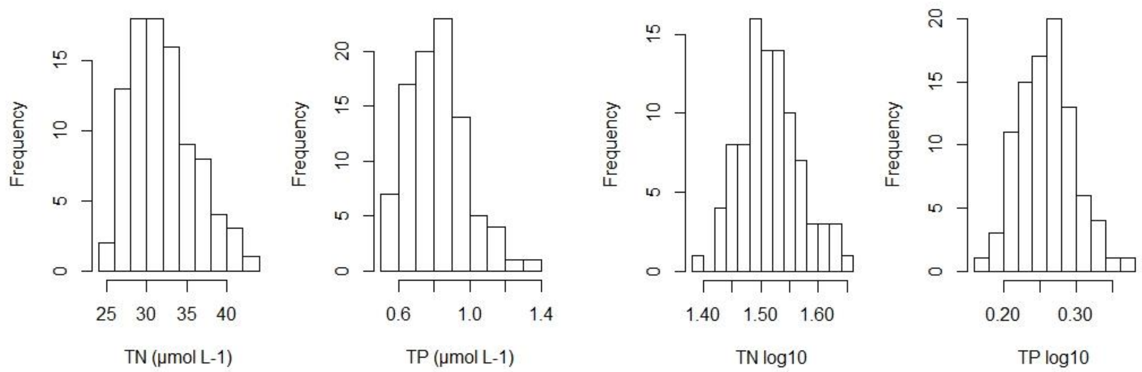



Figure 52. Range distribution of nutrient concentrations Total Nitrogen (TN) and Total Phosphorus (TP).

**i. Outliers**

Outliers in the data (Figure 53) will be removed if justified but only after xy relationships evaluation. From the Cleveland plots it can be observed that the three outliers in the EQR (with values >1.0) deviate considerably from the rest of the data.

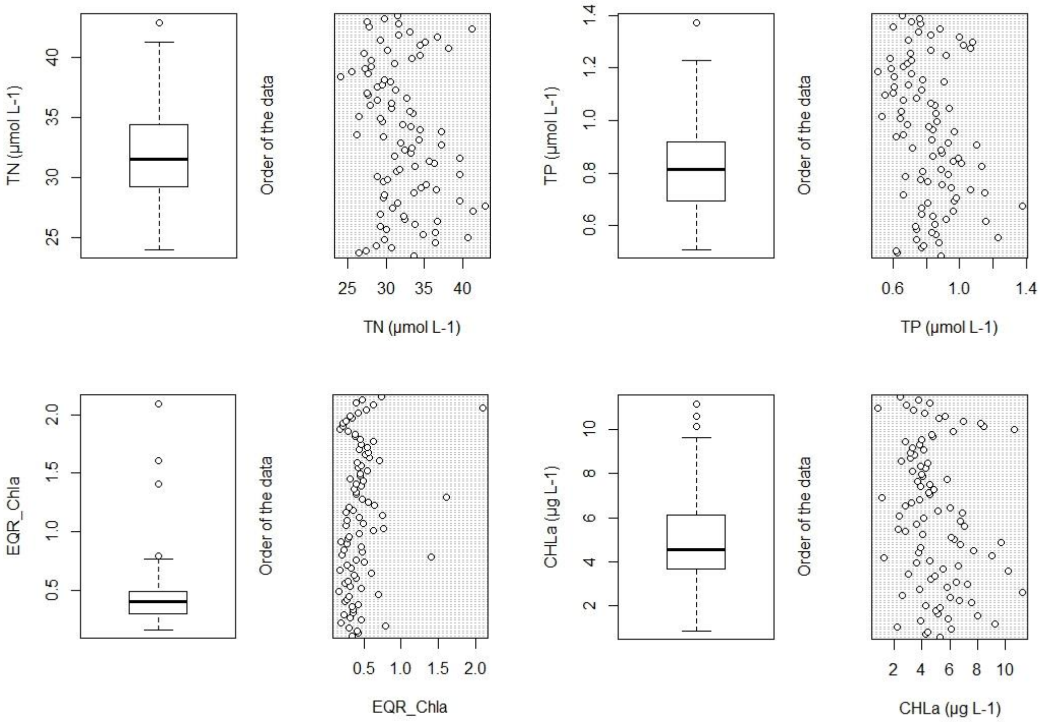



Figure 53. Outliers' verification in the predictors (TN and TP) and response (EQR, Chl*a*) variables.

### ii. Collinearity among covariates

Pearson correlation between covariates TP and TN is relatively high (r= 0.777, Figure 54 – left graph), but VIF = 2.525 does not indicate collinearity between these predictors.

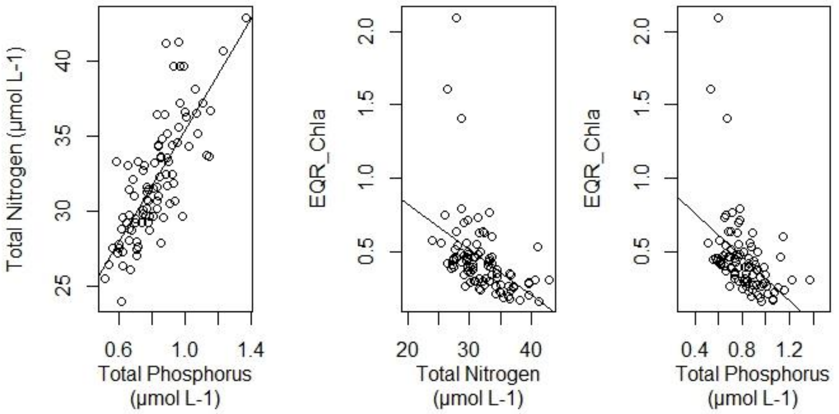



Figure 54. Scatterplots between explanatory variables TN and TP (µmol $L^{-1}$), and between these nutrients and EQR.

### iii. Relationship between X & Y

The nutrient variables Total Phosphorus (TP) and Total Nitrogen (TN) show a moderate negative correlation (Pearson correlation, r) with the EQR_Chl*a*: EQR vs. TN r=-0.437; EQR vs. TP r=-0.437. Data shows a slight wedge-shape distribution (Figure 54 – centre and right graphs). After *xy* relationship inspection, the outliers proposed to be removed from the analysis are those beyond the 0.975 quantile, representing six outliers for TN and five outliers for

TP (Figure 55). This implies removing all EQR values corresponding to High status from the relationship with TN, and leaving only one for the relationship with TP. Also, after the outliers removal from the analysis the slope of the relationship changes (Figure 55), and so does the shape of the data distribution, and this might interfere with the stressor–response real signal.

Therefore, for this dataset, categorical and linear regression results should ideally be compared to those obtained with a quantile approach as suggested in the Guidance (2017) for when the "relationship is significant, $r^2$ is not high; information on confounding variables is not available, i.e. relationship is "wedge-shaped" (i.e. assymetrical with respect to "line of best fit")".

The xy scatterplots show also that the linear trend of the relationship gets lost towards higher nutrient concentrations, approximately at 35 µmol $L^{-1}$ for TN and 1.0 µmol $L^{-1}$ for TP (Figure 54 – centre and right graphs). In this sense, a GAM model with segmented regression was fitted to the relationship of EQR vs TN to identify whether there are significant changes in the slope of the relationship at the end of the TN range, and help selecting linear regions of the data. For 1 break point method the break point at 38.5 µmol $L^{-1}$ was obtained, together with its lower (36.1 µmol $L^{-1}$) and upper (41.1 µmol $L^{-1}$) confidence limits (Figure 55).

Regarding EQR vs. TP, the GAM model with segmented regression would remove most of the data points since the break point has been identified at 0.624 µmol $L^{-1}$ (lower 0.615 µmol $L^{-1}$ and upper 0.633µmol $L^{-1}$ confidence limits, Figure 55). All data points with higher TP concentrations would be left out of the analysis and wouldn't allow deriving proper nutrient boundaries. Using the excel toolkit we visually identified the higher end of TP concentrations outside the linear range at TP >=1.1 µmol $L^{-1}$. See results of the analysis in section 4.

In any case, as mentioned before, the overlap between G/M classes is a problem within this dataset (Figure 55 bottom right graph).

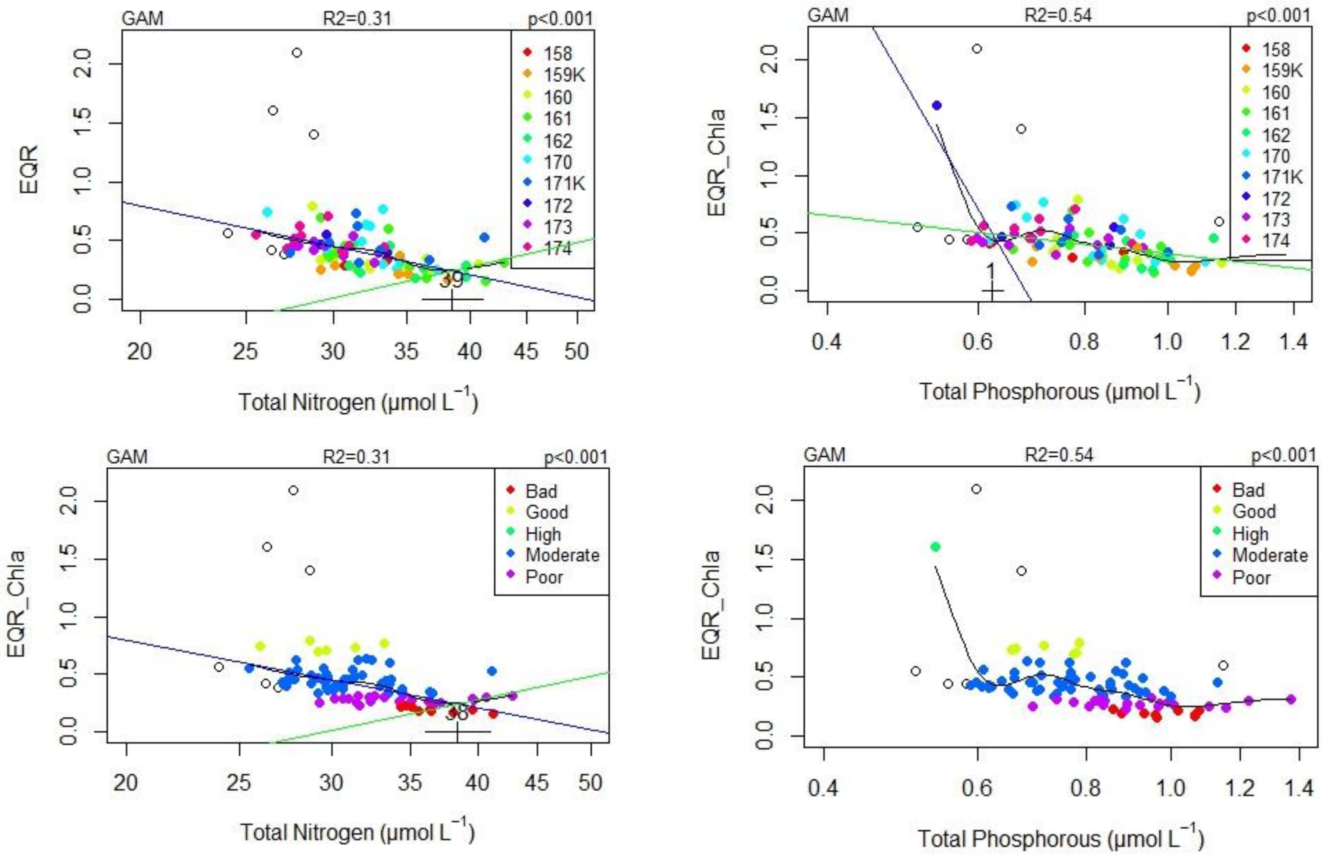



Figure 55. Scatterplot showing relationship between EQR and nutrients (left: TN; right: TP) with fitted GAM model. Points coloured by Station (top) and EQS class (bottom), open circles outliers not used to fit model.

### b) Statistical analysis

The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below.

For Latvia, due to the weak results (TN: r=0.533; TP: r=0.555; r <0.6) obtained for the linear regression analysis (Table 16 and Table 17), the categorical approaches should be used to confirm and derive nutrient boundaries from IC EQR_Chla boundaries for Total Nitrogen. Details of the regression analysis results are presented in Figure 56.

The exploratory analyses revealed a wedge-shape distribution of the data (EQR~ Total Phosphorus; Figure 56) which indicates that the linear regression approach might not be the most adequate for deriving nutrient boundaries from this data. Categorical approaches results should be taken into consideration. Other approaches such as regression quantiles would need to be tested for this dataset. See the Guidance by Phillips *et al.* (2017) for advice and developments regarding regression quantiles (RQ), namely test R scripts included in the toolkit.

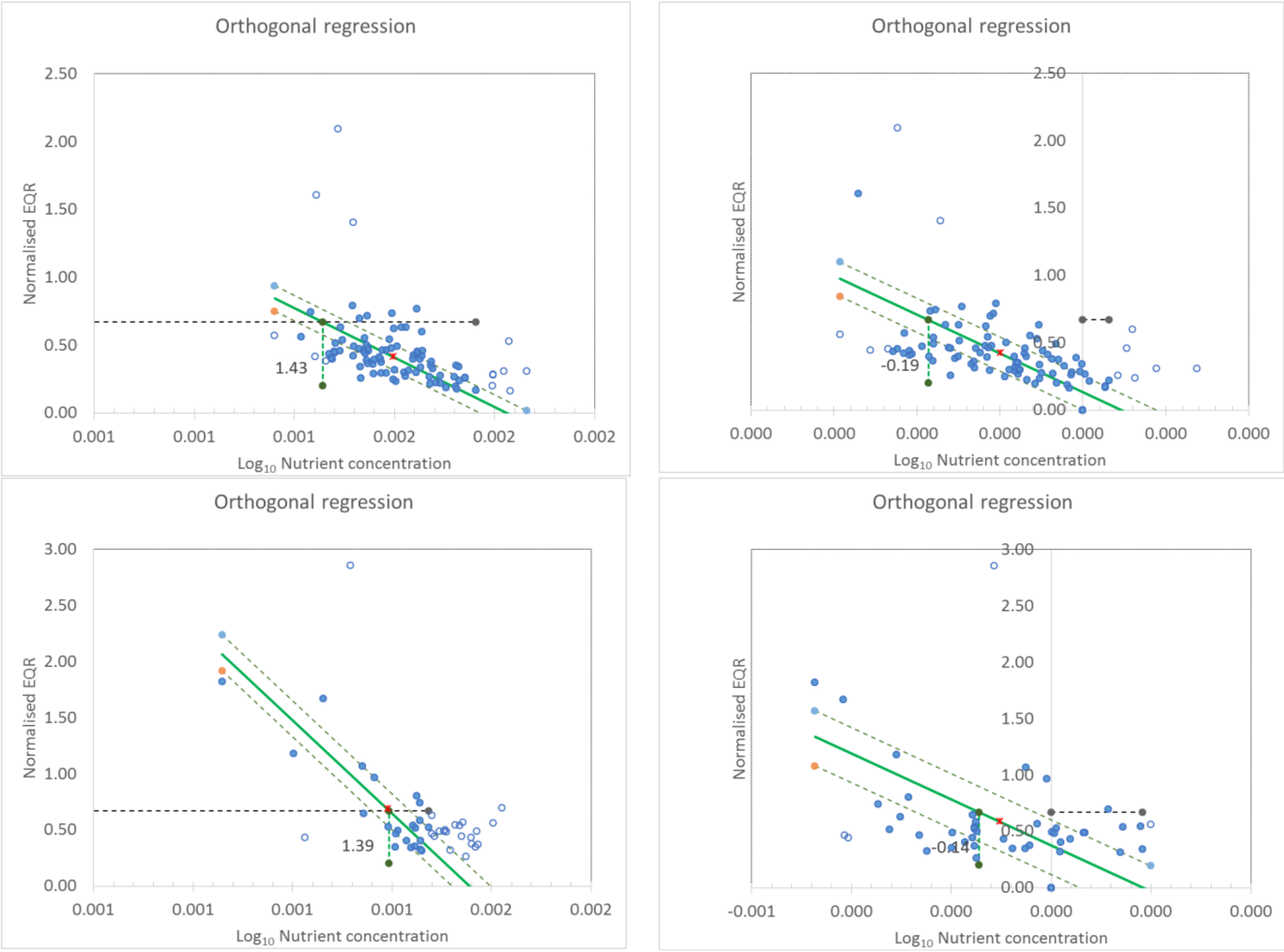



Figure 56. CWBALBC4 Latvia: Orthogonal type II regression of EQR v log10 Total Nitrogen concentration (left) and EQR v $\log_{10}$ Total Phosphorus concentration (right). Solid points values used for models, open circles excluded data (outliers or values beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

#### 6.1.1.2. Estonia (ds6_CWBALBC4)

##### a) Data check

The EQR values range a disturbance gradient suitable for deriving nutrient boundaries for nutrients, from High to Poor (Figure 57). However there is great overlap between classes and, in particularly for TN, the Good do not distinguish from the Moderate sites in terms of this nutrient concentration. One possible outlier at TN 16.8 µmol $L^{-1}$, and another for TP at 1.58 µmol $L^{-1}$.

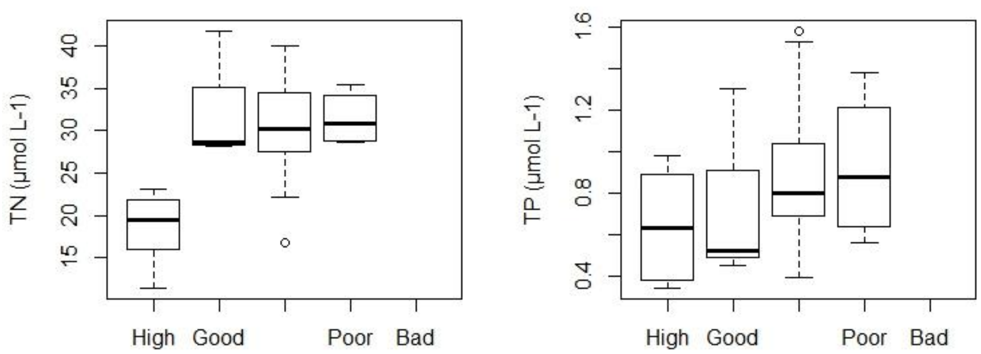



Figure 57. Range of Total Nitrogen (TN) and Total Phosphorus (TP) concentrations for Estonia sites grouped by ecological quality status (EQS) class: High, Good, Moderate, Poor and Bad.

Total Phosphorus has a slightly left skewed distribution that approximates normal after $log_{10}$ transformation (Figure 58).

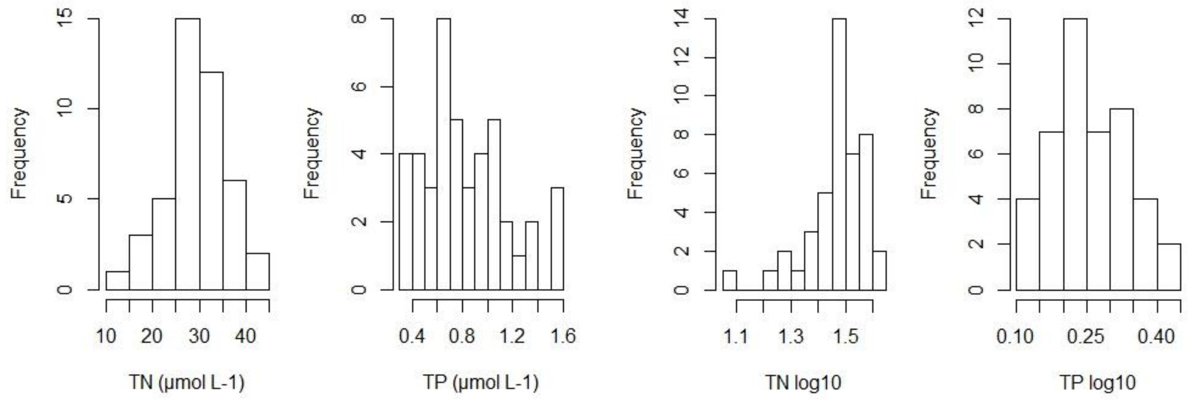



Figure 58. Range distribution of nutrient values: Total Nitrogen (TN) and Total Phosphorus (TP) before and after log 10 transformation.

## i. Outliers

Outliers for the variables of interest in this dataset are shown in Figure 59. There is an outlier in Total Nitrogen (<15 µmol $L^{-1}$), two outliers in Chl*a* (<1 and >7 µmol $L^{-1}$) and six in the EQR variable. Some EQR outliers are beyond the expected range of [0-1.0], with a maximum EQR registered at 2.5. The Cleveland plots distribution show however that, except for EQR, these outliers may not be problematic and will only be removed after analysing the xy relationships, following the nutrient Guidance protocol (2017). However caution is needed when removing EQR outliers, to avoid losing signal response to nutrient pressure. The outliers finally removed from the analysis are presented in section 4.

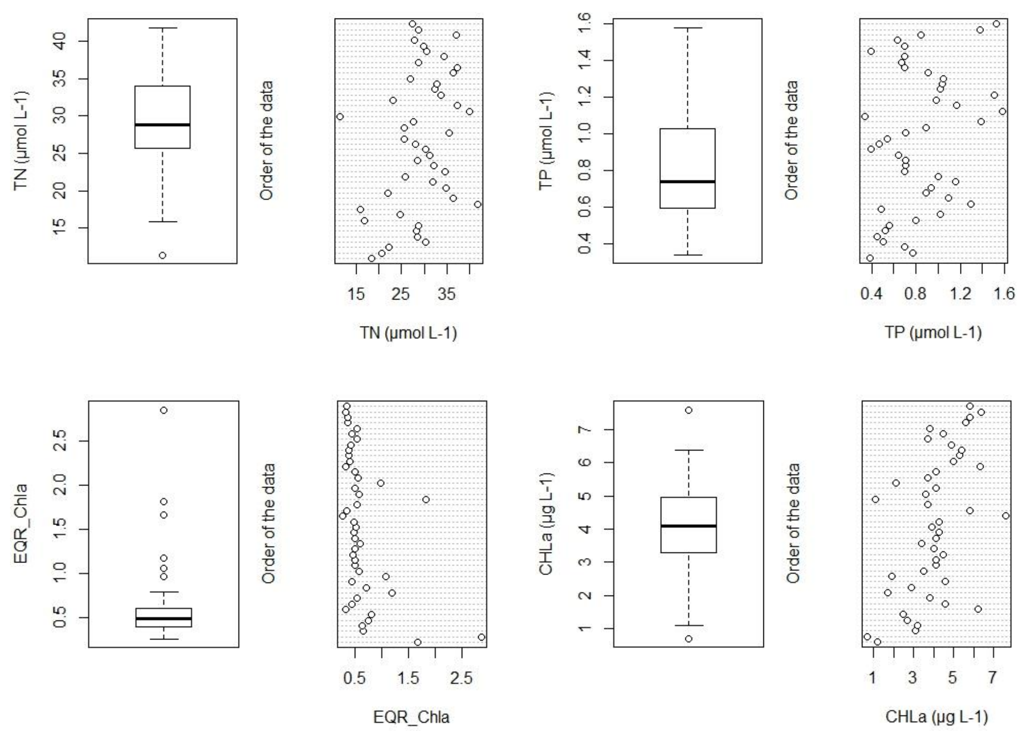



Figure 59. Outliers' verification in the predictors (TN, TP) and response (EQR, Chl*a*) variables, using Boxplot and Cleveland plots.

### ii. Collinearity among covariates

There is no collinearity (r=0.395, n=44) between covariates Total Phosphorus (TP) and Total Nitrogen (TN) (Figure 60).

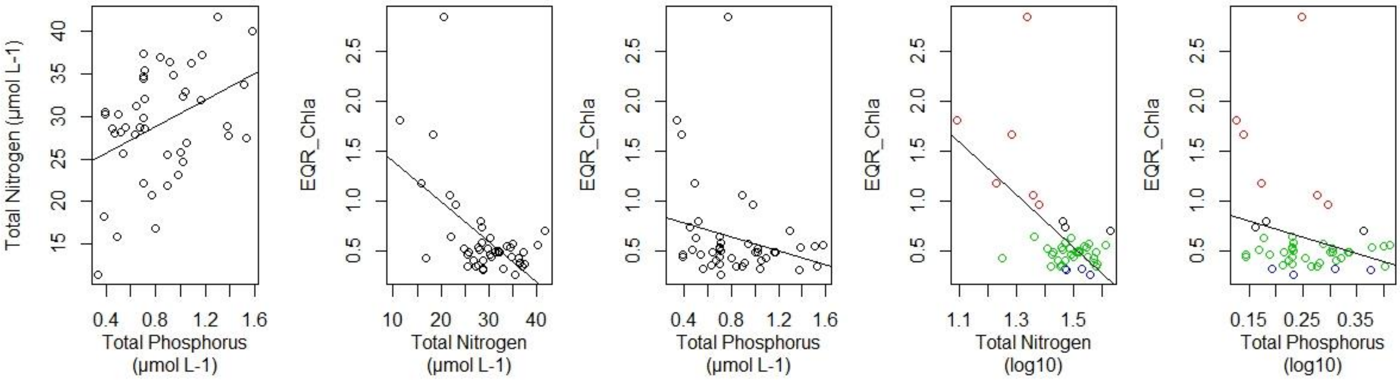



Figure 60. Scatterplots between nutrient explanatory variables Total Nitrogen and Total Phosphorus (left), and between these nutrients and EQR, before (centre) and after (right) nutrient log $_{10}$ transformation. In the right hand graph, dots are coloured after EQS classification: High-black; Good-red; Moderate-green, and Poor-blue.

### iii. Relationship between X & Y

The correlation (Pearson r) between nutrients and EQR is acceptable (EQR vs TN r=-0.56, n=44; EQR vs TP r=-0.25, n=44) but do not follow clear linear patterns (Figure 60), with TP presenting a wedge shape distribution of the data (wedge shape). The relationship does not change significantly with $\log_{10}$ transformed nutrient variables (EQR vs $\log_{10}$TN r=-0.60, n=44; EQR vs $\log_{10}$TP r=-0.27, n=44). For TP another type of approach, such as quantile regression, might be more appropriate.

The graphs reinforce that EQRs >1.0 bring relevant information for this analysis, and outliers removal will depend on other evidences. These EQR values might reflect however some need for reference conditions adjustment. After xy relationship inspection, the outliers proposed to be removed from the analysis are those beyond the 0.975 quantile, representing three outliers for TN and four outliers for TP (see results in section 4).

As mentioned previously, and also visible in Figure 60, class overlap is a problem in this dataset.

Coplots (not presented here) did not show evidence of interaction between nutrient variables.

The xy scatterplots also show some evidence that the linear trend of the data gets lost for TN values around 30 µmol $L^{-1}$. In this sense, a GAM model with segmented regression was fitted to the relationship of EQR vs TN to identify whether there are significant changes in the slope of the relationship at the end of the TN range, and help selecting linear regions of the data. For 2 break points method at 18.4 and 25.6 µmol $L^{-1}$ there would be too few datapoints left below the High/Good boundary, and points at the Good/Moderate boundary would fall out of this range. For 1 break point method, with the break point estimated at 26.5 µmol $L^{-1}$ (with upper confidence limit at 28.1 µmol $L^{-1}$), the Good/Moderate boundary would still be left out of the selected linear range (Figure 61). Therefore, to run the linear analysis, we have visually set the break point at 30 µmol $L^{-1}$, in order to have the range of the Good/Moderate status samples included in the analysis. However few data points are left above that boundary (results presented in section 4).

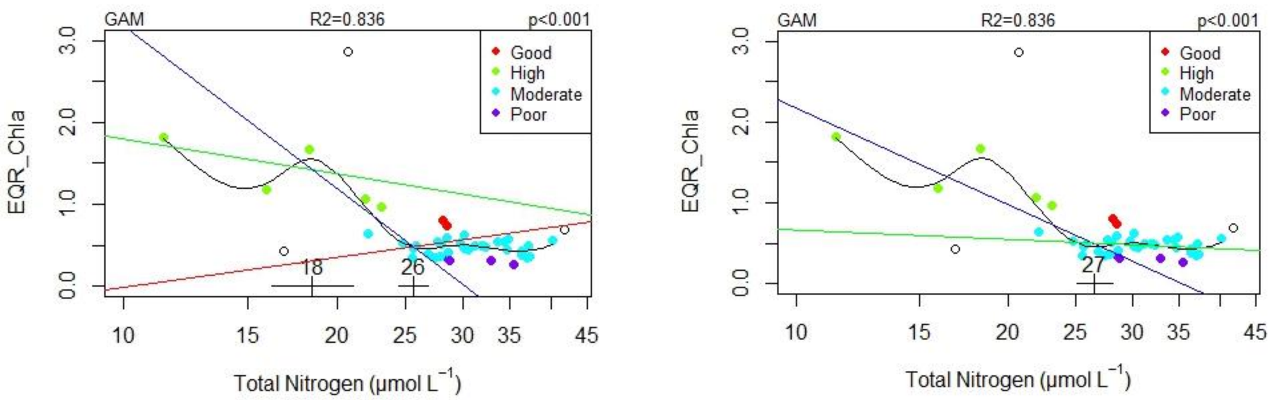



Figure 61. Scatterplot showing relationship between EQR and TN with fitted GAM model, with segmented lines. The graph shows linear trend for two estimated break points at 18 and 26 µmol L$^{-1}$ (left), and for one break point at 26.5 µmol L-1 (right). Samples coloured by EQS class, open circles outliers not used to fit model.

## b) Statistical analysis

The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below.

The linear regression results obtained for EQR~TN are robust to derived boundaries for this nutrient (r=0.87 >0.6). Details of the regression analysis results are presented in Figure 62. For Phosphorus, the linear regression results are weaker (r< 0.6: Estonia r=0.501) and the categorical approaches results should also be taken into consideration.

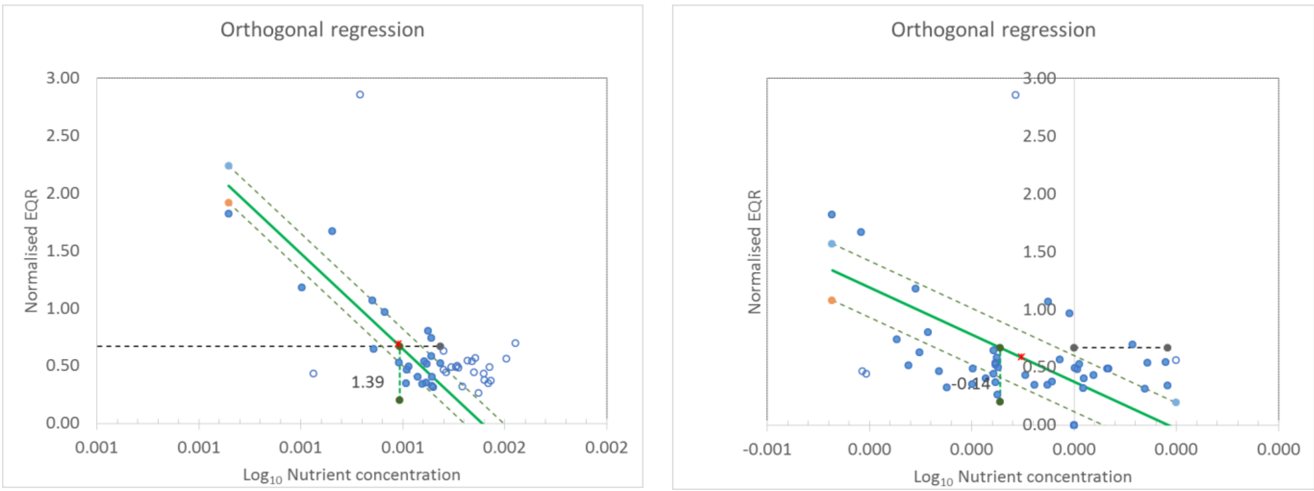



Figure 62. CWBALBC4 - Estonia: Orthogonal type II regression of EQR v log10 Total Nitrogen concentration (left) and EQR v log$_{10}$ Total Phosphorus concentration (right). Solid points values used for models, open circles excluded data (outliers or values beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

### 6.1.1.3. Nutrient boundaries

Below are the summary tables of nutrient boundaries obtained for Total Nitrogen (TN; Table 16) and Total Phosphorus (TP; Table 17) for the MS reporting data in this common type: Latvia and Estonia.

Table 16. Summary of predicted values of Total Nitrogen concentration (µmol $L^{-1}$) at the Good/Moderate and High/Good boundaries for Latvia and Estonia in common type CWBALBC4, results obtained by linear regression and categorical analyses.

| CWBALBC4 Total Nitrogen | Phytoplankton Models | $R^2$ *p-value* n | Nutrient range µmol $L^{-1}$ | Most likely boundary GM µmol $L^{-1}$ | | | HG µmol $L^{-1}$ | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| EQR_Chl*a* Boundaries: HG 0.82 GM 0.67 MP 0.33 | EQR v TN (RMA) | **0.284** *p<0.001* n=79 | 25.5 - 38.1 | 26.8 | 23.3 | 28.9 | 24.4 | 19.5 | 27.5 | GM 20-32 HG 16-30 |
| **Latvia** | Average adjacent quartiles | n=79 (86) | (25.5 - 43) | 30.4 | | | 29 | | | |
| | Average adjacent classes | | | 30.4(30.5) | | | 29.4 | | | |
| | Average 75th quartile | | | 31.9 | | | 28.5 | | | |
| | Minimise class difference | | | 26.9 | | | 25.5 | | | |
| EQR_Chl*a* Boundaries: HG 0.83 GM 0.67 MP 0.33 | EQR v TN (RMA) | **0.756** *p<0.001* n=22 | 11.4 - 29.8 | 24.78 | 24.76 | 24.8 | 22.7 | 22.4 | 22.9 | GM 22.6-28.5 HG 20.4-25.2 |
| **Estonia** | Average adjacent quartiles | n=22 (41) | (11.4 - 40.1) | 27(31) | | | 25.2(25.3) | | | |
| | Average adjacent classes | | | 27.8(29.4) | | | 24.8(24.9) | | | |
| | Average 75th quartile | | | 28.5(34.6) | | | 22.2 | | | |
| | Minimise class difference | | | 23.5 | | | 22.5 | | | |

Table 17. Summary of predicted values of Total Phosphorus concentration (µmol $L^{-1}$) at the Good/Moderate and High/Good boundaries for Latvia and Estonia in common type CWBALBC4, results obtained by linear regression and categorical analyses.

| CWBALBC4 Phosphorus | Phytoplankton Models | $R^2$ *p-value* n | Nutrient range µmol $L^{-1}$ | Most likely boundary GM µmol $L^{-1}$ | | | HG µmol $L^{-1}$ | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| EQR_Chl*a* Boundaries: HG 0.82 GM 0.67 MP 0.33 | EQR v TP (RMA) | **0.043** *p<0.001* n=81 | 0.5 - 1.1 | 0.65 | 0.55 | 0.71 | 0.58 | 0.45 | 0.67 | GM 0.47-0.81 HG 0.38-0.75 |
| **Latvia** | Average adjacent quartiles | n=81 (86) | (0.5 - 1.4) | 0.72 | | | 0.68 | | | |
| | Average adjacent classes | | | 0.77 | | | 0.70 | | | |
| | Average 75th quartile | | | 0.78 | | | 0.65 | | | |
| | Minimise class difference | | | 0.62 | | | 0.53 | | | |
| EQR_Chl*a* Boundaries: HG 0.83 GM 0.67 MP 0.33 | EQR v TP (RMA) | **0.260** *p<0.001* n=40 | 0.3 - 1.5 | 0.72 | 0.65 | 0.43 | 0.60 | 0.46 | 0.69 | GM 0.43-0.95 HG 0.30-0.89 |
| **Estonia** | Average adjacent quartiles | n=40 | | 0.76 | | | 0.69 | | | |
| | Average adjacent classes | | | 0.67 | | | 0.64 | | | |
| | Average 75th quartile | | | 0.82 | | | 0.89 | | | |
| | Minimise class difference | | | 0.55 | | | 0.48 | | | |

### 6.1.2. Common Type: CWBALBC5

Within the common type BC5, Latvia and Lithuania have defined an EQR based on the Chl*a* metric, and each MS has different intercalibrated EQR boundaries (Table 3). The datasets for these MS, each with 104 and 65 samples respectively, will be analysed separately.

We have kept the nutrient units for this dataset in mg $L^{-1}$ as provided by the MS. However to compare nutrient boundaries proposals with results obtained in other CW, the units presented in Figure 3 and Figure 4were converted to µmol $L^{-1}$.

#### 6.1.2.1. Latvia (ds7_CWBALBC5)

a) Data check

The EQR values cover the full gradient of disturbance from High to Bad ecological quality status (EQS), which is suitable for deriving nutrient boundaries (Figure 63). The main issue is that there seems to be some overlap between classes of interest: the Good/Moderate classes overlap in TN and the High/Good classes overlap in TP.

There are a few outliers that come out of the data (Figure 63), in particular those very high TN values in the High status classes: 0.56 and 0.44 mg $L^{-1}$. Also for TP some outliers should be looked at: 0.0298; 0.0293; 0.0325; and 0.0346 mg $L^{-1}$.

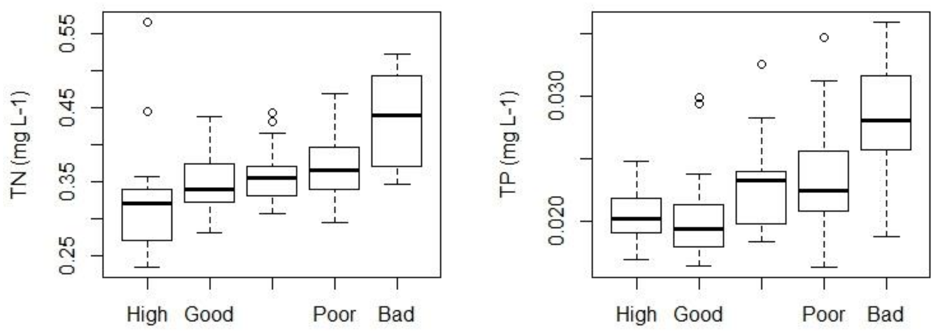



Figure 63. Range of Total Nitrogen (TN) and Total Phosphorus (TP) concentrations for Latvia sites grouped by EQS class: High, Good, Moderate, Poor and Bad.

The Total Phosphorus data presents a left-skewed distribution (Figure 64), which still maintains even when $log_{10}$ transformation is applied.

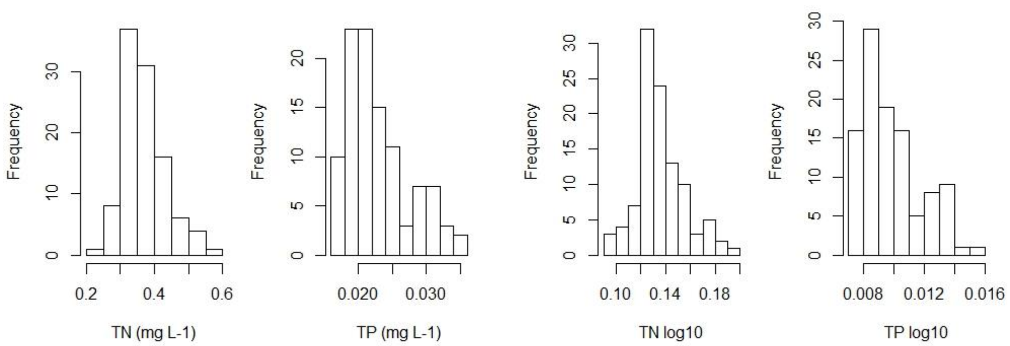



Figure 64. Range distribution of nutrient values: Total Nitrogen (TN) and Total Phosphorus (TP), before and after $log_{10}$ transformation.

### i. Outliers

Potential outliers in the variables of interest in this dataset are shown in Figure 65. The Cleveland plots distribution show however that these outliers may not be problematic outliers and will only be removed after analysing the *xy* relationships, following the nutrient Guidance protocol (2017). To avoid losing signal response, the EQR observations highlighted as outliers (e.g. 2.45) will only be removed if justifiable by other evidence. The outliers finally removed from the analysis are reported in section 4.

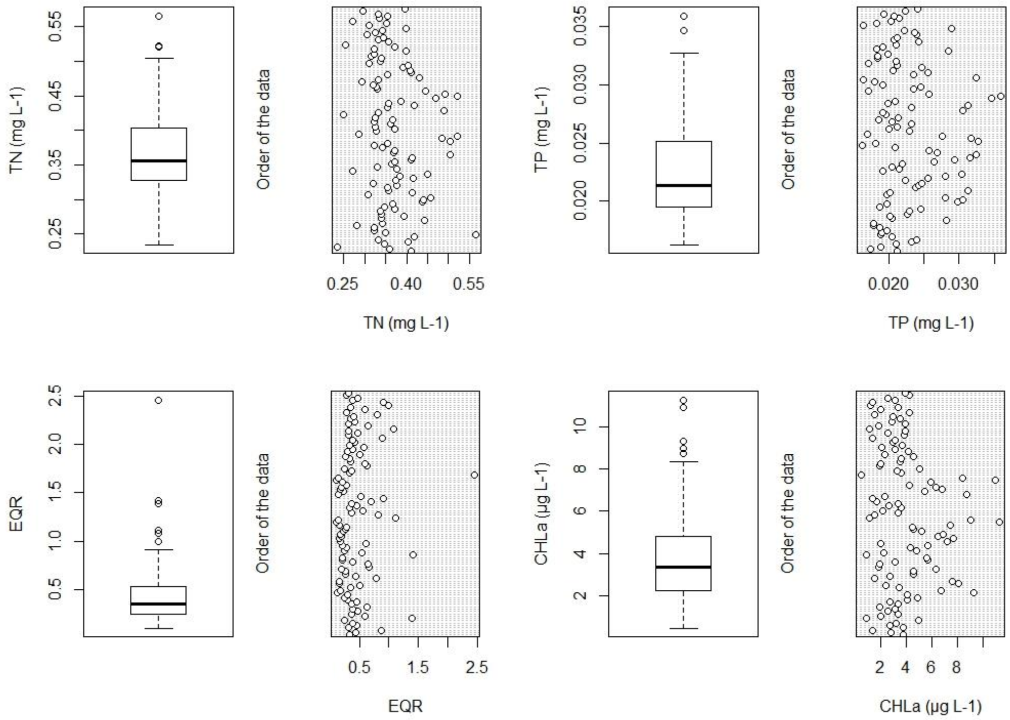



Figure 65. Outliers' verification in the predictors (TN, TP) and response (EQR, Chl*a*) variables, using box plots and Cleveland plots.

### ii. Collinearity among covariates

There is no collinearity (r=0.644, n=104; VIF= 1.709) between covariates Total Phosphorus and Total Nitrogen (Figure 66).

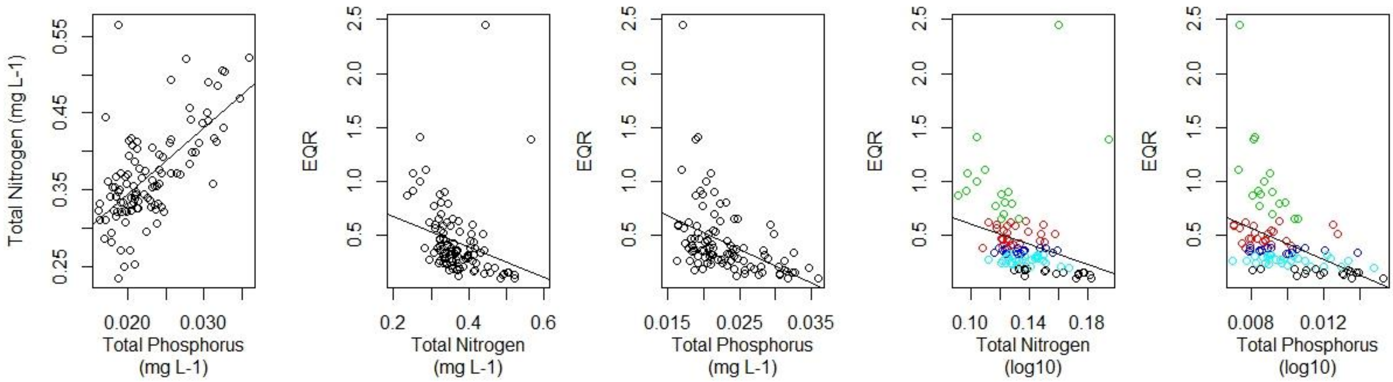



Figure 66. Scatterplots between nutrient explanatory variables Total Nitrogen and Total Phosphorus (left), and between these nutrients and EQR, before (centre) and after (right) nutrient $log_{10}$ transformation. In the right hand graph, dots are coloured after EQS classification: High-green; Good-red; Moderate-dark blue, Poor-blue, and Bad-black.

### iii. Relationship between X & Y

The correlation between nutrients and EQR is weak (EQR vs TN r= -0.264, n=104) to Moderate (EQR vs TP r= -0.433, n=104), not changing significantly after $\log_{10}$ transformed variables (EQR vs TN r= -0.275, n=104; EQR vs TP r= -0.433, n=104). The data shows a clear wedge shape distribution (Figure 66), more evident in TP, indicating that other unknown confounding effects besides the nutrient pressure are taking place. As such, quantile regression, instead of linear, might be a better approach to this dataset.

No sign of an eventual interaction was found between explanatory variables TN and TP (coplots not shown here).

In the univariate regressions (presented in the next section), outliers will be dealt separately for each nutrient. The univariate regression models to be applied will use $\mathrm{Log}_{10}$ transformation of the nutrient concentration.

## b) Statistical analysis

The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below. For Latvia the orthogonal type II regression of EQR v log10 TN concentration presented an acceptable correlation (r=0.693, n=98) (Figure 67), despite that data present a slight wedge shape distribution (see model validation in Figure 68). Nutrient boundary proposals should therefore be compared with those obtained using categorical approaches (Table 18), until better models are developed for this dataset.

Phosphorus presented a weak relationship with EQR (

Table 19), below the advisable r=0.6 when using linear regression models (Latvia r=0.507, n=98). The scattered pattern of the data can be observed in Figure 67. To be able to establish nutrient boundaries for TP using the EQR boundaries of Phytoplankton, the values proposed by the categorical approaches should therefore be taken into consideration. However, the categorical method based on the "Average 75th quartile" is more sensitive to the considerable overlap and "inversion" between High and Good EQS classes that can be observed for Phosphorus in the data of both countries within this type (check Figure 63 and Figure 69). This poses a problem since this region of the quality/disturbance gradient is one of the ranges where nutrient boundaries need to be established.

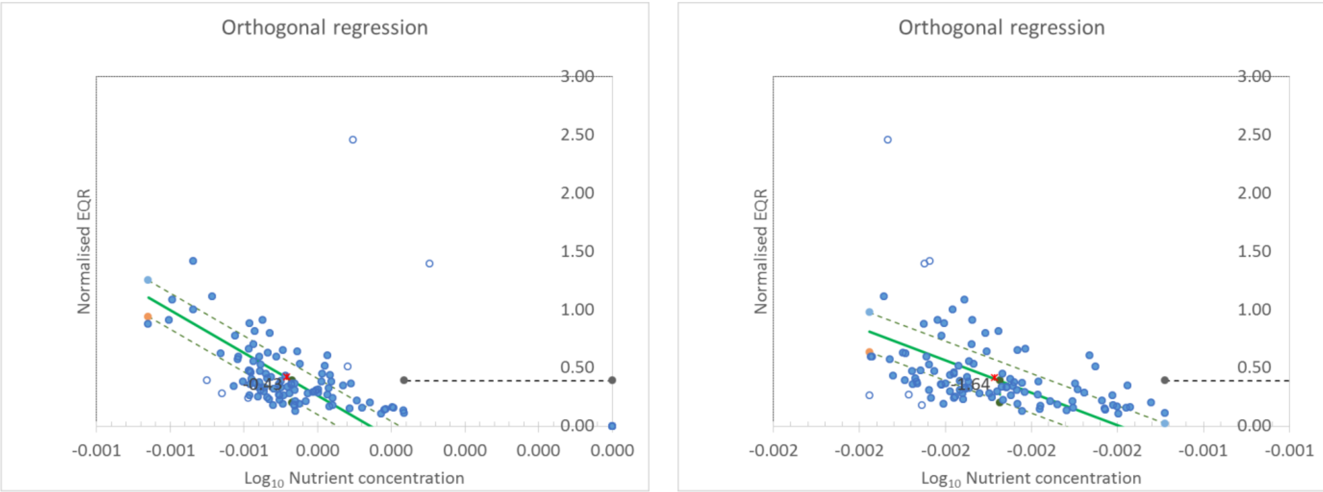



Figure 67. CWBALBC5 Latvia: Orthogonal type II regression of EQR v log10 Total Nitrogen concentration (left) and EQR v log10 Total Phosphorus concentration (right). Solid points values used for models, open circles excluded data (outliers or values beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

**Model validation**

Despite the acceptable r>0.6 obtained for the Latvian dataset regarding the Total Nitrogen regression approach (Table 18), model validation indicates a lack of suitability of this linear regression model (Figure 68). The residuals frequency distribution has an approximate normal distribution; however the residuals show a pattern of increasing variability along the fitted values of this nutrient (no homogeneity observed).

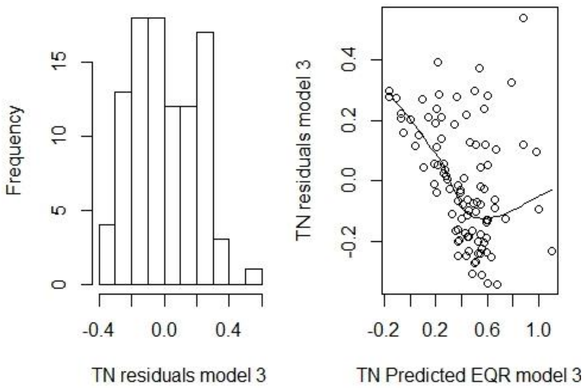



Figure 68. Model diagnosis of residual's normal distribution (histograms) and homoscedasticity (scatterplots), using ordinary residuals, for Latvian data in the Baltic coastal waters common type BC5. Graphs are presented only for regression model 3 (RMA Orthogonal type II regression of EQR versus log10 of TN concentrations (which is an average of model 1 – OLS EQR vs nutrient and model 2 OLS nutrient vs. EQR).

### 6.1.2.2. Lithuania (ds8_CWBALBC5)

a) Data check

For this MS the EQR values cover the full gradient of disturbance, from High to Bad EQS, as required to derive nutrient boundaries. However there is great overlap between all quality classes for TN (Figure 69), and also for TP the High class does not distinguish from the Good and Moderate classes. In both cases, the Good status class presents lower TP values than the High status one. Such an inversion and overlaps may compromise the derivation of nutrient boundaries from EQR values.

There are two outliers identified for TN in the Good (0.85 mg $L^{-1}$) and Moderate classes (0.82 mg $L^{-1}$), and four outliers in the Moderate class for TP (at 0.0725; 0.0710; 0.0685; and 0.0745 mg $L^{-1}$).

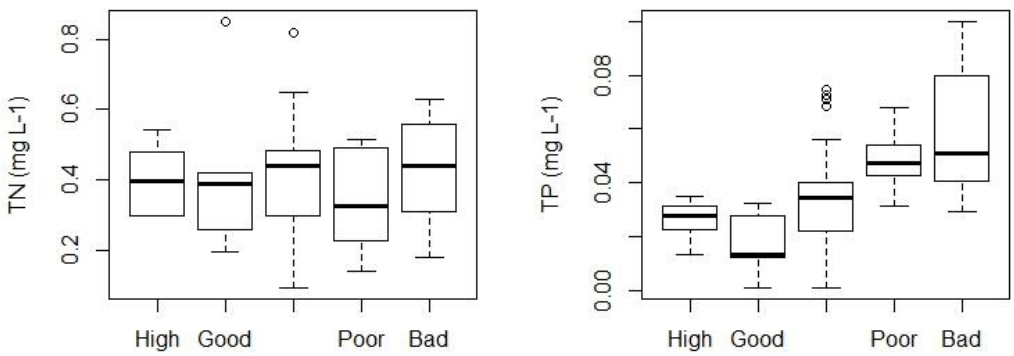



Figure 69. Range of Total Nitrogen (TN) and Total Phosphorus (TP) concentrations for Lithuania sites grouped by ecological quality status (EQS) class: High, Good, Moderate, Poor and Bad.

Nutrient data present a slightly left-skewed distribution, which improved towards a normal distribution after $\log_{10}$ transformation (Figure 70).

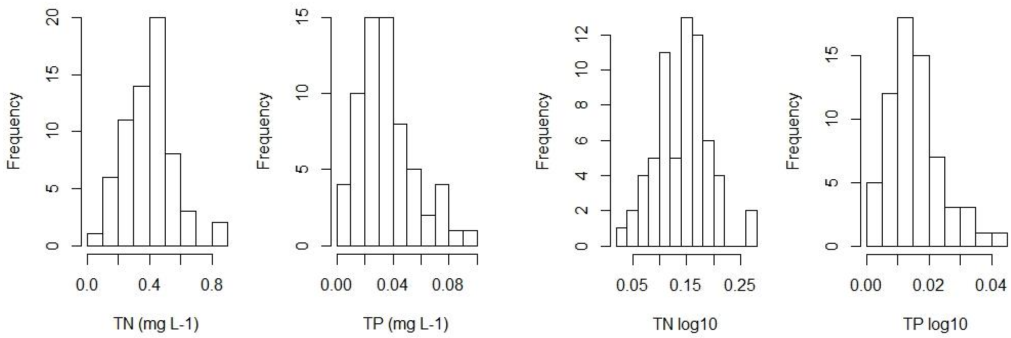



Figure 70. Range distribution of nutrient values Total Nitrogen (TN) and Total Phosphorus (TP) before and after $\log_{10}$ transformation.

#### i. Outliers

Potential outliers in the variables of interest in this dataset are shown in Figure 71. The Cleveland plots distribution show however that these outliers may be particularly problematic for EQR observation >3. Outliers will only be removed after analysing the *xy* relationships, following the nutrient Guidance protocol (2017). The outliers finally removed from the analysis are reported in section 4.

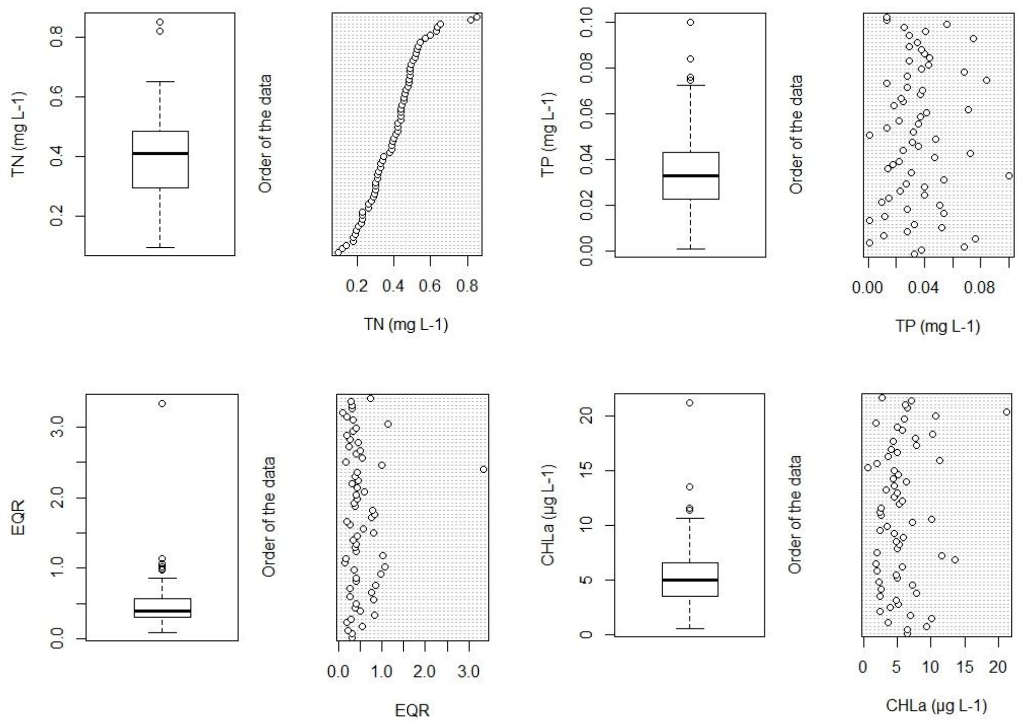



Figure 71. Outliers' verification in the predictors (TN, TP) and response (EQR, Chl*a*) variables, using box plots and Cleveland plots.

### ii. Collinearity among covariates

There is no sign of collinearity (r = -0.026, n=65) between covariates Total Phosphorus (TP) and Total Nitrogen (TN) (Figure 72).

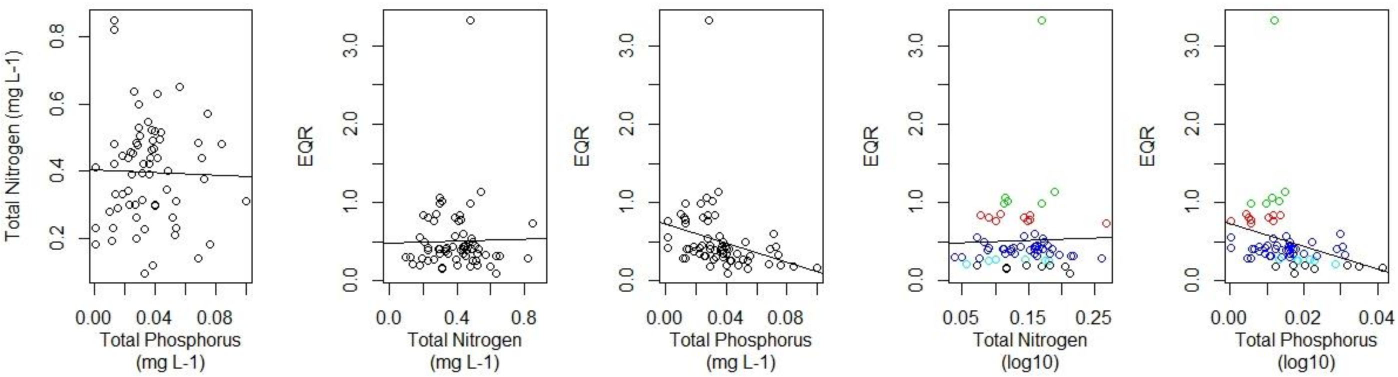



Figure 72. Scatterplots between nutrient explanatory variables Total Nitrogen and Total Phosphorus (left), and between these nutrients and EQR, before (centre) and after (right) nutrient $log_{10}$ transformation and outlier removal (EQR>3). In the right hand graph, dots are coloured after EQS classification: High-black; Good-red; Moderate-green, Poor-dark blue, and Bad-light blue.

### iii. Relationship between X & Y

The correlation between nutrients and EQR is very low for Total Nitrogen (r= 0.02, n=65), with no clear trend in the data, and moderate for Total Phosphorus (r= -0.29, n=65) (Figure 72). Transforming ($log_{10}$) the nutrient variables only improves the relationship for Phosphorus (TN: r= -0.03, n=65 ; TP: r= -0.29, n=65; Figure 72 right) if in addition the EQR oultier >3 is removed (TN: r= -0.05, n=64 ; TP: r= -0.43, n=64). However, for the EQR vs. TP relationship, the data presents a wedge-shape distribution, and therefore the linear regression models do not seem very adeqaute for this dataset.

Also, there is some evidence of confounding factors, not accounted for in the available explanatory variables (Figure 73). At both Phosphorus and Nitrogen lowest concentrations (first two bottom panels in Figure 73) there are several low EQR values observed.

For the above reasons, linear regression models might not be appropriate for this dataset and, as suggested in the guidance, quantile regression approaches migh be more adequate.

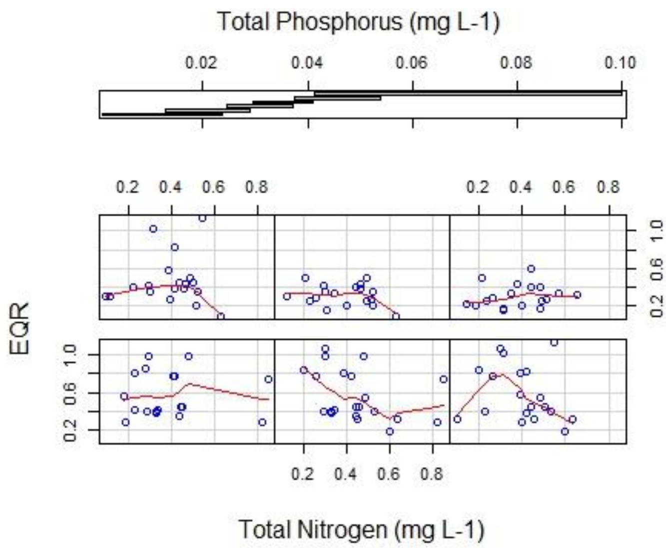



Figure 73. Coplot between EQR and nutrient explanatory variables.

### b) Statistical analysis

The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below.

For Lithuania, due to the weak results obtained in the linear regression analyses for Total Nitrogen, only categorical approaches can be used to derive boundaries for this nutrient using the IC EQR_Chla boundaries. The comparison of the three types of regressions for TN (Lithuania) is shown in Figure 67. But even within the categorical approaches only the methods “Average adjacent classes” and “Minimise class difference” are less sensitive to the considerable overlap between EQS classes in the High - Moderate gradient range, exactly where nutrient boundaries need to be established (check Figure 69). The other two categorical methods propose higher nutrient values for the H/G boundary than for the G/M one (Figure 24).

For Phosphorus presented a weak relationship with EQR (

Table 19), below the advisable r=0.6 when using linear regression models (Lithuania r=0.462, n=61). The scattered pattern of the data can be observed in Figure 74. To be able to establish nutrient boundaries for TP using the EQR boundaries of Phytoplankton, the values proposed by the categorical approaches should be therefore taken into consideration. However, the categorical method based on the "Average 75th quartile" is more sensitive to the considerable overlap and "inversion" between High and Good EQS classes that can be observed for Phosphorus in the data of both countries within this type (check Figure 63 and Figure 69). This poses a problem since this region of the quality/disturbance gradient is one of the ranges where nutrient boundaries need to be established.

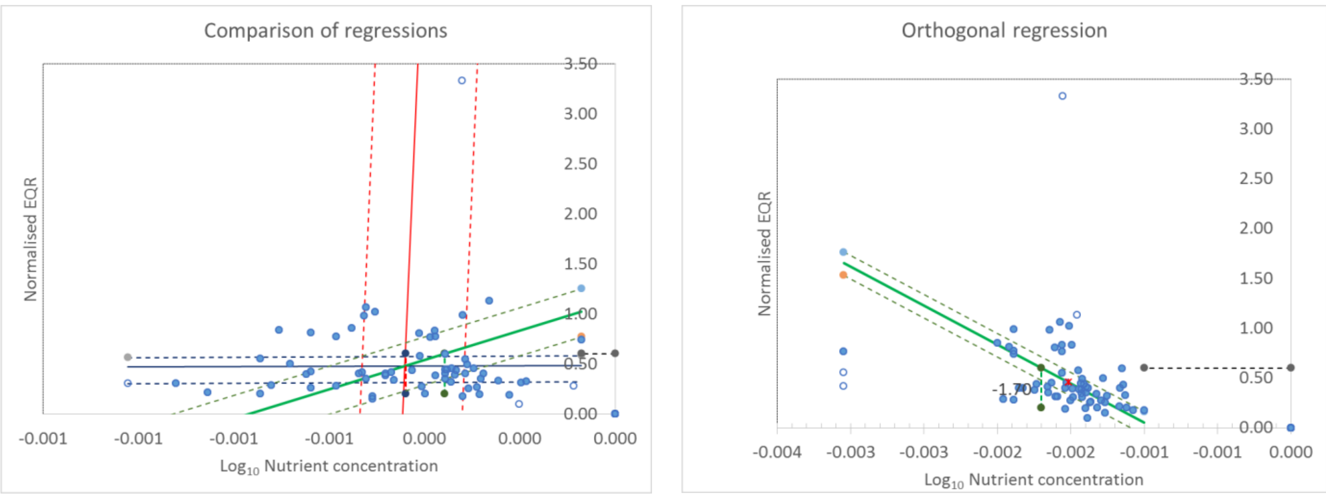



Figure 74. CWBALBC5 Lithuania: Orthogonal type II regression of EQR v log10 Total Nitrogen concentration (left) and EQR v log10 Total Phosphorus concentration (right). For TN (left) the comparison of the three types of regressions is shown: OLS regression of EQR v log10 nutrient concentration (blue lines); OLS regression of log10 nutrient concentration v EQR (red lines); and Orthogonal type II regression of EQR v log10 nutrient concentration (green lines). Solid points values used for models, open circles excluded data (outliers or values beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

### 6.1.2.3. Nutrient boundaries

Below are the summary tables of nutrient boundaries obtained for Total Nitrogen (TN; Table 18) and Total Phosphorus (TP; Table 19) for the MS reporting data in this common type: Latvia and Lithuania. Details of the regression analyses results for both MS are presented in Figure 67.

Table 18. Summary of predicted values of Total Nitrogen concentration (mg $L^{-1}$) at the Good/Moderate and High/Good boundaries for Latvia and Lithuania in common type CWBALBC5, results obtained by linear regression and categorical analyses.

| **CWBALBC5 Total Nitrogen** | **Phytoplankton Models** | **$R^2$** *p-value* n | **Nutrient range mg $L^{-1}$** | **Most likely boundary** | | | | | | **Possible Range** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | **GM mg $L^{-1}$** | | | **HG mg $L^{-1}$** | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| EQR_Chl*a* Boundaries: HG 0.65 GM 0.39 MP 0.33 | EQR v TN (RMA) | **0.480** *p<0.001* n=98 | 0.23 - 0.52 | 0.368 | 0.366 | 0.370 | 0.312 | 0.292 | 0.327 | GM 0.33-0.41 HG 0.26-0.36 |
| **Latvia** | Average adjacent quartiles | n=98 | 0.23 - 0.52 | 0.353 | | | 0.332 | | | |
| | Average adjacent classes | | | 0.347 | | | 0.331 | | | |
| | Average 75$^{th}$ quartile | | | 0.375 | | | 0.339 | | | |
| | Minimise class difference | | | 0.340 | | | 0.320 | | | |
| EQR_Chl*a* Boundaries: HG 0.87 GM 0.6 MP 0.28 | EQR v TN (RMA) | **0.0002** *p=0.919* n=61 | 0.12 - 0.85 | 0.44 | 0.36 | -- | 0.7 | 0.4 | -- | GM 0.3- -- HG 0.2- -- |
| **Lithuania** | Average adjacent quartiles | n=61 | 0.12 - 0.85 | 0.358 | | | 0.37 | | | |
| | Average adjacent classes | | | 0.409 | | | 0.388 | | | |
| | Average 75$^{th}$ quartile | | | 0.419 | | | 0.480 | | | |
| | Minimise class difference | | | 0.285 | | | 0.190 | | | |

Table 19. Summary of predicted values of Total Phosphorus concentration (mg $L^{-1}$) at the Good/Moderate and High/Good boundaries for Latvia and Lithuania in common type CWBALBC5, results obtained by linear regression and categorical analyses.

| **CWBALBC5 Total Phosphorus** | **Phytoplankton Models** | **$R^2$** *p-value* n | **Nutrient range mg $L^{-1}$** | **Most likely boundary** | | | | | | **Possible Range** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | **GM mg $L^{-1}$** | | | **HG mg $L^{-1}$** | | | |
| EQR_Chl*a* Boundaries: HG 0.65 GM 0.39 MP 0.33 **Latvia** | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | GM 0.019-0.026 HG 0.012-0.023 |
| | EQR v TP (RMA) | **0.257** *p<0.001* n=98 | 0.016 - 0.036 | 0.023 | 0.023 | 0.023 | 0.019 | 0.015 | 0.021 | |
| | Average adjacent quartiles | n=98 | | 0.021 | | | 0.020 | | | |
| | Average adjacent classes | | | 0.022 | | | 0.020 | | | |
| | Average $75^{th}$ quartile | | | 0.021 | | | 0.022 | | | |
| | Minimise class difference | | | 0.021 | | | 0.018 | | | |
| EQR_Chl*a* Boundaries: HG 0.87 GM 0.6 MP 0.28 **Lithuania** | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | GM 0.005-0.035 HG 0.001-0.030 |
| | EQR v TP (RMA) | **0.214** *p<0.001* n=61 | 0.0009 - 0.10 | 0.020 | 0.012 | 0.025 | 0.009 | 0.002 | 0.017 | |
| | Average adjacent quartiles | N=61 | | 0.025 | | | 0.021 | | | |
| | Average adjacent classes | | | 0.025 | | | 0.020 | | | |
| | Average $75^{th}$ quartile | | | 0.028 | | | 0.030 | | | |
| | Minimise class difference | | | 0.023 | | | 0.013 | | | |

## 6.2. Mediterranean Sea

In the Mediterranean coastal waters data has been reported for three common types: MED I, II, and III (Table 2). Mediterranean datasets included data from two MS, mostly from Italy and some from Greece. Within common type MEDII there are two different datasets, one from the Adriatic coastal region and another from the Tyrrhenian coastal region.

The EQR method reported is based only on Chl*a.* These two MS reported their EQR values and respective IC boundaries, along with several nutrient parameters (Table 2): Ammonia (NH4), Nitrates (NO3), Nitrites (NO2), Total Nitrogen (TN), Orthophosphates (PO4), Total Phosphorus (TP), and Silica (Si). The exploratory analysis presented below includes them all.

However, for the next stage of the analyses (regressions and categorical approaches) we focused only on the nutrient parameters most commonly assessed across other CW and also TRW: TN and TP (µmol $L^{-1}$). This will allow further comparisons of results obtained across larger regions. The exception is for the common type CWMEDIII where neither TN, TP nor DIN are available. In this case NO3 was used, since there were national boundaries that allow further comparison of the toolkit results.

These Mediterranean datasets are also very comprehensive on the supporting parameters presented and have a relatively higher number of observations than most of the CTRW gathered for this work. Therefore, these datasets may allow more robust and complex regression models to be applied and compare the results obtained with the simpler univariate linear regression models.

Below are presented some of the exploratory analyses to these datasets per type (and region when justified by the type of data, e.g. different IC EQR boundaries).

### 6.2.1. Common Type: CWMEDI

#### a) Data check

Italy is the sole MS presenting data within this common type (IT ds9_CWMEDI). The data distribution covers three EQS classes in the range where nutrient boundaries need to be established: High to Moderate (Figure 75). However, for both nutrients some overlap is observed across EQS classes, in particular between High and Good status. Only TN and TP results are fully presented since these will be the ones modelled in section 4. Some outliers can be seen, especially in TP (1.376, 1.360, 1.780, and 4.180 µmol $L^{-1}$).

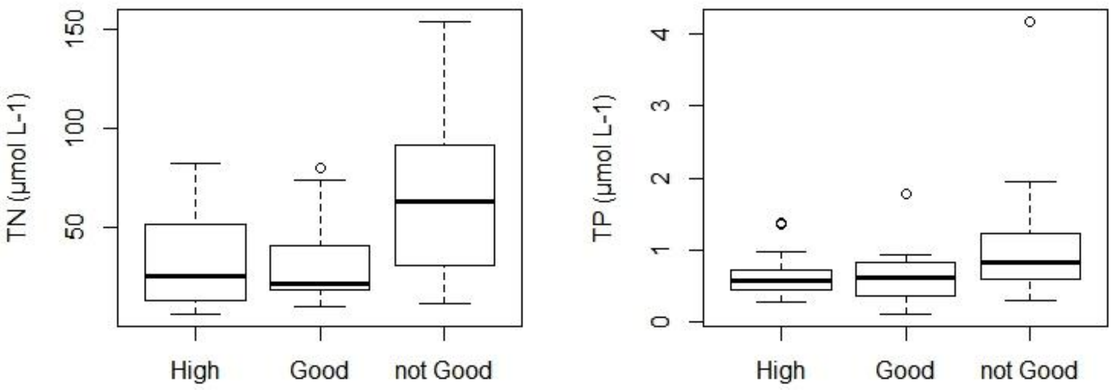
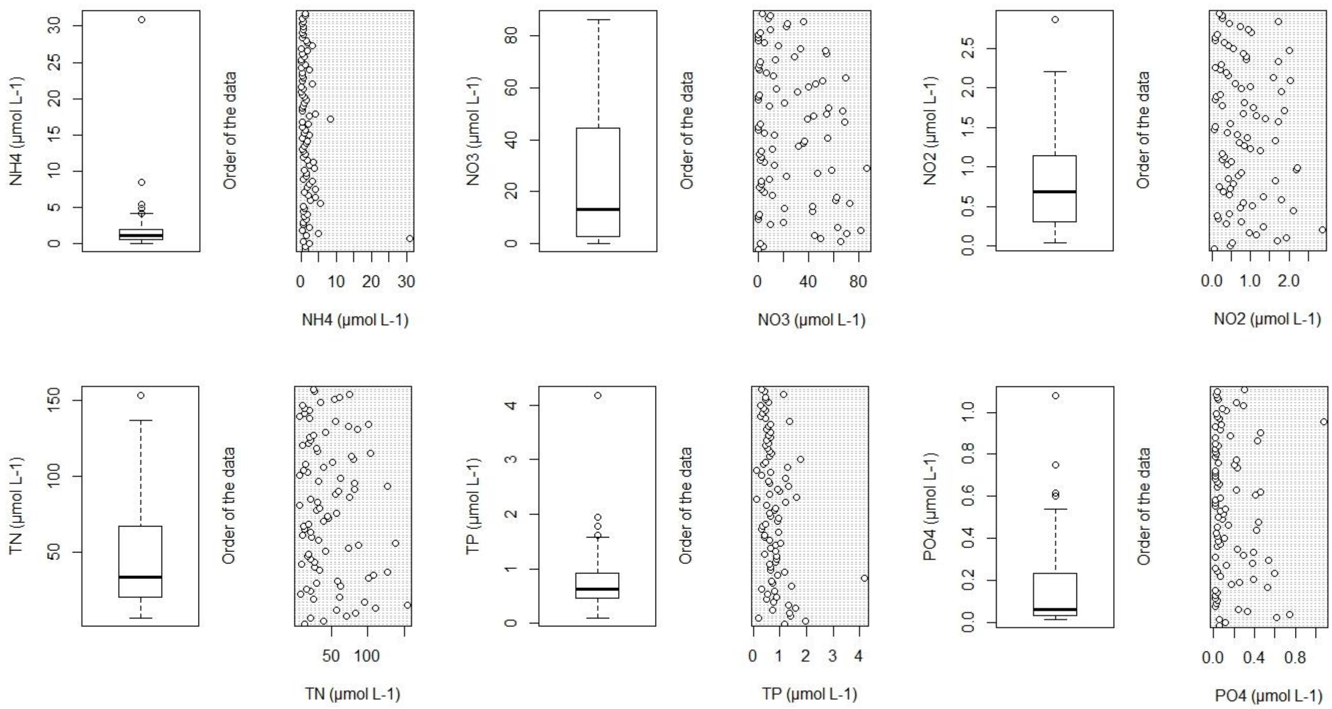



Figure 75. Range of TN and TP concentrations for CWMEDI sites grouped by ecological quality status (EQS) class: High, Good and below Good status.

### i. Outliers

Boxplots in Figure 76 point out the outliers in the data, however from the Cleveland plots it can be observed that some of those values present themselves more disperse from the main data than others. Special attention should be given to Ammonia outlier at 30.93 µmol $L^{-1}$, TP outlier >4 µmol $L^{-1}$, and EQR values of 8 and 12.

EQR in this dataset are ranging up to 12, with a large proportion of the sites falling beyond 1, so this should be checked with data providers, since it evidences inadequacy of reference conditions adopted.



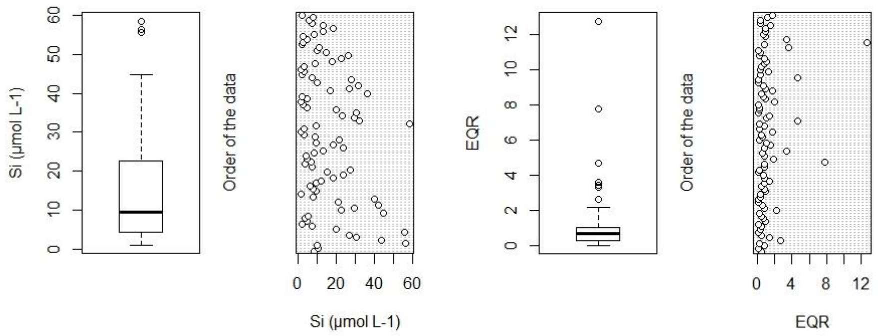



Figure 76. Outliers' verification in the predictors and response variable (EQR).

### ii. Collinearity among covariates

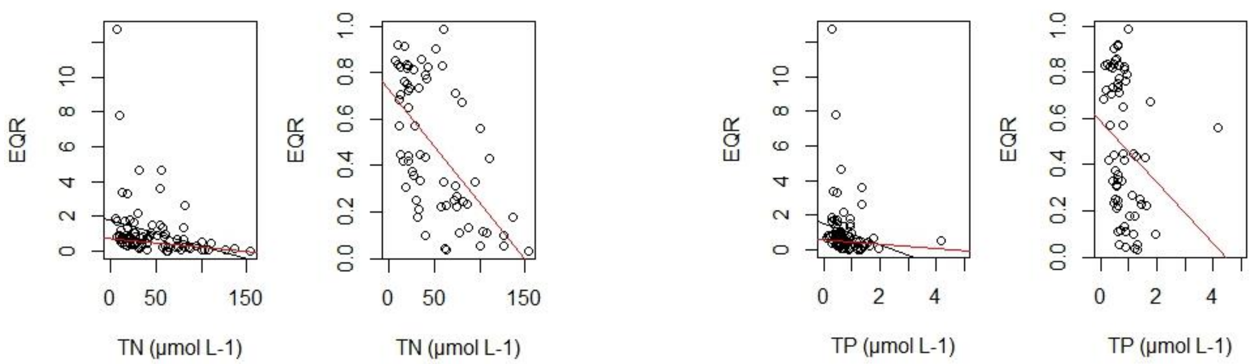
Some collinearity might be observed between Nitrates with TN, Nitrites, and Silica (Table 20). Calculating the variance inflation factor (VIF) for all explanatory variables (using vifstep with threshold set at vif=8) showed that Nitrates has collinearity problems. After excluding the collinear variable, the linear correlation coefficients would range between a minimum correlation of 0.116 for Orthophosphates ~ Nitrites and a maximum correlation of 0.684 for Silica ~ Nitrites. (Full results of the analysis are given in the R scripts). In case of multiple regression is attempted variable selection should be carefully considered.

Table 20. Pearson correlations for checking for collinearity between nutrient explanatory variables.

| | Ammonia | Nitrates | Nitrites | Total Nitrogen | Orthophosphates | Total Phosphorus | Silica |
|---|---|---|---|---|---|---|---|
| **Ammonia** | 1 | 0.18 | 0.185 | 0.182 | 0.413 | 0.237 | 0.271 |
| **Nitrates** | 0.18 | 1 | 0.756 | 0.917 | 0.187 | 0.475 | 0.81 |
| **Nitrites** | 0.185 | 0.756 | 1 | 0.674 | 0.116 | 0.265 | 0.684 |
| **Total Nitrogen** | 0.182 | 0.917 | 0.674 | 1 | 0.145 | 0.502 | 0.676 |
| **Orthophosphates** | 0.413 | 0.187 | 0.116 | 0.145 | 1 | 0.325 | 0.42 |
| **Total Phosphorus** | 0.237 | 0.475 | 0.265 | 0.502 | 0.325 | 1 | 0.544 |
| **Silica** | 0.271 | 0.81 | 0.684 | 0.676 | 0.42 | 0.544 | 1 |

### iii. Relationship between X & Y

XY relationships are presented below for all nutrients with EQR (Figure 77). Two plots are presented, one for all range of EQR in the data (up until EQR=12) and another where EQR is truncated at 1. However for the analyses, even if some EQR outliers are removed, data won't be truncated at 1, to avoid loose the signal with nutrient relationships. Both TN and TP present a weak (TN r= -0.291; TP r= -0.187) and clearly wedge shape distribution with EQR, more evident for TN. The same trend can be observed for other EQR ~ nutrient relationships.



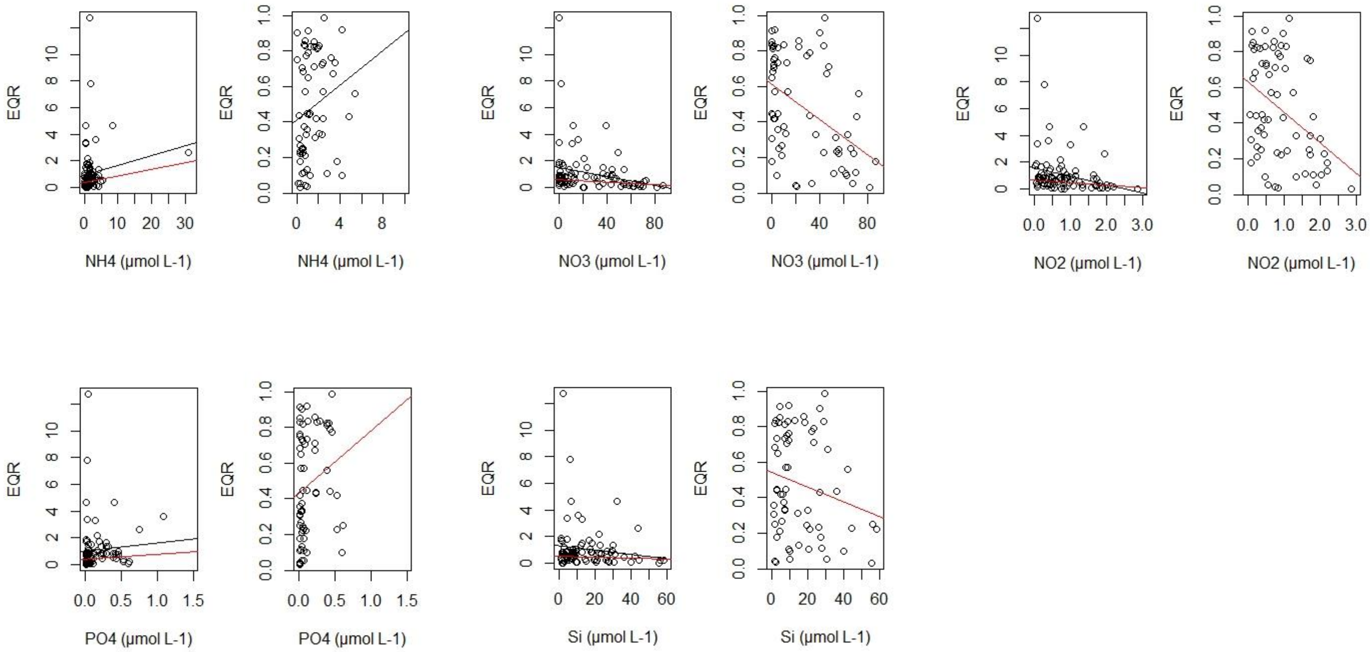


Figure 77. Scatterplots of nutrients relationships with EQR (based on Chl*a*), for all range of EQR in the data (left graph), and for EQR truncated at 1 (right graph). Black regression line for all data, red regression line for regression considering only EQR values <1.

### b) Statistical analysis

The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables below.

Both for Total Nitrogen (TN) and Total Phosphorus (TP), due to the weak results obtained for univariate linear regression analyses (Table 21; Figure 78), only categorical approaches should be used to derive nutrient boundaries from IC EQR_Chl*a*.

However due to the great overlap between High and Good EQS classes, particularly for TN (Figure 75), not all categorical analyses provide useful results. For TN, the “Average adjacent quartiles” and the “Average 75th quartile” methods indicate a higher nutrient value for the H/G boundary than for the G/M (Table 21).

Has was expected from the exploratory analyses in section 3, the wedge-shape of data distribution does not allow to use linear regression approaches to derive nutrient boundaries for TN or TP from IC EQR boundaries (Figure 78).
Other approaches such as quantile regression would need to be tested in this dataset.

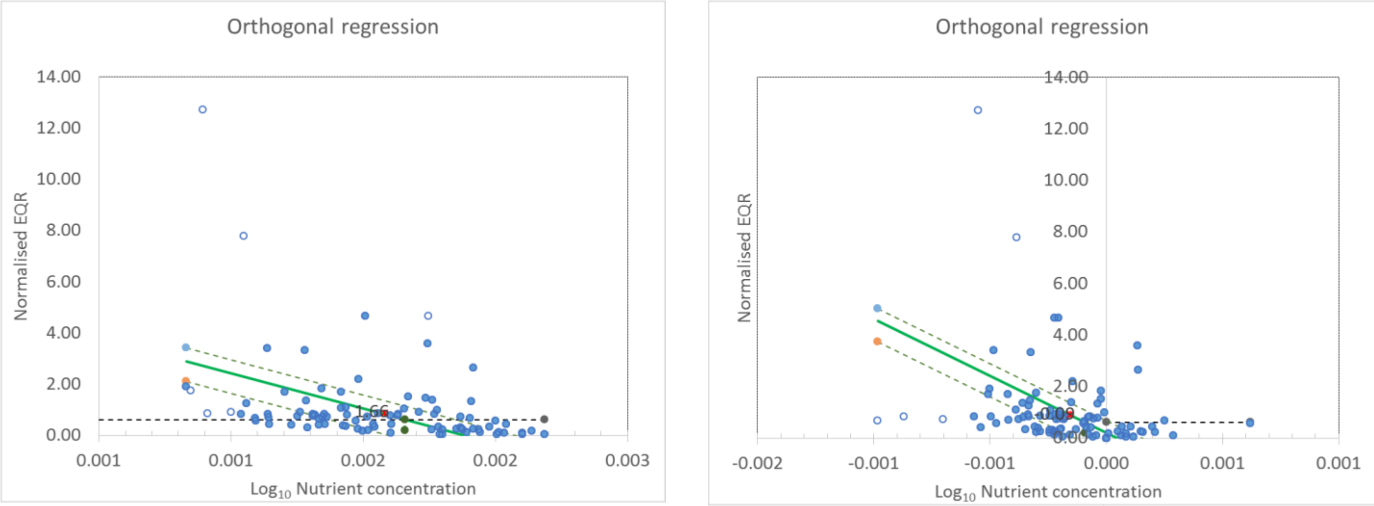



Figure 78. CWMEDI: Orthogonal type II regression of EQR v log10 Total Nitrogen concentration ($R^2$=0.09). Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

### 6.2.1.1. Nutrient boundaries

Below are the predicted nutrient boundaries obtained for Total Nitrogen (TN) and Total Phosphorus (TP) (Table 21) for the MS reporting data in this common type: Italy (IT).

Table 21. Summary of predict values of nutrient concentration at the Good/Moderate and High/Good boundaries of TN and TP (µmol $L^{-1}$), for common type CWMEDI, results obtained by regression and categorical analyses.

| CWMEDI (IT) | Phytoplankton Models | $R^2$ *p-value* n | Nutrient range µmol $L^{-1}$ | Most likely boundary | | | | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | GM µmol $L^{-1}$ | | | HG µmol $L^{-1}$ | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | |
| EQR_Chl*a* Boundaries: HG 0.85 GM 0.62 | EQR v TP (RMA) | **0.098** *p=0.004* n=82 | 7 - 154 | 45 | 40 | 67 | 37 | 36 | 38 | GM 22-94 HG 12-64 |
| **Total Nitrogen** | Average adjacent quartiles | | | 36 | | | 36 | | | |
| | Average adjacent classes | n=82 (88) | | 43 | | | 26(24) | | | |
| | Average $75^{th}$ quartile | | | 41 | | | 52 | | | |
| | Minimise class difference | | | 39(40) | | | 24 | | | |
| | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | |
| **Total Phosphorus** | EQR v TP (RMA) | **0.043** *p=0.06* n=83 | 0 - 4 | 0.81 | 0.72 | 1.38 | 0.72 | 0.71 | 0.78 | GM 0.32-1.93 HG 0.18-1.08 |
| | Average adjacent quartiles | | | 0.72(0.70) | | | 0.58(0.54) | | | |
| | Average adjacent classes | n=83 (88) | | 0.72 | | | 0.62(0.59) | | | |
| | Average $75^{th}$ quartile | | | 0.81 | | | 0.71 | | | |
| | Minimise class difference | | | 0.76 | | | 0.57(0.54) | | | |

### 6.2.2. Common Type: CWMEDII

The data gathered for this common type was provided by Italy, and regards the Adriatic (no. obs = 336) and the Tyrrhenian (no. obs = 245). The EQR method presented is based solely on the Chl*a*, and the two coastal regions reported different EQR boundaries from the intercalibration exercise. In this sense, the data will be analysed separately. Results are presented below for each region.

#### 6.2.2.1. **Adriatic** (IT ds10_CWMEDIIAdriatic)

a) Data check

The Adriatic dataset, despite covering a gradient of disturbance that allows deriving nutrient boundaries, shows a great overlap between quality classes along the nutrient concentrations (Figure 79). For TP, there is hardly any distinction between the EQR quality classes, with the High status sites presenting even higher mean TP concentrations than the lower quality sites (High= 0.918 µmol $L^{-1}$; Good= 0.582 µmol $L^{-1}$; not Good= 0.731 µmol $L^{-1}$). This does not allow deriving nutrient boundaries for this nutrient from this dataset. Consider checking for interaction between nutrients.

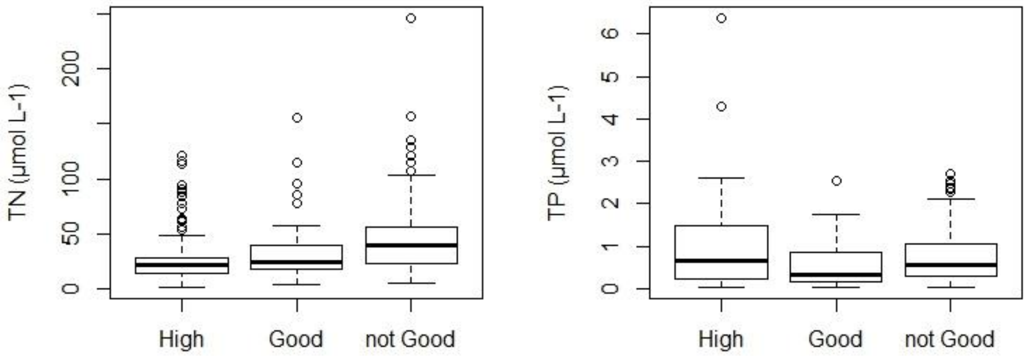



Figure 79. Range of TN and TP concentrations for CWMEDII in the Adriatic sites grouped by ecological quality status (EQS) class: High, Good and below Good status.

Nutrient data present a left-skewed distribution, and only Total Nitrogen (TN) improved towards a normal distribution after $\log_{10}$ transformation (Figure 80).

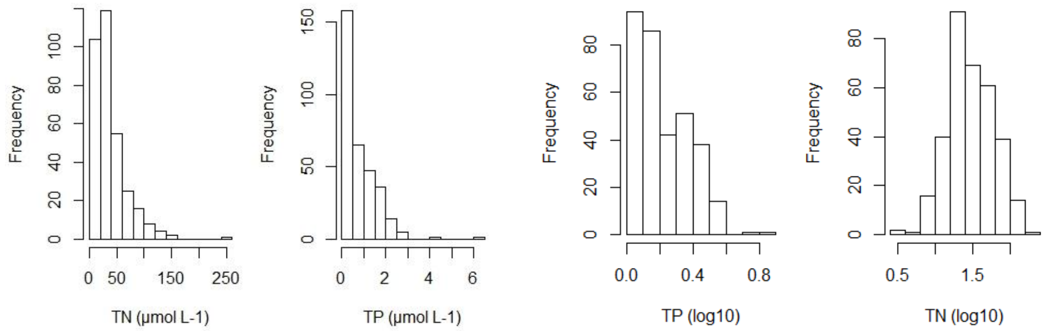



Figure 80. Range distribution of nutrient values Total Nitrogen (TN) and Total Phosphorus (TP) before and after $\log_{10}$ transformation.

### i. Outliers

Outliers in the variables of interest are shown in the boxplots and Cleveland plots below. The outliers will be removed if justified after analysing the xy relationships between the response (EQR) and nutrient variables. These will be reported in section 4.

This dataset presents 25% of the EQR values above the expected range [0-1], and therefore reference conditions adjustment to these sites should also be checked.

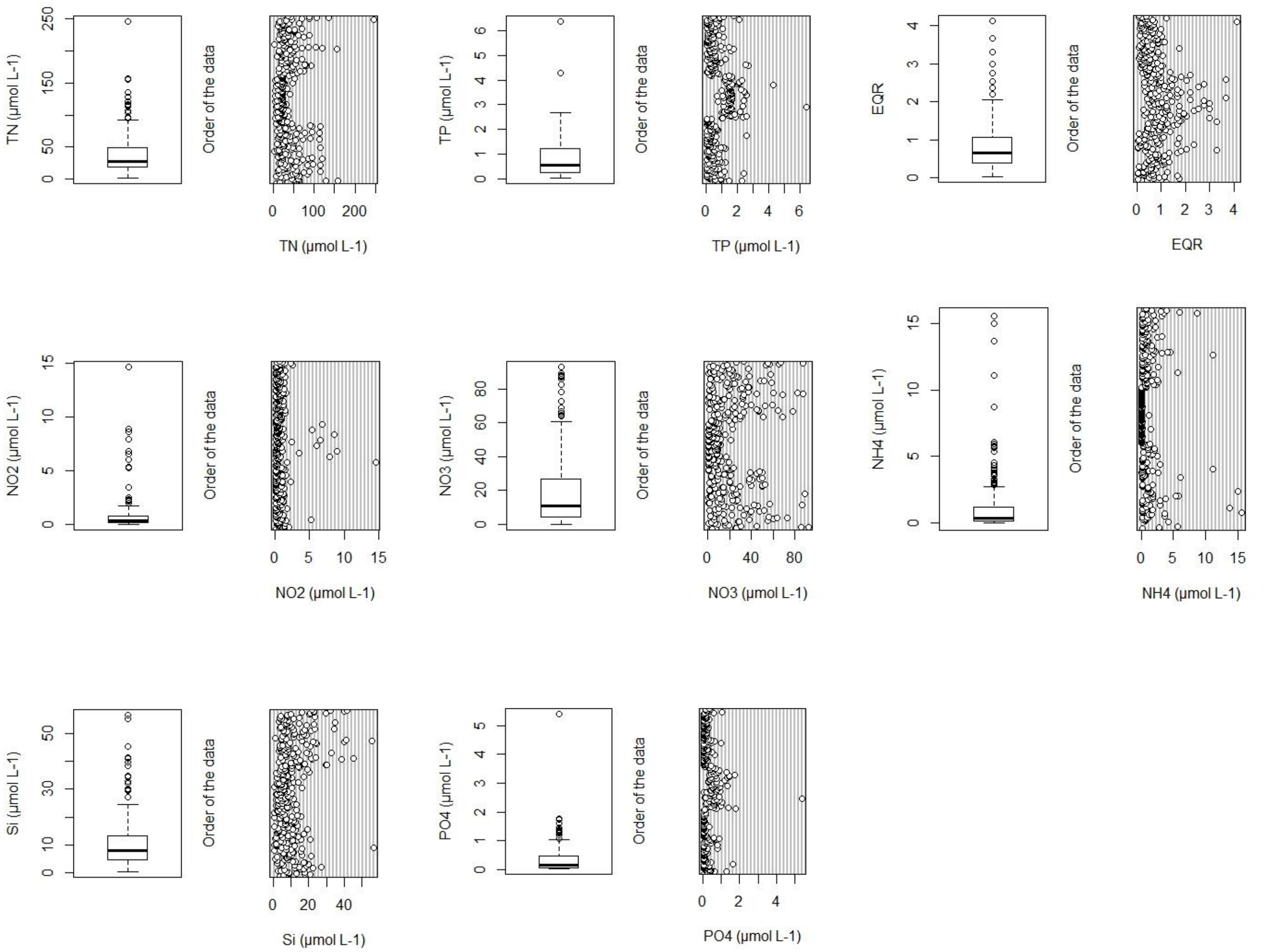



Figure 81. Outliers' verification in the predictors and response variable (EQR), in the CWMEDII Adriatic dataset.

### ii. Collinearity among covariates

Despite some higher correlations between explanatory variables (Table 22, e.g. Total Nitrogen ~ Nitrates), the VIF showed that no variable from the seven input variables has collinearity problem (VIFs<8).

Table 22. Pearson correlation coefficients for nutrients (higher values observed highlighted in red) and variance inflation factor (VIF) of the nutrient variables.

| | Ammonia | Nitrates | Nitrites | Total_Nitrogen | Orthophosphates | Total_Phosphorus | Silica | VIF |
|---|---|---|---|---|---|---|---|---|
| Ammonia | 1 | 0.228 | -0.009 | 0.308 | -0.029 | -0.106 | 0.26 | 1.219725 |
| Nitrates | 0.228 | 1 | 0.013 | 0.873 | -0.117 | -0.061 | 0.651 | 5.898099 |
| Nitrites | -0.009 | 0.013 | 1 | 0.091 | 0.291 | 0.254 | 0.16 | 1.179558 |
| Total_Nitrogen | 0.308 | 0.873 | 0.091 | 1 | -0.054 | -0.011 | 0.549 | 4.476825 |
| Orthophosphates | -0.029 | -0.117 | 0.291 | -0.054 | 1 | 0.786 | -0.032 | 2.832673 |
| Total_Phosphorus | -0.106 | -0.061 | 0.254 | -0.011 | 0.786 | 1 | 0.025 | 2.72572 |
| Silica | 0.26 | 0.651 | 0.16 | 0.549 | -0.032 | 0.025 | 1 | 2.401561 |

### iii. Relationship between X & Y

In general the relationship of EQR with nutrients is weak. Silica, Nitrates and Total Nitrogen (TN) present the stronger relationships observed, which are presented below for all nutrients: Total Nitrogen (TN), Total Phosphorus (TP), Silica, Nitrates, Ammonia, Nitrites, and Orthophosphates (Figure 82). Some of the nutrients, namely TN, present a wedge shape distribution, which might compromise the use of linear regression approaches. Other nutrients do not show a relationship with EQR, data presents no trend, being too dispersed with a cloud shape.

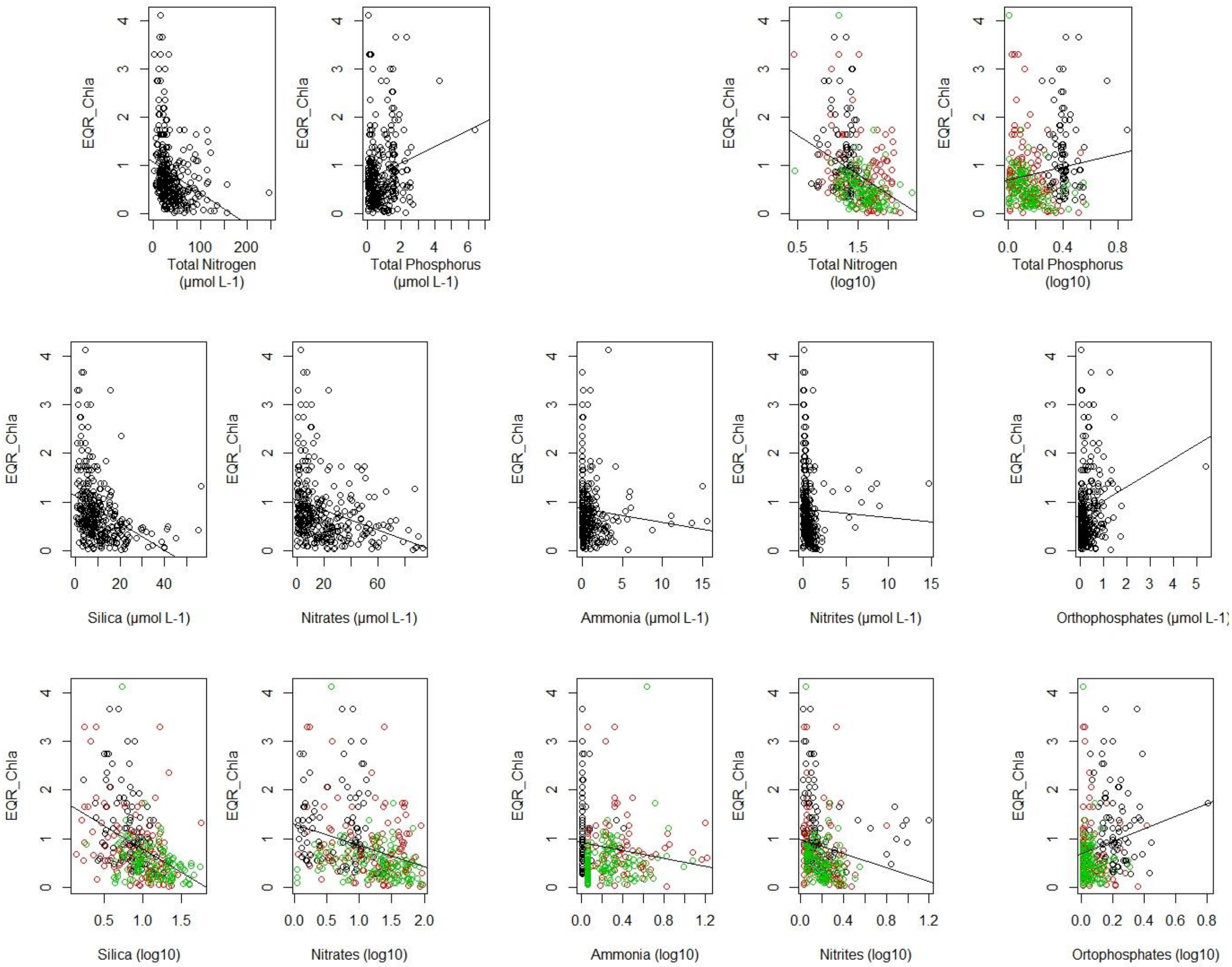



Figure 82. Scatterplots of relationships between nutrient explanatory variables (Total_Nitrogen (TN), Total_Phosphorus (TP), Silica, Nitrates, Ammonia, Nitrites, Orthophosphates) and response variable EQR. Relationship of EQR with log10 transformed variables is also presented (scatterplots with coloured points according to Adriatic coastal region: Abruzzo, Marche, and Veneto).

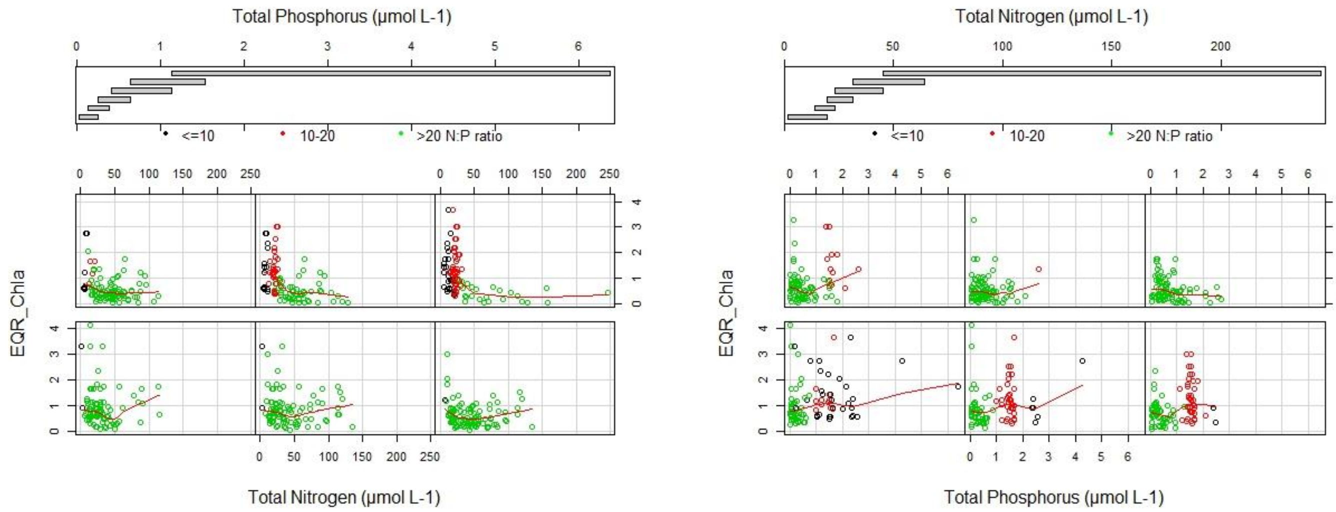



Figure 83. Coplots for checking possible interactions between TN and TP. Points are coloured in relation to the N:P ratio (≤10; 10-20; >20).

## b) Statistical analysis

The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Bivariate regressions EQR ~ TN+TP
- Quantile linear regressions EQR ~ TN
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables in section 6.2.2.3.

Due to the weak results obtained in the linear regression analyses, the categorical approaches should be preferred to derive boundaries for TN. For TP, both the regression and the categorical approaches are compromised as there is a great overlap of nutrient concentrations between quality classes. Furthermore, higher mean TP concentrations were observed in high quality sites comparatively to lower quality ones (see section 3; Figure 79). The categorical approaches are thus proposing boundaries at higher nutrient concentrations for the H/G boundary than for the G/M.

### *Univariate regressions EQR ~ TN and EQR ~ TP*

Has was expected from the exploratory analyses in section 3, for TP there is no observed trend of EQR with this nutrient and therefore it is very difficult to derive nutrient boundaries from IC EQR boundaries (Figure 84). For TN the wedge-shape of data distribution does not allow to use the linear regression approaches proposed, and therefore other approaches such as quantile regression would need to be tested in this dataset. Outliers identified beyond the 0.975 quantile have been removed and are identified in the Figure below.

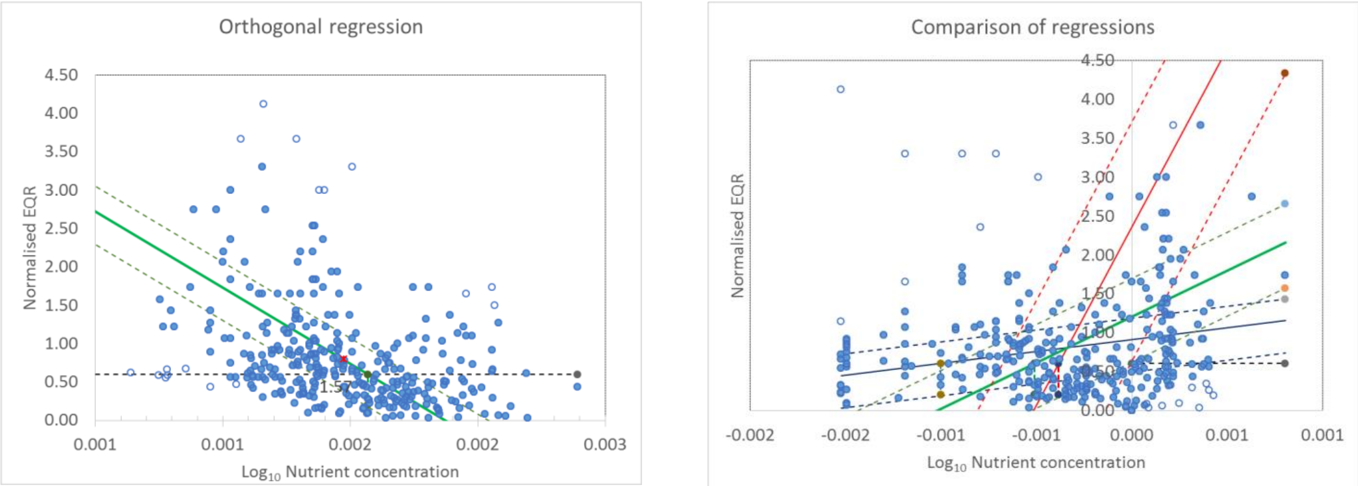



Figure 84. CWMEDII Adriatic: Orthogonal type II regression of EQR ~ log10 Total Nitrogen concentration (left; r=0.466) and for EQR ~ log10 Total Phosphorus concentration (right; r=0.258) the comparison of the three types of regressions is shown: OLS regression of EQR v log10 nutrient concentration (blue lines); OLS regression of log10 nutrient concentration v EQR (red lines); and Orthogonal type II regression of EQR v log10 nutrient concentration (green lines). Solid points values used for models, open circles excluded data (outliers or beyond linear region). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

### *Bivariate linear regression EQR ~ TN + TP*

The bivariate model does not improve much from the univariate model for TN presented previously, with $R^2$ still below advised (Table 25; N+P $R^2$= 0.2338). The Q-Q plot (Figure 85), for model validation, indicates that there is some skewness left in the data, which is probably related to TP; whose distribution was not normal even after log transformed (as seen in Figure 80). In addition, the nutrient boundaries proposed by the multivariate approach, both for TN and TP (Table 25 and Table 26), are not coherent since the proposed H/G nutrient boundaries are higher than the G/M ones for both nutrients.

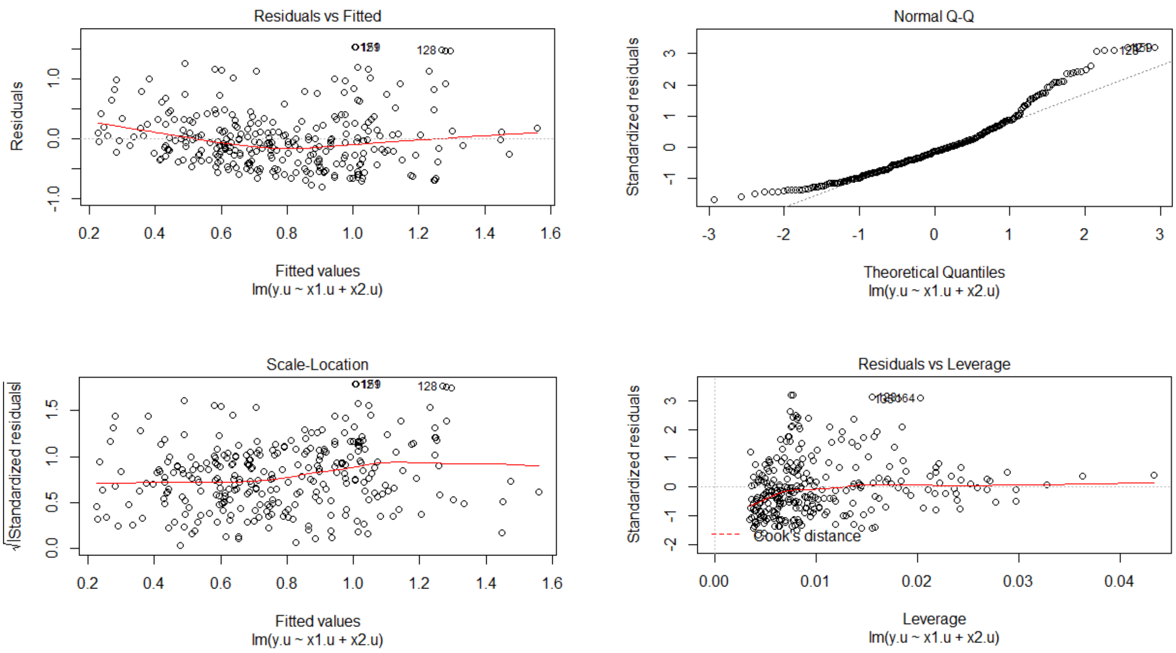



Figure 85. Model validation plots for multivariate model EQR ~ TN + TP, which presented the lowest AIC in comparison to the univariate models (AIC: NP mod = 407.4; N mod = 428.6; P mod = 464.8).

There is too much spread in TP data, and no evidence of clear interaction between the nutrients that would explain the EQR observed in this data set. Also, observing the wedge-shape distribution of the TN data (Figure 84), we should consider that other factors may be constraining the Phytoplankton in this dataset (i.e. with lower EQR values than would be expected for the TN values found). This is also observed for other nutrients in this dataset (Figure 82). In these cases, a quantile regression approach could be an alternative to derivate nutrient boundaries from the upper quantiles of the distribution (see details in Guidance 2017). That would be a nutrient concentration which would be relatively certain of causing a downgrade of biological status (i.e. EQR decrease, see Phillips *et al.* 2017).

***Linear Quantile Regression EQR ~ TN*** *(using Quantile_v2.xlsx not included in the Guidance)*

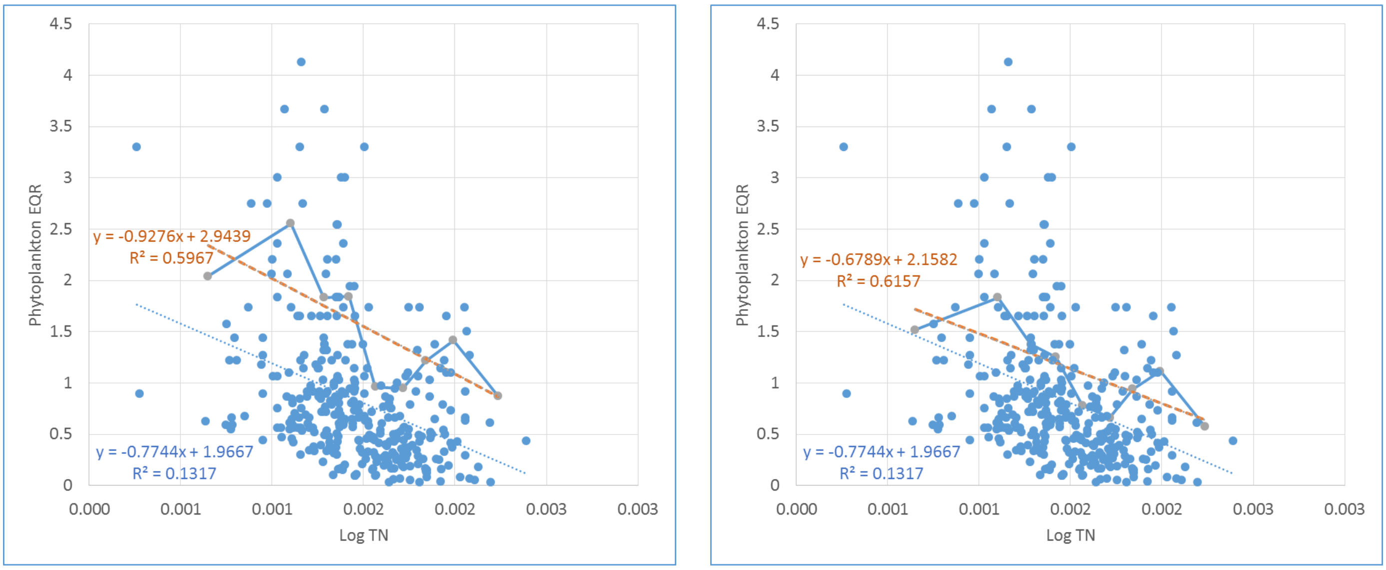



Figure 86. Linear quantile regression for EQR ~ TN in the Adriatic dataset: scatter plot of the data, together with a fitted regression line for all data together with the quantiles for each category of nutrient (orange line): 90$^{th}$ (left) and 80$^{th}$ (right).

Table 23. Predicted nutrient concentrations at high/good and good/moderate boundaries using univariate linear quantile regression approach at 80$^{th}$ and 90$^{th}$ quantiles.

| | | **Predicted nutrient concentrations** | | | |
|---|---|---|---|---|---|
| | | *80$^{th}$ quantile* | | *90$^{th}$ quantile* | |
| **Boundary** | **EQR boundary** | **TN** *$R^2$=0.615* | **TP** | **TN** *$R^2$=0.597* | **TP** |
| **High/Good** | 0.81 | 97 | -- | 200 | -- |
| **Good/Mod** | 0.60 | 197 | -- | 336 | -- |

### 6.2.2.2. **Tyrrhenian** (IT ds11_CWMEDIITyrrhenian)

a) Data check

**i. Outliers**

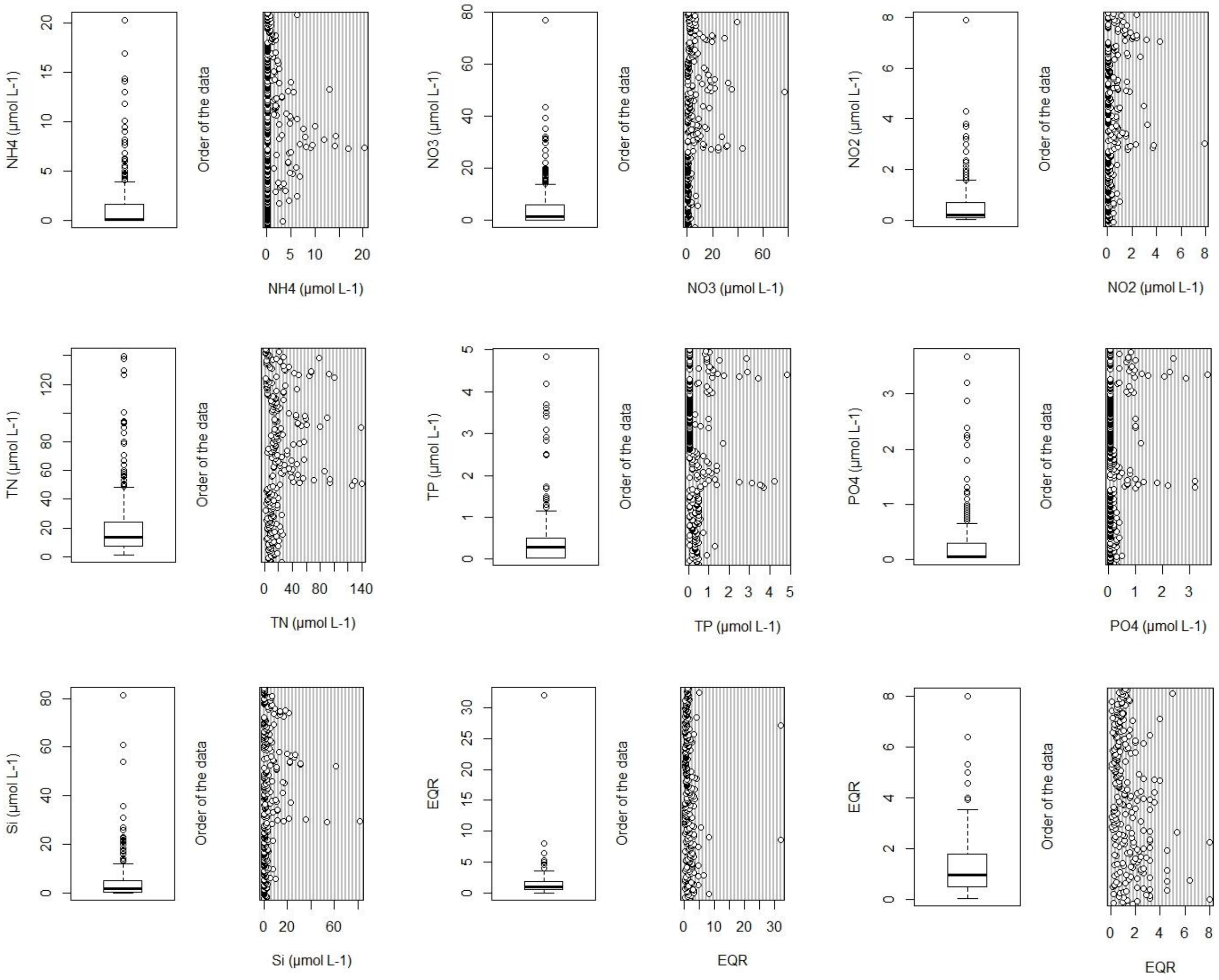



Figure 87. Outliers verification CWMEDIII Tyrrhenian.

EQR values indicate poor definition of reference conditions, with an extremely high EQR_Chla value of 32 when normal EQR range is [0-1]. If these two more extreme values are removed, the EQR range still extends well beyond the expected EQR range, until EQR = 8 (Figure 87).

### ii. Collinearity among covariates

Attention to Orthophosphates - Total Phosphorus, and Nitrates - Total Nitrogen (see Figure 88 and Table 24).

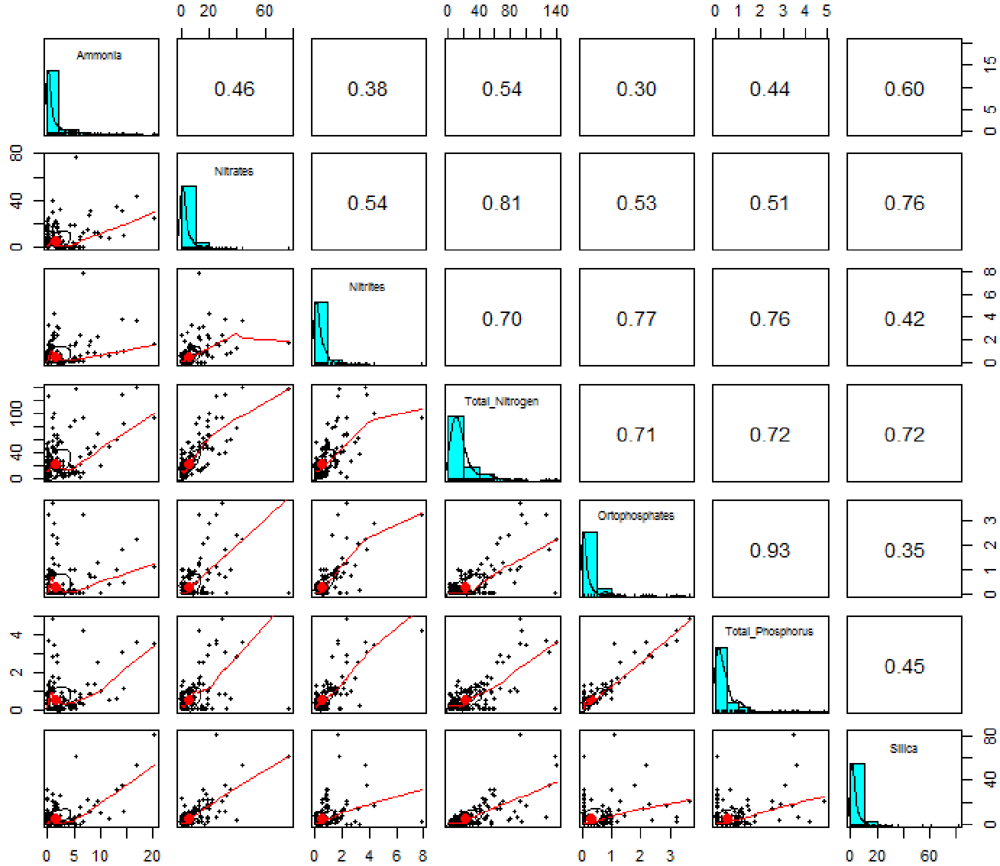



Figure 88. Pearson correlations between nutrients.

Table 24. Pearson correlations between nutrients, with collinear variables highlighted.

| | Ammonia | Nitrates | Nitrites | Total_Nitrogen | Orthophosphates | Total_Phosphorus | Silica |
|---|---|---|---|---|---|---|---|
| Ammonia | 1 | 0.46 | 0.385 | 0.537 | 0.298 | 0.437 | 0.604 |
| Nitrates | 0.46 | 1 | 0.537 | 0.813 | 0.527 | 0.507 | 0.756 |
| Nitrites | 0.385 | 0.537 | 1 | 0.701 | 0.775 | 0.763 | 0.424 |
| Total_Nitrogen | 0.537 | **0.813** | 0.701 | 1 | 0.71 | 0.719 | 0.719 |
| Orthophosphates | 0.298 | 0.527 | 0.775 | 0.71 | 1 | 0.928 | 0.348 |
| Total_Phosphorus | 0.437 | 0.507 | 0.763 | 0.719 | **0.928** | 1 | 0.453 |
| Silica | 0.604 | 0.756 | 0.424 | 0.719 | 0.348 | 0.453 | 1 |

### iii. Relationship between X & Y

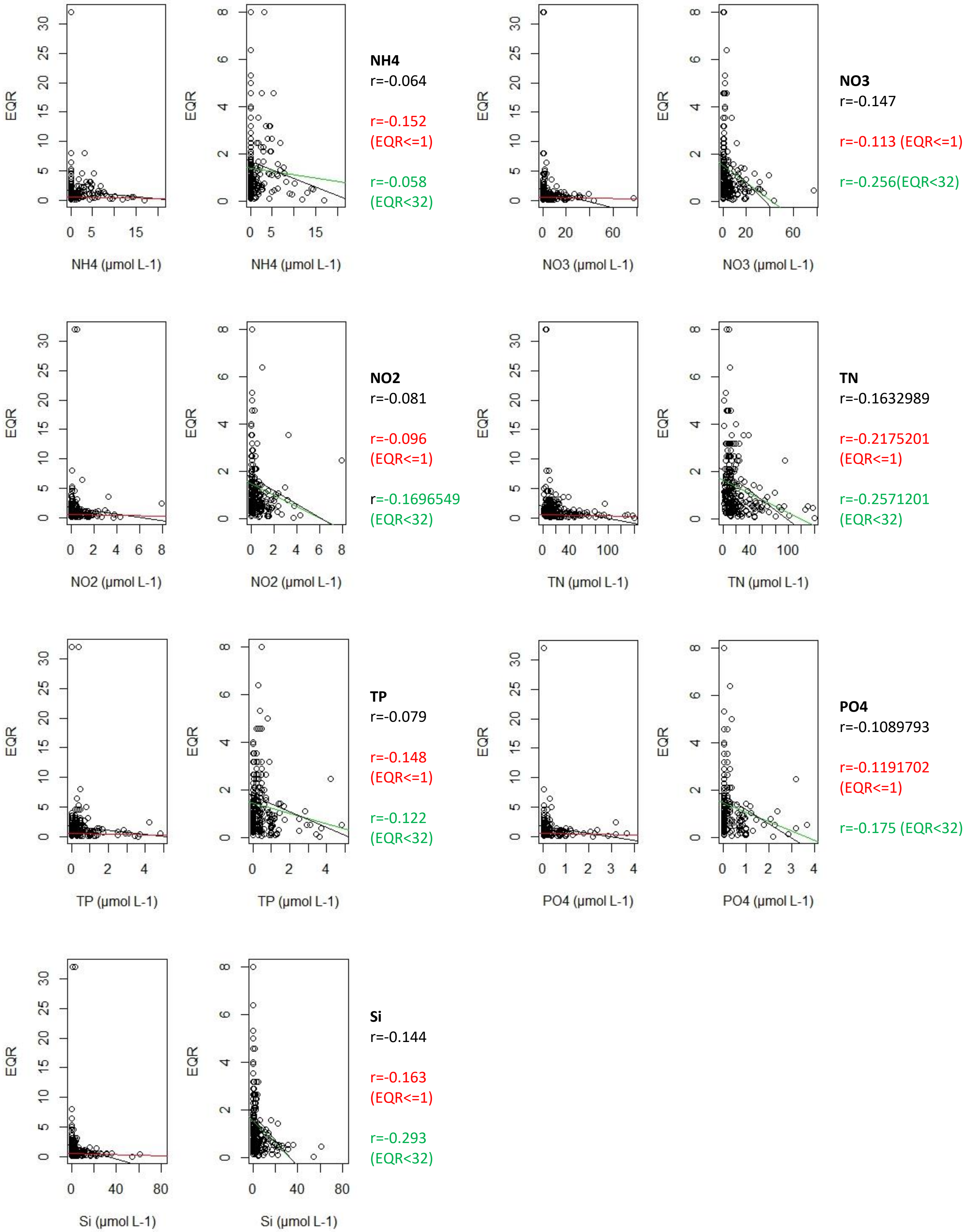



Figure 89. Scatterplot between nutrients and response variable EQR.

b) Statistical analysis

The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Total Nitrogen (TN)
- Univariate regressions EQR ~ Total Phosphorus (TP)
- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary tables in section 6.2.2.3. For details in the analyses performed check Annex files.

***Univariate regressions EQR ~ TN and EQR ~ TP***

For both nutrients (TN and TP) the wedge-shape of data distribution (Figure 90) does not allow to use the linear regression approaches proposed in the toolkit, and therefore other approaches would need to be tested in this dataset, such as quantile regression (included in the toolkit as alternative approach, still under development). Outliers identified beyond the 0.975 quantile have been removed and are identified in the Figure below.

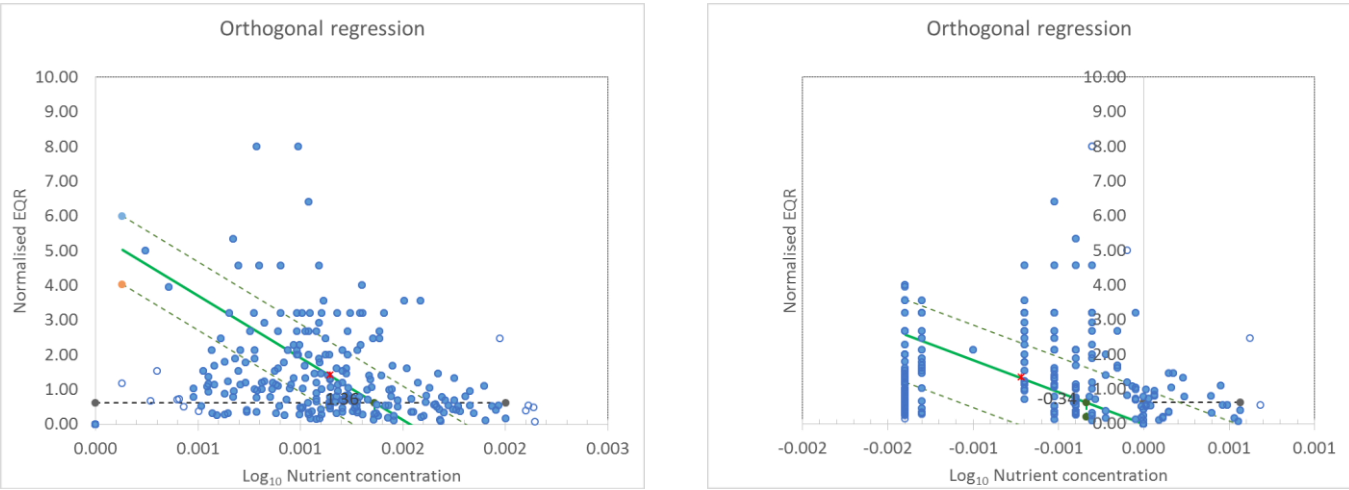



Figure 90. CWMEDII Tyrrhenian: Orthogonal type II regression of EQR ~ log10 Total Nitrogen concentration (left; r=0.297) and EQR ~ log10 Total Phosphorus concentration (right; r=0.068). Solid points values used for models, open circles excluded data (outliers or beyond linear region, with two outliers at EQR= 32 for both nutrients not shown in the graph). Red cross marks mean of data. Solid lines show fitted regression, broken lines upper and lower 25th 75th quantiles of residuals. Good/Moderate boundary point marked.

### 6.2.2.3. Nutrient boundaries

Below are the summary tables of nutrient boundaries obtained for Total Nitrogen (TN; Table 25) and Total Phosphorus (TP; Table 26) for the Adriatic and Tyrrhenian regions.

Table 25. Summary of predicted values of Total Nitrogen concentration (µmol $L^{-1}$) at the Good/Moderate and High/Good boundaries for the Adriatic and Tyrrhenian in common type CWMEDII, results obtained by linear regression and categorical analyses.

| **CWMEDII Total Nitrogen** | **Phytoplankton Models** | **$R^2$** *p-value* n | **Nutrient range µmol $L^{-1}$** | **Most likely boundary** | | | | | | **Possible Range** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | **GM µmol $L^{-1}$** | | | **HG µmol $L^{-1}$** | | | |
| EQR_Chl*a* Boundaries: HG 0.81 GM 0.60 | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | |
| | EQR v TN *(RMA)* | **0.217** *p<0.001* n=316 | 1.82 - 245.7 | 37.1 | 33.03 | 47.59 | 29.06 | 28.19 | 29.48 | GM 19.5-78 HG 11.6-46.2 |
| **Adriatic** | EQR v TN + TP *(Bivar. R)* | **0.228** *p<0.001* n=294 | 5.68 - 245.7 | 25 | 32 | 21 | 29 | 37 | 25 | -- |
| | EQR v TN *(L Quantile R)* | **0.597/0.615** $90^{th}/80^{th}$ n=332 | 3 - 245.7 | 336/197 $90^{th}/80^{th}$ | | | 200/97 $90^{th}/80^{th}$ | | | |
| | Average adjacent quartiles | | | 33.19 | | | 23.71 | | | |
| | Average adjacent classes | n=316 | | 33.18 | | | 24.78 | | | |
| | Average $75^{th}$ quartile | | | 43.36 | | | 28.57 | | | |
| | Minimise class difference | | | 30 | | | 23 | | | |
| EQR_Chl*a* Boundaries: HG 0.84 GM 0.62 | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | |
| | EQR v TP (RMA) | **0.088** *p<0.001* n=228 | 1.75 - 100.6 | 23.02 | 16.22 | 74.63 | 19.98 | 15.56 | 46.39 | GM 9.8-190.6 HG 8.7-118.5 |
| **Tyrrhenian** | Average adjacent quartiles | | | 19.62 | | | 15.39 | | | |
| | Average adjacent classes | n=228 | | 17.96 | | | 13.76 | | | |
| | Average $75^{th}$ quartile | | | 28.25 | | | 16.80 | | | |
| | Minimise class difference | | | 20 | | | 15.30 | | | |

Table 26. Summary of predicted values of Total Phosphorus concentration (µmol $L^{-1}$) at the Good/Moderate and High/Good boundaries for the Adriatic and Tyrrhenian in common type CWMEDII, results obtained by linear regression and categorical analyses.

| CWMEDII Total Phosphorus | Phytoplankton Models | $R^2$ *p-value* n | Nutrient range µmol $L^{-1}$ | Most likely boundary: GM µmol $L^{-1}$ | | | HG µmol $L^{-1}$ | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| EQR_Chl*a* Boundaries: HG 0.81 GM 0.60 **Adriatic** | EQR v TP (RMA) | **0.066** *p<0.001* n=309 | 0.03 - 6.38 | 0.31 | 0.10 | 0.41 | 0.46 | 0.46 | 0.49 | GM 0.01-2.26 HG 0.06-10.99 |
| | EQR v TN +TP (Multivar. R) | **0.228** *p<0.001* n=294 | 0.03 - 6.38 | 0 | 2 | 0 | 1 | 22 | 0 | -- |
| | EQR v TP (L Quantile R) | --- | | | | | | | | |
| | Average adjacent quartiles | n=309 | | 0.55 | | | 0.84 | | | |
| | Average adjacent classes | | | 0.45 | | | 0.49 | | | |
| | Average $75^{th}$ quartile | | | 0.81 | | | 1.48 | | | |
| | Minimise class difference | | | 0.58 | | | 0.35 | | | |
| EQR_Chl*a* Boundaries: HG 0.81 GM 0.60 **Tyrrhenian** | | | | Pred | 25th | 75th | Pred | 25th | 75th | |
| | EQR v TP (RMA) | **0.005** *p=0.307* n=228 | 0.04 - 3.7 | 0.46 | 0.2 | 7.7E+04 | 0.35 | 0.2 | 1.4E+03 | GM 0.04-3.4E+08 HG 0.001-5.9E+06 |
| | Average adjacent quartiles | n=228 | | 0.46 | | | 0.22 | | | |
| | Average adjacent classes | | | 0.20 | | | 0.15 | | | |
| | Average $75^{th}$ quartile | | | 0.87 | | | 0.40 | | | |
| | Minimise class difference | | | 0.42 | | | 0.34 | | | |

### 6.2.3. Common Type: CWMEDIIIE

a) Data check

Two MS, Cyprus and Greece, share this common type of coastal waters not influenced by freshwater input, in the Eastern Basin of the Mediterranean (MED IIIE), and have provided data for this report (n=99 obs). The nutrient data provided does not include TN, TP or DIN, as in other CW types in Europe. Therefore, from the nutrients available (see Table 2), nitrates NO3 (µmol $L^{-1}$) were analysed as an example, also because national boundaries from Greece were available for comparison with the results here obtained. Some results for PO4 are also presented.

The data distribution covers three EQS classes in the range where nutrient boundaries need to be established (Figure 91), although some overlap is observed for NO3 (mean NO3 concentration in High EQR class is 0.856 and 0.861 for the Good class).

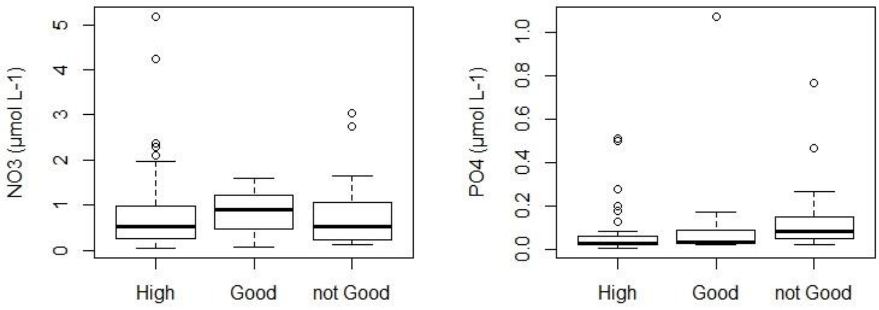



Figure 91. Range of NO3 and PO4 concentrations for CWMEDIIIE sites grouped by ecological quality status (EQS) class: High, Good and below Good status.

### i. Outliers

Attention to EQR values beyond expected range 0-1 (Figure 92).

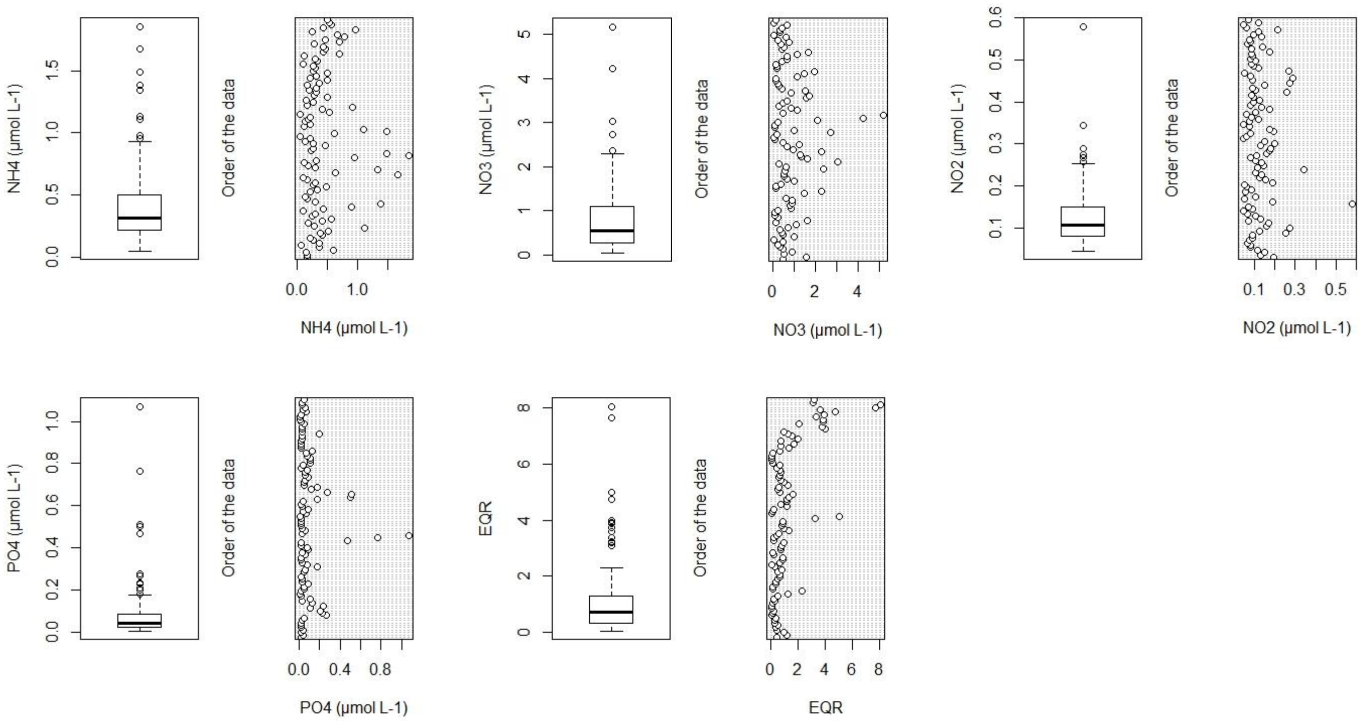



Figure 92. Outliers verification for common type CWMEDIII.

### ii. Collinearity among covariates

Collinearity not observed between nutrient variables available in this dataset (Figure 93).

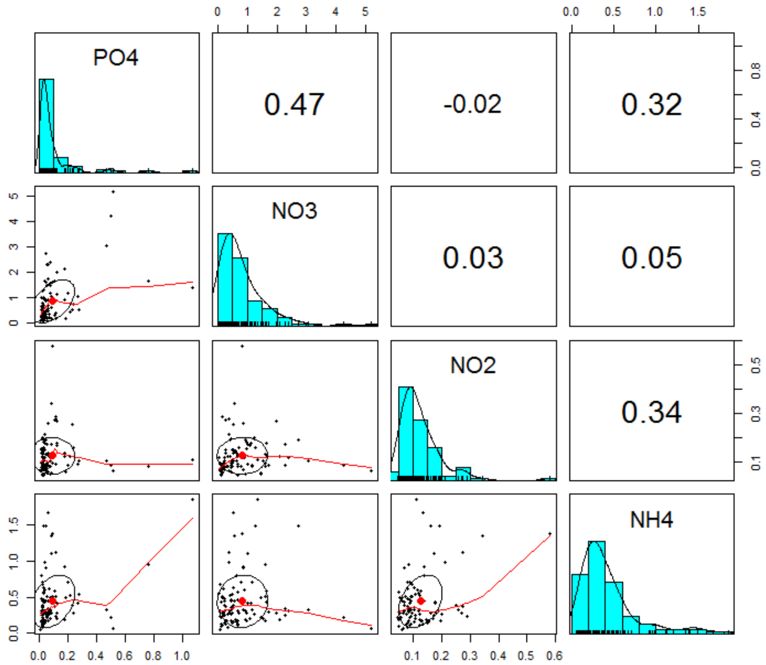
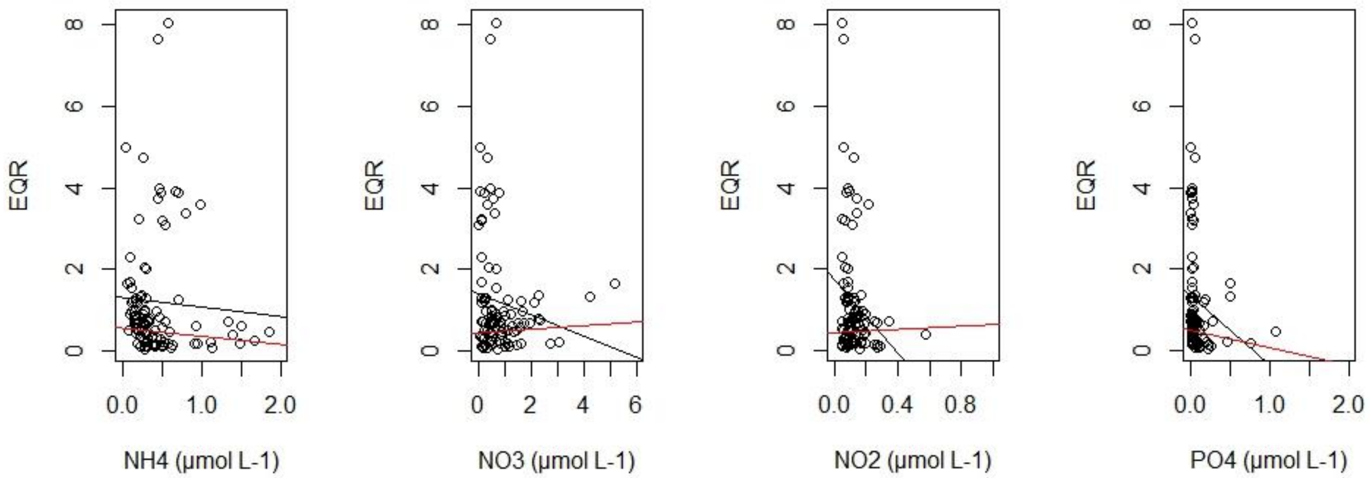



Figure 93. Correlation matrix (Pearson r) between nutrient variables in CW MEDIIIE.

### iii. Relationship between X & Y

Pressure-response relationships considering all data, or truncating EQR values >1 (Figure 94).




| | | | |
|---|---|---|---|
| r=-0.05729611 | r=-0.1480609 | r=-0.2301623 | r=-0.175353 |
| r=-0.2852787 (EQR<=1) | r=0.09699548 (EQR<=1) | r=0.05325166 (EQR<=1) | r=-0.2548219 (EQR<=1) |

Figure 94. Scatterplots of the relationship of nutrients and response variables, for all data and for EQR values <1.

## b) Statistical analysis

The following analyses were performed on this dataset, and presented below:

- Univariate regressions EQR ~ Nitrates (NO3)

- Categorical Analyses

Boundaries and respective ranges predicted by these analyses are presented in the summary table below. See Annex toolkit files for details in the analyses.

#### 6.2.3.1. Nutrient boundaries

| CWMEDIIIE (GR/CY) | Phytoplankton Models | $R^2$ *p-value* n | Nutrient range µmol $L^{-1}$ | Most likely boundary | | | | | | Possible Range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | GM µmol $L^{-1}$ | | | HG µmol $L^{-1}$ | | | |
| EQR_Chl*a* Boundaries: HG 0.66 GM 0.37 | | | | Pred | 25th | 75th | Pred | 25th | $75^{th}$ | |
| | EQR v TP (RMA) | **0.132** *P<0.001* n=92 | 0.048 - 4.23 | 1.0 | 0.69 | 2.8 | 0.79 | 0.63 | 1.47 | GM 0.35-5.77 HG 0.30-3.03 |
| **NO3** | Average adjacent quartiles | | | 0.75 | | | 0.77 | | | |
| | Average adjacent classes | n=92 | | 0.72 | | | 0.74 | | | |
| | Average $75^{th}$ quartile | | | 1.25 | | | 0.99 | | | |
| | Minimise class difference | | | 1.06 | | | 0.74 | | | |

## 6.3. North East Atlantic

In the North East Atlantic coastal waters data has been reported for five common types (Table 2). Within each common type several MS reported their EQR values and respective IC boundaries, along with the nutrient parameter commonly assessed in all NEA CW: DIN (µmol $L^{-1}$). For the analyses we will keep the unit used by all these MS in order to facilitate the comparison with their own established nutrient boundaries.

If within common type there are differences in EQR method/IC boundaries, then the dataset is split for analysis.

*(The exploratory and statistical analyses for these NEA CW types were not conducted at this stage as effort was devoted to work previous examples for feedback into the Guidance development. MS will nevertheless be able to run the toolkit analyses following examples discussed here for other common types.)*

### 6.3.1. Common Type: CWNEA1-26C

a) Data check

Attention with this dataset: very few data points (n=8 complete obs), furthermore, data may still need to be split since DK and DE have different IC EQR boundaries. The full spectrum of ecological quality (EQR) is not covered, and the range needed to derive nutrient boundaries for Good/Moderate High/Good status is not represented in this dataset (the maximum EQR is 0.54).

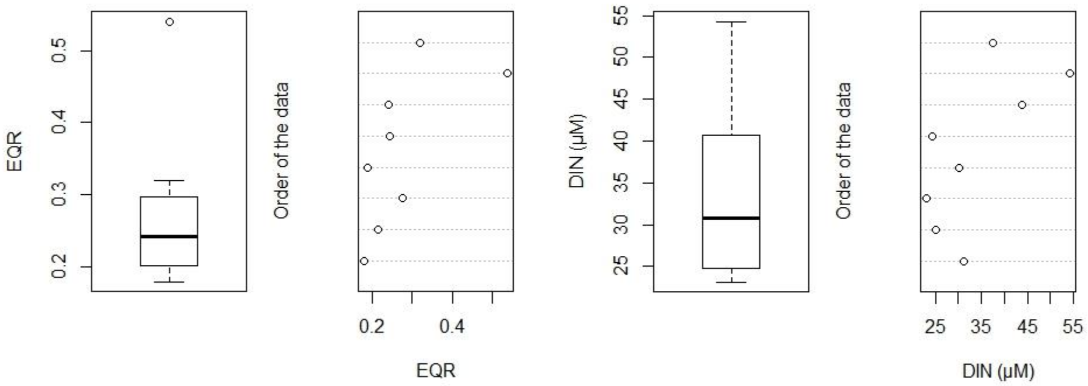


Figure 95. Outliers' verification in the predictor (DIN) and response (EQR, Chl*a*) variables.

### b) Statistical analysis

The *n* of observations is too low (n=8), and they do not cover the range of EQR needed to allow deriving nutrient boundaries for the High/Good and Good/Moderate spectrum of the gradient.

In addition, the MS (Denmark and Germany) within this type have established different EQR_Chl*a* boundaries during the WFD intercalibration exercise (Table 3), therefore datasets would need to be further split, decreasing even more the *n* available for each analysis.

### 6.3.2. Common Type: CWNEA3-4

No EQR intercalibrated boundaries were available for running analyses for this dataset at the moment of data analyses. Moreover the number of observations in this dataset is rather low (n=14 complete obs).

## 6.4. Black Sea

No data was made available for this exercise from the Black Sea. Nevertheless, Romania MS experts a have run the toolkit analyses, with positive feedback.

# 7. References

Decision 2013/480/EU, Commission Decision of 20 September 2013 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise and repealing Decision 2008/915/EC.

Dworak, T., Berglund, M., Haider, S., Leujak, W., Claussen, U., 2016. A comparison of European nutrient boundaries for transitional, coastal and marine waters. Working Group on ecological Status ECOSTAT.

Phillips, Birk, Böhmer, Kelly & Willby, 2016. The use of pressure response relationships between nutrients and biological quality elements as a method for establishing nutrient supporting element boundary values for the Water Framework Directive. Unpublished report for DG Environment. Available from CIRCA BC.

Phillips, G., Kelly, M., Teixeira, H., and Salas, Fuensanta, Free, G., Leujak, W., Solheim, A.L., Várbíró, G., 2017. Best Practice Guide on establishing nutrient concentrations to support good ecological status. Draft submitted to EU COM in December 2017.

# 8. Annexes

All files used for the analyses here presented are provided in a zip file:

1. BPG excel toolkit templates;
2. R scripts
3. And original datasets files

All files are identified with the dataset code as indicated in Table 4 and Table 5 (e.g. ds1_ or ds10_).

### *Relevant references mentioned in the Annex supporting files:*

R v2.9.0 statistical programming language (R Development Core 2008).

Zuur, Alain F., Elena N. Ieno, and Chris S. Elphick. 2010. “A Protocol for Data Exploration to Avoid Common Statistical Problems.” Methods in Ecology and Evolution 1 (1): 3–14. doi:10.1111/j.2041-210X.2009.00001.x.

Feld, Christian K, Pedro Segurado, and Cayetano Gutiérrez-Cánovas. 2016. “Analysing the Impact of Multiple Stressors in Aquatic Biomonitoring Data: A ‘cookbook’ with Applications in R.” Article. Science of The Total Environment, August. doi:10.1016/j.scitotenv.2016.06.243.

Naimi, B., 2015. Usdm: Uncertainty Analysis for Species Distribution Models. R Package Version 1.1-15. http://CRAN.R-project.org/package=usdm (accessed July 2016).

***See Phillips et al. 2017 for further details on all R packages used.***