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# Relative contribution of multiple stressors on copepod density and diversity dynamics in the Belgian part of the North Sea

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## ABSTRACT

The effect of multiple stressors on marine ecosystems is poorly understood. To partially bridge this knowledge gap we investigated the relative contribution of environmental variables to density and diversity dynamics of the zooplankton community in the Belgian part of the North Sea. We applied multimodel inference on generalized additive models to quantify the relative contribution of chlorophyll *a*, temperature, nutrients, salinity and anthropogenic chemicals (i.e. polychlorinated biphenyls and polycyclic aromatic hydrocarbons) to the dynamics of calanoid copepod species in the Belgian part of the North Sea. Temperature was the only predictor consistently showing a high importance in all models predicting the abundances of the selected copepod species. The relative contribution of other predictors was species-dependent. Anthropogenic chemicals were important predictors for three out of six species indicating that chemical mixtures at low concentrations should not be left unattended when performing risk assessments in a natural environment.

## 1. Introduction

As human population is expanding, coastal and shallow marine ecosystems are increasingly experiencing multiple disturbances (Airoldi and Beck, 2007; Crain et al., 2008). Ongoing climatic changes are leading to changes in sea water temperature, salinity and pH (IPCC, 2014; Wiltshire and Manly, 2004). Several effects of climate change on marine ecosystems and species distributions have already been reported: latitudinal shifts in plankton and fish species, local species extinctions, invasion of non-native species, changes in community structures and mismatches between successive trophic levels (Pitois et al., 2012; Wernberg et al., 2012). In addition to these physical stressors, marine ecosystems have to deal with a wide range of chemical pollutants that end up in the marine environment due to human activities (Weis, 2014). In the Belgian part of the North Sea (BPNS), multiple micropollutants are present and some of them exceed their corresponding Environmental Quality Standard (EQS) indicating their potential harmful effects (Janssen et al., 2010). These chemical mixtures can cause adverse effects to organisms, even when the individual components of the mixture are present at concentrations below their individual no effect concentrations (Janssen et al., 2010). Current risk characterization has shown to be insufficient to integrate the effects of these chemical mixtures (Janssen et al., 2010) and does not take into

account the fact that stressors resulting from climate change are likely to affect contaminant exposure and toxic effects and vice versa (Moe et al., 2013; Schiedek et al., 2007). To guarantee a sustained biodiversity and thus ecosystem functioning, the understanding of the relative importance of the main factors in marine ecosystems is crucial (European Marine Board, 2013; Crain et al., 2008). Laboratory studies have shown that synergistic effects are common when multiple stressors are acting together on an ecosystem (Crain et al., 2008). However, laboratory tests do not necessarily reflect the conditions prevailing in nature, making field observations crucial to validate the results of laboratory tests to more natural conditions (Galic et al., 2010; Holmstrup et al., 2010).

Zooplankton populations are good indicators of environmental stress (Hays et al., 2005; Taylor et al., 2002). Zooplankton consists of drifting organisms with limited swimming capacities who largely depend on currents in the water column. This makes their responses to environmental stressors relatively straightforward to interpret (Richardson, 2008). Most zooplankton species are short-lived (< 1 year), allowing for a tight coupling between environmental changes and population dynamics (Hays et al., 2005; Taylor et al., 2002). Subtle environmental perturbations are even known to be amplified within this community (Taylor et al., 2002). At the base of the marine pelagic food web, they form the plant-animal interface and any

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change in the structure of this community is therefore propagated to higher trophic levels. Impacts of stressors on zooplankton communities are expressed through changes in species distributions and abundances, changes in timing of life cycle events and modified community structures (Richardson, 2008). An increased understanding of the relative importance of different stressors for this functional group is a good step forward to identify the main drivers of changes in the marine ecosystem. One specific taxonomic group, Copepoda (Crustacea), comprises 66% of total zooplankton abundances in the BPNS and is part of the holoplankton (organisms spending their entire life as plankton in the water column), making them suitable indicators for changes in the zooplankton community (Van Ginderdeuren et al., 2014).

The BPNS is among the most intensively exploited marine areas in the world, with a large variety of activities concentrated in a small region (Douve et al., 2007). Even though regulations about the release of toxicants in the environment are becoming more stringent, several persistent organic pollutants (POPs) are still present in this region at concentrations exceeding international quality standards (Everaert et al., 2014; Ghekiere et al., 2013; Janssen et al., 2010). Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are intensively studied POPs as they are part of the OSPAR List of Chemicals for Priority Action (OSPAR, 2007) and are often used as POPs model substances (Desaules et al., 2008; Everaert et al., 2014, 2015a; Schuster et al., 2010; Wania and Mackay, 1999). Our study aims to quantify the relative contribution of chlorophyll *a*, temperature, nutrients, salinity and POPs (more specifically PCBs and PAHs) to density and diversity dynamics of the zooplankton community in the BPNS. To do so, we applied multimodel inference to generalized additive models on calanoid copepod abundance and diversity data collected monthly from February 2015 to February 2016 at three stations in the BPNS.

## 2. Materials and methods

### 2.1. Data collection

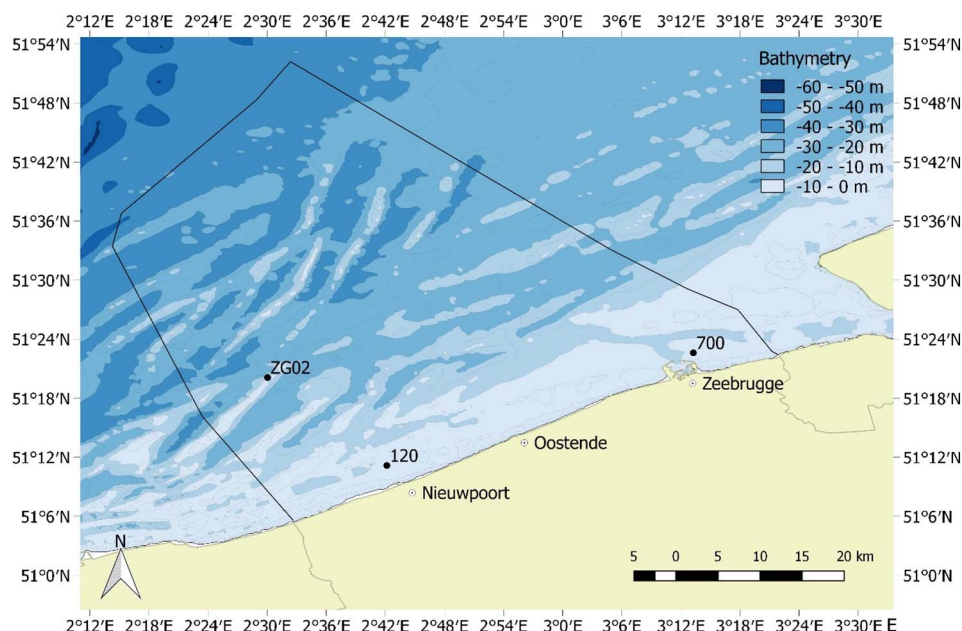
Simultaneous zooplankton and water samples have been collected on a monthly basis from February 2015 to February 2016 at three stations in the BPNS. These stations were selected as we expect differences in their zooplankton dynamics (Van Ginderdeuren et al., 2014). Two nearshore stations were selected: station 700 located close to the

harbour of Zeebrugge, situated near the mouth of the Scheldt River, and station 120 close to the harbour of Nieuwpoort, together with the off-shore station ZG02 which is situated within the Flemish banks in the western part of the BPNS (Fig. 1).

In total 100 zooplankton and 32 water samples were collected. Triplicate zooplankton samples were collected as in Van Ginderdeuren et al. (2014) with a WP2 zooplankton net (70 cm diameter, 200 µm mesh size) fitted with a flow meter that was towed in an oblique haul from bottom to surface at each station. Zooplankton was fixed and stored in a 4% formaldehyde solution. Calanoid copepods (Crustacea, Copepoda, Calanoida) were identified in the lab to the lowest taxonomic level possible using a stereomicroscope (Leica MZ 10) to identify the abundances of the different taxonomic groups. At each sampling station, CTD (conductivity – temperature – depth profile, using a Seabird 19plusV2 CTD) data were collected, together with measurements of dissolved oxygen and oceanographic, meteorological and navigational data using measurement devices on board the RV Simon Stevin (Flanders Marine Institute, 2017a). Water samples were taken with Teflon-coated Niskin bottles at a depth of 3 m. Nutrient and pigment concentrations were measured in these samples next to the concentration of a selection of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (Table 1). The concentration of these toxicants was determined with gas chromatography–mass spectrometry (Trace GC and Trace DSQ, Thermo Electron Cooperation) after liquid-liquid extraction of the filtered (0.45 µm) water samples. To do so, internal standards were added to 4 L of water, which was extracted three times with dichloromethane. The extract was dried on Na<sub>2</sub>SO<sub>4</sub>, and subsequently concentrated to 0.2 mL using a rotavapor and a concentration apparatus with N<sub>2</sub>. Before the final concentration anthracene-d10 was added as recovery standard. All solvents used were of purity suitable for organic residue analysis. Nutrient and pigment concentration data collected during our sampling campaigns were provided by the LifeWatch observatory as part of the Flemish contribution to the LifeWatch ESFRI by Flanders Marine Institute (2017b).

### 2.2. Model construction

Generalized additive modelling (GAM) (Zuur et al., 2009; Wood, 2006) was used to determine the main drivers of the abundance and distribution of calanoid copepod species in the BPNS. As opposed to generalized linear models, who are limited to the assumption that the



**Fig. 1.** Sampling locations in the Belgian part of the North Sea. Station 120: 51° 11' 1" N, 2° 42' 07" E, station 700: 51° 22' 6" N, 3° 12' 2" E, station ZG02: 51° 20' 0" N, 2° 30' 0" E.

**Table 1**

Highest measured concentrations (HMC), average concentration (Average) and standard error (SE) of PAHs and PCBs, together with Environmental Quality Standards (annual average EQS values set by the European Commission and the Flemish Government (EC, 2008; Vlaamse regering, 2010)) and the octanol/water partition coefficient ( $\log K_{ow}$ ).

	HMC (ng/L)	Average	SE (n = 95)	EQS (ng/L)	$\log K_{ow}$
Acenaphthylene	1.100	0.258	0.003	4000	3.94
Acenaphthene	7.840	1.176	0.015	60	4.15
Fluorene	10.060	1.736	0.018	2000	4.02
Phenanthrene	34.590	4.537	0.061	100	4.35
Anthracene	1.320	0.251	0.004	100	4.35
Fluoranthene	9.770	1.057	0.017	100	4.93
Pyrene	4.700	0.644	0.008	40	4.93
Benzo(a)anthracene	30.830	1.172	0.057	300	5.52
chrysene	1.990	0.179	0.004	1000	5.52
Benzo(b)fluoranthene + Benzo(k)fluoranthene	0.450	0.055	0.001		6.11
Benzo(a)pyrene	0.280	0.027	0.001	$\Sigma = 30$	6.11
Indeno(1,2,3-c,d)pyrene	0.990	0.085	0.002	50	6.11
Benzo(g,h,i)perylene	0.000	0.000	0.000	2	6.7
Dibenzo(a,h)anthracene	0.090	0.003	0.000	2	6.7
$\Sigma$ PCB <sub>7</sub>	0.210	0.015	0.000	500	6.7
PCB 28	7.280	1.620	0.016	2	
PCB 52	1.530	0.285	0.003	NA	5.69
PCB 101	1.470	0.319	0.003	NA	6.34
PCB 118	1.830	0.263	0.003	NA	6.98
PCB 153	0.500	0.053	0.001	NA	6.98
PCB 138	1.280	0.304	0.003	NA	7.62
PCB 180	1.450	0.259	0.003	NA	7.62
	1.360	0.139	0.003	NA	8.27

response variable is linked with a linear combination of all explanatory variables, GAMs are able to deal with nonlinear, nonmonotonic relationships between the set of explanatory variables and response variables by using nonparametric smooth functions of the explanatory variables (Wood, 2006; Van Echelpoel et al., 2015; Zuur et al., 2009) (Eq. (1)).

$$g^{-1}[E(Y_i | X_{ij})] = \alpha + \sum_{j=1}^k f_j(X_{ij}) \quad (1)$$

where  $g$  specifies the link function between the expected value of the response variable ( $Y_i$ ) and the covariates ( $X_{ij}$ ), with  $i$  = the index number of the observation ( $i = 1$  to  $n$ ) and  $j$  = the index number of the covariate ( $j = 1$  to  $k$ ) (Wood, 2006; Everaert et al., 2014). The smooth function  $f_j(X_{ij})$  quantifies the effect of the  $j$ th covariate on  $Y_i$  and  $\alpha$  represents the model intercept. Smooth functions represent an assembly of polynomials joined together. The points at which the polynomials join are known as the knots of the smooth functions (Wood, 2006). Increasing the number of knots allows more flexibility, but may result in overfitting. The number of knots was limited to four for all variables, which gives a good compromise between model flexibility and overall fit (Keele, 2008). GAMs were fitted using the *mgcv* package in R (Wood and Wood, 2016). All values are reported as average  $\pm$  standard error.

### 2.2.1. Covariate selection

To avoid collinearity, variables with a variance inflation factor (VIF)  $> 3$  were removed from the analysis after evaluating their potential ecological significance in the model. The VIF is based on the proportion of variance in the  $j$ -th independent variable that is associated with the other independent variables in the model ( $R_j^2$ ) and is calculated as:  $1/(1 - R_j^2)$  (Zuur et al., 2009; Zuur et al., 2010). The variables that were selected for constructing the GAMs include chlorophyll  $a$  concentration (chl  $a$ ), water temperature (temp), the ratio of dissolved inorganic nitrogen concentration over silicate concentration (DIN/SiO<sub>4</sub>), salinity (sal) and the sum of PCB and PAH concentrations

(sumtox) which were first multiplied with their corresponding octanol/water partition coefficient ( $K_{ow}$ ) and converted to molar concentration ( $W_m$  = molecular weight ( $\text{g}\cdot\text{mol}^{-1}$ )) (Eq. (2)).

$$\text{sumtox} = \sum_i ((\text{conc}_i \times K_{ow,i})/w_m) \quad (2)$$

The octanol/water partition coefficient ( $K_{ow}$ ) is defined as the ratio of the concentration of a chemical in octanol over water at equilibrium at a specified temperature. Substances with high  $K_{ow}$  values tend to adsorb more readily to organic matter and accumulate more readily in lipid tissue because of their low affinity for water and high affinity for lipids (Chiou, 1985; ECHA, 2008). PCB and PAH concentrations were summed after multiplying them with their  $K_{ow}$  values, assuming non-polar narcosis to be the main mode of action for both groups of toxicants (McCarty and Mackay, 1993). Prior to the modelling, we detected four outliers (1 outlier for chlorophyll  $a$ , 2 outliers for DIN/SiO<sub>4</sub> and 1 for sumtox) by means of Cleveland dotplots (Fig. S1) as explained in Zuur et al. (2010). These four data points were not included in the statistical analysis.

### 2.2.2. Step 1: Multimodel inference technique

The multimodel inference (MMI) technique from Burnham and Anderson (2003) was applied to GAMs. By using the MMI technique, inferences are based on weighted support from the exhaustive set of possible model configurations. The main advantage of using MMI is that it takes into account model selection uncertainty. The latter is a better approach instead of selecting one model as being the “best” one (Johnson and Omland, 2004; Holguin-Gonzalez et al., 2013).

Models containing all possible combinations of the selected variables were constructed and ranked according to the second-order Akaike's information criterion (AICc), which corrects for small sample size (Hurvich and Tsai, 1989). Based on the AICc, the Akaike weight ( $w_i$ ) of each model was calculated as the probability of each model being the best model:

$$w_i = \frac{\exp\left(-\frac{1}{2}\Delta_i\right)}{\sum_{r=1}^R \exp\left(-\frac{1}{2}\Delta_r\right)} \quad (3)$$

where  $\Delta_i$  = AICc best model – AICc model of interest  $i$ ,  $\Delta_r$  = AICc best model – AICc model  $r$  and where the denominator is the sum of  $\Delta_r$  of all  $R$  models that have been tested (Lukacs et al., 2010; Symonds and Moussalli, 2011).

The evidence ratio (ER =  $w_{ib}/w_{im}$ ) was calculated to check whether the model with the highest  $w_i$  ( $w_{ib}$ ) was indeed a better model than the models with a lower  $w_i$  ( $w_{im}$ ) (Symonds and Moussalli, 2011; Burnham and Anderson, 2003). A cut-off value of 2 was chosen, as models with an ER  $> 2$  were considered as negligible with respect to the best model (Symonds and Moussalli, 2011). Variables that occurred in the best model and in models with an ER  $\leq 2$  were selected as they were considered important contributors for explaining the variability of the dependent variable.

### 2.2.3. Step 2: Model fit

All selected models were checked for overfitting by fitting the model on a random subset of 80% of the data and by plotting the predicted remaining 20% of the data against the observed values (Wood, 2006). The residuals of the fitted model were used to test for underlying assumptions of normality and homogeneity (Zuur et al., 2009). When the variability of the residuals was similar across the range of fitted values, homogeneity was concluded. Graphical diagnostics were used to evaluate normality. Normality was assumed if the QQplot showed a straight line of residuals and when the histogram of the residuals showed a bell-shaped curve (Everaert et al., 2014; Wood, 2006; Zuur et al., 2009). The deviance explained was used to indicate the amount of variability in the data explained by the models (Zuur et al., 2009).

### 2.2.4. Step 3: Relative importance of the different stressors

In order to quantify the relative importance of the selected variables (so the variables occurring in models with an  $ER \leq 2$ , selected in Step 1), full-model averaging (Lukacs et al., 2010; Symonds and Moussalli, 2011) was used to calculate the weighted estimates for each of the basic functions forming the smoother functions of each variable (Wood, 2006). As we fixed the number of knots to four, three estimates ( $\beta_{1,2,3}$ ) were calculated to construct the smooth function. The relative importance of each variable ( $V$ ) was calculated as:

$$\text{Relative importance } V = \frac{\sum (\beta_{1V}, \beta_{2V}, \beta_{3V})/3}{\sum_{V=1}^V \sum (\beta_{1V}, \beta_{2V}, \beta_{3V})/3} \quad (4)$$

after which the relative importance was averaged over all selected models. We predicted the relative importance of the variables on calanoid copepod community metrics (number of individuals, Hill diversity ( $N_1$ ,  $N_2$ )) and on the densities of the most common calanoid copepod species in the BPNS. Hill  $N_1$  and  $N_2$  are both indications of diversity, but  $N_2$  gives more weight to the common species, and is thus less influenced by the occurrence of rare species than  $N_1$  ( $N_1 = \exp[-\sum(p_i \ln p_i)]$ ,  $N_2 = (\sum p_i^2)^{-1}$ , where  $p_i$  is the proportion of the  $i$ th taxa in a sample) (Heip et al., 1998). Only adult copepods were taken into consideration, as the nauplii and copepodites of some of the species could not always be identified at species level.

### 2.2.5. Step 4: Visual inspection of the smoothers + predicted versus observed values

Smoothers of the different variables (Figs. S2 to S6) were constructed based on models containing all selected variables (see Step 1) for each diversity index or species. These smoothers were used for visual inspection of the shape of the effect of the different variables on the densities of the copepods species. Lastly, graphs displaying the predicted versus observed values were created for stepwise constructed models, with variables being added to the model in decreasing order of relative importance (Fig. 5, Figs. S13–S17). These stepwise constructed models give us an idea of the amount of extra deviance that is explained by adding a variable to the model, providing us a quality check of the results acquired through the MMI technique.

## 3. Results

### 3.1. Copepod community composition

The number of copepod species across all samples ranged from 5 (station 120, April 2015) to 11 (station 120, September–October 2015). The most common species found (with percentage of samples

containing the species between brackets) were *Temora longicornis* (100%), *Acartia* sp. (100%), *Paracalanus parvus* (100%), *Centropages hamatus* (95%), *Pseudocalanus elongatus* (64%), *Calanus* sp. (56%) and *Pseudodiaptomus marinus* (21%). *T. longicornis*, *Acartia* sp., *P. parvus* and *C. hamatus* can be considered as abundant species all year round and at all stations. *P. marinus* is an invasive species (Brylinski et al., 2012) absent in winter and spring but reaching relatively high densities from July till October (up to  $110 \pm \text{SE of } 41 \text{ ind m}^{-3}$  in September 2016, station 700). Densities of *Calanus* sp. adults were very low during the entire year with maximum densities of  $3 \pm 1 \text{ ind m}^{-3}$  (station 700, June 2016). Total copepod abundances peaked in spring and early summer, with a second smaller peak late summer and early autumn (Fig. 2). The timing and size of the peak in copepod abundances and corresponding changes in species composition differed between stations (Fig. 2). *T. longicornis* and *Acartia* sp. contributed most to the spring peak of copepod abundance, reaching maximum densities of  $973 \pm 133 \text{ ind m}^{-3}$  (station 700, May 2016) and  $833 \pm 70 \text{ ind m}^{-3}$  (station ZG02, May 2016), respectively. The autumn peak was associated with increased abundance of *T. longicornis* and reduced abundance of *Acartia* sp.

### 3.2. Models on community metrics

#### 3.2.1. Model selection

Based on the MMI results, two models were retained (models with an  $ER < 2$ ) for the total number of calanoid copepods ( $N$ ) and for diversity index Hill  $N_1$ . For diversity index Hill  $N_2$ , three models were retained (Table 2). The models explained  $75.9 \pm 1.7\%$  of the variability of  $N$ . The models for  $N_1$  and  $N_2$  explained  $23.4 \pm 9.1\%$  and  $42.5 \pm 3.2\%$  of the variability, respectively (Table 3). Temperature is the only variable that was included in all selected models for  $N$ ,  $N_1$  and  $N_2$ . The other variables only occurred in one of the selected models for  $N$  and  $N_1$  and in one or two of the selected models for  $N_2$ .

#### 3.2.2. Relative importance of the different stressors

Temperature is an important predictor for  $N$ ,  $N_1$  and  $N_2$ , with a relative importance of up to  $0.77 \pm 0.23$ . The importance of temperature as a predictor for these indices is also reflected by the shape of its smoothers (Fig. S3), showing a positive effect of water temperatures between approximately  $12^\circ\text{C}$  and  $18^\circ\text{C}$  and an optimal temperature of  $16^\circ\text{C}$ .

Even though nutrients ( $\text{DIN}/\text{SiO}_4$ ) are not that important for  $N$  (not included in any of the best models) and  $N_1$  (relative importance of  $0.10 \pm 0.10$ ), they have a high relative importance in explaining the variability in  $N_2$  ( $0.47 \pm 0.03$ ). The shape of the effect is non-monotonic, with positive effects of the  $\text{DIN}/\text{SiO}_4$  ratio below  $\pm 1$  and

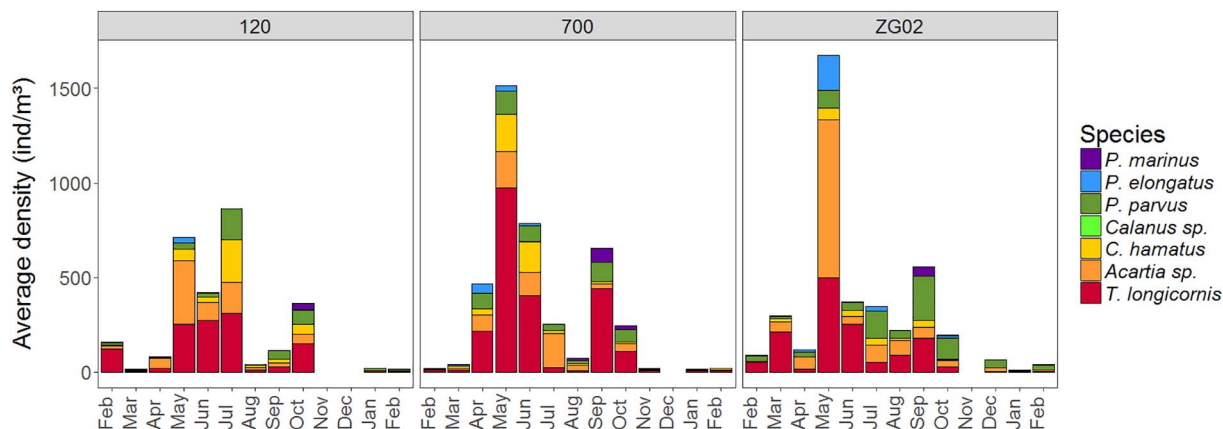


Fig. 2. Average densities ( $n = 3$ ) of the most common calanoid copepod species in the BPNS at station 120, 700 and ZG02 from February 2015 to February 2016.



**Table 2**

Results of the multimodel inference for the calanoid copepod community metrics ( $N$  = total densities of calanoid copepods,  $N_1$  = Hill's diversity index  $N_1$ ,  $N_2$  = Hill's diversity index  $N_2$ ) and for the abundances of the most common calanoid species (*Temora longicornis*, *Acartia* sp., *Centropages hamatus*, *Paracalanus parvus*, *Pseudocalanus elongatus*, *Pseudodiaptomus marinus*). The variables that were selected for constructing the models include chlorophyll *a* concentration (chl *a*), water temperature (temp), the ratio of dissolved inorganic nitrogen concentration over silicate concentration (DIN/SiO<sub>4</sub>), salinity (sal) and the sum of PCB and PAH concentrations (sumtox).  $W_i$  = Akaike weight (Eq. (3)), ER = evidence ratio (ER =  $w_i$  best model/ $w_i$  model). M1, M2 & M3: models which are almost equally likely to be the best model (ER < 2).

		AICc	Model	$W_i$	ER
N	M1	44.69	temp + sal	0.25	1.00
	M2	45.95	chl <i>a</i> + temp + sumtox	0.13	1.87
Hill $N_1$	M1	173.35	chl <i>a</i> + temp + DIN/SiO <sub>4</sub> + sal	0.26	1.00
	M2	174.73	temp	0.13	2.00
Hill $N_2$	M1	173.39	chl <i>a</i> + temp + DIN/SiO <sub>4</sub> + sal	0.33	1.00
	M2	173.42	temp + DIN/SiO <sub>4</sub> + sal	0.32	1.02
	M3	174.53	chl <i>a</i> + temp + DIN/SiO <sub>4</sub> + sal + sumtox	0.19	1.77
<i>T. longicornis</i>	M1	67.49	chl <i>a</i> + temp + sal + sumtox	0.52	1.00
	M2	68.86	chl <i>a</i> + temp + DIN/SiO <sub>4</sub> + sal + sumtox	0.26	1.99
<i>Acartia</i> sp.	M1	98.97	temp + sumtox	0.24	1.00
	M2	99.51	temp + sal + sumtox	0.18	1.31
	M3	99.67	chl <i>a</i> + temp + sumtox	0.17	1.42
<i>C. hamatus</i>	M1	90.74	temp + sal	0.31	1.00
	M2	91.72	temp + DIN/SiO <sub>4</sub> + sal	0.19	1.63
<i>P. parvus</i>	M1	67.82	temp + DIN/SiO <sub>4</sub>	0.25	1.00
	M2	68.51	temp + DIN/SiO <sub>4</sub> + sal	0.18	1.42
	M3	68.76	temp + DIN/SiO <sub>4</sub> + sal + sumtox	0.16	1.60
<i>P. elongatus</i>	M1	110.65	temp + DIN/SiO <sub>4</sub> + sal	0.29	1.00
	M2	110.86	temp + DIN/SiO <sub>4</sub> + sal + sumtox	0.26	1.11
<i>P. marinus</i>	M1	61.73	chl <i>a</i> + temp + DIN/SiO <sub>4</sub> + sal + sumtox	0.46	1.00
	M2	62.20	chl <i>a</i> + temp + DIN/SiO <sub>4</sub> + sumtox	0.36	1.27

between  $\pm 2$  and 3 (Fig. S4). The other variables are less important for all indices, ranging from no contribution at all to a relative importance of  $0.11 \pm 0.11$  (see Fig. 3).

### 3.3. Models on copepod species abundances

#### 3.3.1. Model selection

For *T. longicornis*, *C. hamatus*, *P. elongatus* and *P. marinus* two models per species were selected based on the MMI results. Three models were selected for *Acartia* sp. and *P. parvus* (Table 2). The models had an overall high fit explaining  $48.2 \pm 1.7\%$  (*P. elongatus*) to  $82.9 \pm 0.5\%$  (*T. longicornis*) of the variability (Table 3). Again, temperature is the only variable included in all models for all species. Chlorophyll *a* is only included in both models for *T. longicornis* and *P. marinus*, and in one out of three models for *Acartia* sp. Salinity is included in one or two models for all species, while sumtox is included in one or two models for all species except for *C. hamatus* and DIN/SiO<sub>4</sub> is included for all species except for *Acartia* sp.

#### 3.3.2. Relative importance of the different stressors

The relative importance of the different variables for explaining the variability in copepod densities is species-dependent. Temperature is the only predictor that consistently shows a high importance in all models predicting the abundances of the selected copepod species. Especially for *T. longicornis*, *Acartia* sp., *C. hamatus* and *P. parvus* temperature is important with relative contributions between  $0.43 \pm 0.00$  and  $0.84 \pm 0.04$ . For *P. elongatus* and *P. marinus*, temperature is not the most important contributor, but still has a relative importance of up to  $0.19 \pm 0.03$ . Temperature smoothers show pronounced effects of temperature on the densities of the different species (Fig. S3). An optimal temperature between  $14^\circ\text{C}$  and  $16^\circ\text{C}$ , together with negative effects below temperatures of  $\pm 11^\circ\text{C}$  was found for every species, except for *P. elongatus* and *P. marinus*. The latter seem to prefer colder

water, as their optimal temperatures lie between  $8^\circ\text{C}$  and  $14^\circ\text{C}$ .

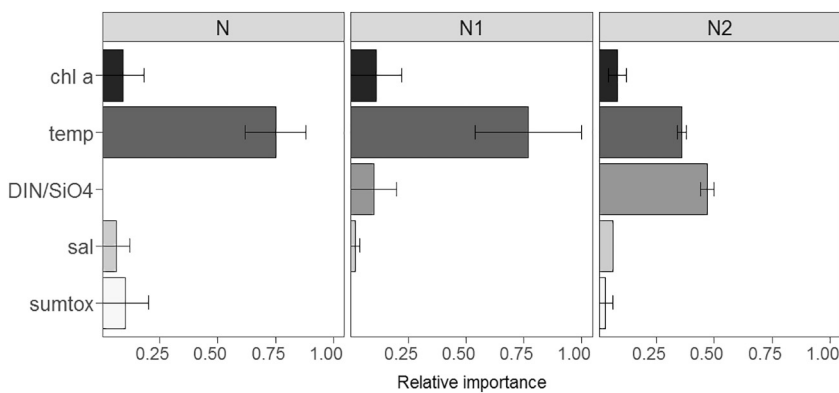
For the other predictors, patterns are less generally applicable as they differ between the different species. For example, chlorophyll *a* shows a low relative importance for all species, except for *T. longicornis* ( $0.21 \pm 0.00$ ), for which chlorophyll *a* concentrations higher than  $\pm 5 \mu\text{g.L}^{-1}$  have a negative effect on the densities of this species (Fig. S2). For most species DIN/SiO<sub>4</sub> has a low relative contribution in the models ( $0.00$  to  $0.09 \pm 0.00$ ) but for *P. elongatus* and *P. marinus* the relative contribution of DIN/SiO<sub>4</sub> is high ( $0.38 \pm 0.02$  and  $0.18 \pm 0.03$ , respectively). DIN/SiO<sub>4</sub> smoothers show a negative effect of intermediate DIN/SiO<sub>4</sub> values for *P. elongatus*, while the opposite is found for *P. marinus* (Fig. S4). Salinity is an important predictor for *C. hamatus* ( $0.34 \pm 0.02$ ), *P. elongatus* ( $0.42 \pm 0.02$ ) and *P. marinus* ( $0.12 \pm 0.12$ ) but is negligible for the other species (maximum relative contribution of  $0.04 \pm 0.04$ ). *C. hamatus* density shows an almost linear effect of salinity with positive effects of salinities lower than  $\pm 32$ , and negative effects of higher salinities. The shape of the effect of salinity on *P. elongatus* and *P. marinus* is more complex (Fig. S5). Finally, the contribution of toxicant concentrations to the models are also species-specific, with high relative contributions for *T. longicornis*, *Acartia* sp. and *P. marinus* ( $0.34 \pm 0.00$  to  $0.39 \pm 0.05$ ) and very low contributions for the other species ( $0$  to  $0.05 \pm 0.05$ ). *T. longicornis*, *Acartia* sp. and *P. marinus* show an inverse u-shaped effect of toxicants, with increased densities at intermediate toxicant concentrations (Fig. S6).

The previous findings on the relative importance of the different predictors are confirmed for most species when displaying the predicted versus observed values of stepwise constructed models, with predictors being added to the model in decreasing order of relative importance (Fig. 5, Figs. S13 to S17). For *T. longicornis* 63.7% of the deviance is explained by a model containing only temperature (Fig. 5). Adding toxicants to the model increases the amount of deviance explained with 11.6%, adding chlorophyll *a* increases the amount of deviance explained with another 7.1%, while salinity and the DIN/SiO<sub>4</sub> ratio together only contribute 1.1%. For *Acartia* sp., *C. hamatus*, *P. parvus*, *P.*

**Table 3**

Relative importance (Eq. (4)) of the chlorophyll *a* concentration (chl *a*), water temperature (temp), ratio between dissolved inorganic nitrogen and silicate (DIN/SiO<sub>4</sub>), salinity (sal) and the sum of PCB and PAH concentrations (sumtox) on the total densities of calanoid copepods ( $N$ ), Hill's diversity index ( $N_1$ ), Hill's diversity index ( $N_2$ ) and the abundances of the most common copepod species found in the Belgian part of the North Sea from February 2015 to February 2016. Deviance explained = the deviance explained by the model containing the indicated variables. (–) indicates that the predictor is not included in the model.

		chl <i>a</i>	temp	DIN/SiO <sub>4</sub>	sal	sumtox	deviance explained
N	M1	–	0.88	–	0.12	–	74.2%
	M2	0.17	0.63	–	–	0.20	77.5%
Hill $N_1$	M1	0.23	0.53	0.20	0.04	–	32.4%
	M2	–	1.00	–	–	–	14.3%
Hill $N_2$	M1	0.12	0.36	0.47	0.06	–	40.9%
	M2	–	0.41	0.53	0.06	–	37.8%
	M3	0.11	0.32	0.42	0.05	0.10	48.7%
<i>T. longicornis</i>	M1	0.21	0.43	–	0.01	0.34	83.4%
	M2	0.21	0.43	0.00	0.01	0.34	82.4%
<i>Acartia</i> sp.	M1	–	0.58	–	–	0.42	61.0%
	M2	–	0.51	–	0.12	0.37	62.6%
	M3	0.24	0.44	–	–	0.32	65.1%
<i>C. hamatus</i>	M1	–	0.64	–	0.36	–	63.9%
	M2	–	0.57	0.12	0.31	–	64.8%
<i>P. parvus</i>	M1	–	0.91	0.09	–	–	66.5%
	M2	–	0.87	0.09	0.04	–	67.2%
	M3	–	0.76	0.08	0.04	0.12	69.2%
<i>P. elongatus</i>	M1	–	0.15	0.40	0.45	–	46.5%
	M2	–	0.14	0.36	0.40	0.10	49.8%
<i>P. marinus</i>	M1	0.10	0.16	0.16	0.24	0.34	73.7%
	M2	0.13	0.21	0.21	–	0.45	70.0%



**Fig. 3.** Average relative importance ( $\pm$  SE) of different stressors for the calanoid copepod community diversity indices, averaged over models with  $ER < 2$ . N = total densities of calanoid copepods,  $N_1$  = Hill's diversity  $N_1$ ,  $N_2$  = Hill's diversity  $N_2$ . Exact values of the average relative importance can be found in supportive information (Table S1).

*elongatus* and *P. marinus*, temperature explains respectively 49.5%, 43.9%, 63.0%, 18.3% and 9.5% of the total deviance. Toxicants are contributing for respectively 10.5%, 0.0%, 1.3%, 3.3% and 7.3% of the deviance explained. For *P. marinus*, the relative importance of the predictors is not confirmed by this graph, as DIN/SiO<sub>4</sub> appears to have a higher relative contribution (36.0% extra deviance is explained when adding DIN/SiO<sub>4</sub>) than temperature and sumtox.

## 4. Discussion

### 4.1. Copepod community composition

Species distributions and abundances of the most common calanoid copepods were consistent with earlier studies in the BPNS (Van Ginderdeuren et al., 2014). The presence of *P. marinus* in the BPNS is however new, as this is the first time this species is recorded in Belgian waters. *P. marinus* is an invasive species that has only recently been found in the southern bight of the North Sea along the coast of France, and that seems to be spreading rapidly (Brylinski et al., 2012).

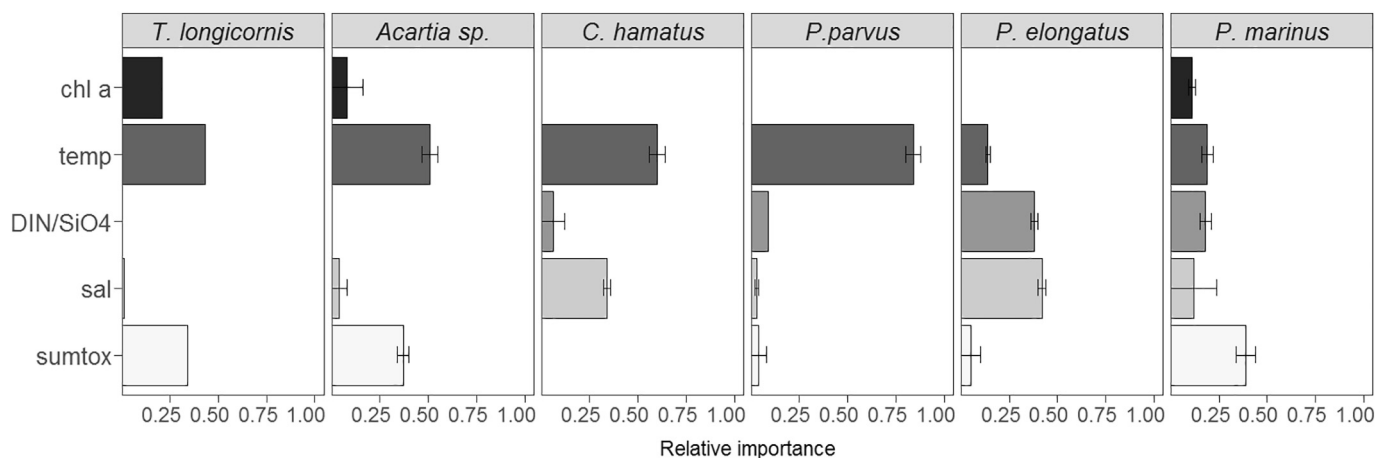
The calanoid copepod species included in our research are known to have different life history strategies (Halsband and Hirche, 2001; Wesche et al., 2007). Both *T. longicornis* and *Acartia* sp. are contributing to the spring peak in copepod abundances, while only *T. longicornis* contributes to the autumn peak. This can be explained by the fact that *Acartia* sp. has an overwintering strategy based on reproductively inactive females which is decoupled from environmental changes during autumn and winter, while *T. longicornis* continuously reproduces throughout the year, allowing them to respond to the increased food levels during autumn (Halsband and Hirche, 2001; Wesche et al., 2007). *C. hamatus* disappears almost completely in wintertime and is

known to form resting eggs, from which they recruit again the next spring (Halsband and Hirche, 2001; Wesche et al., 2007). This difference in life history strategy allows *T. longicornis* to react to changing environmental conditions faster than *Acartia* sp. and *C. hamatus* (Van Ginderdeuren et al., 2014; Wesche et al., 2007). *P. elongatus* is able to reproduce throughout the year as well, but as the percentage of spawning females remains low this species is not able to respond very fast to changing conditions either (Halsband and Hirche, 2001).

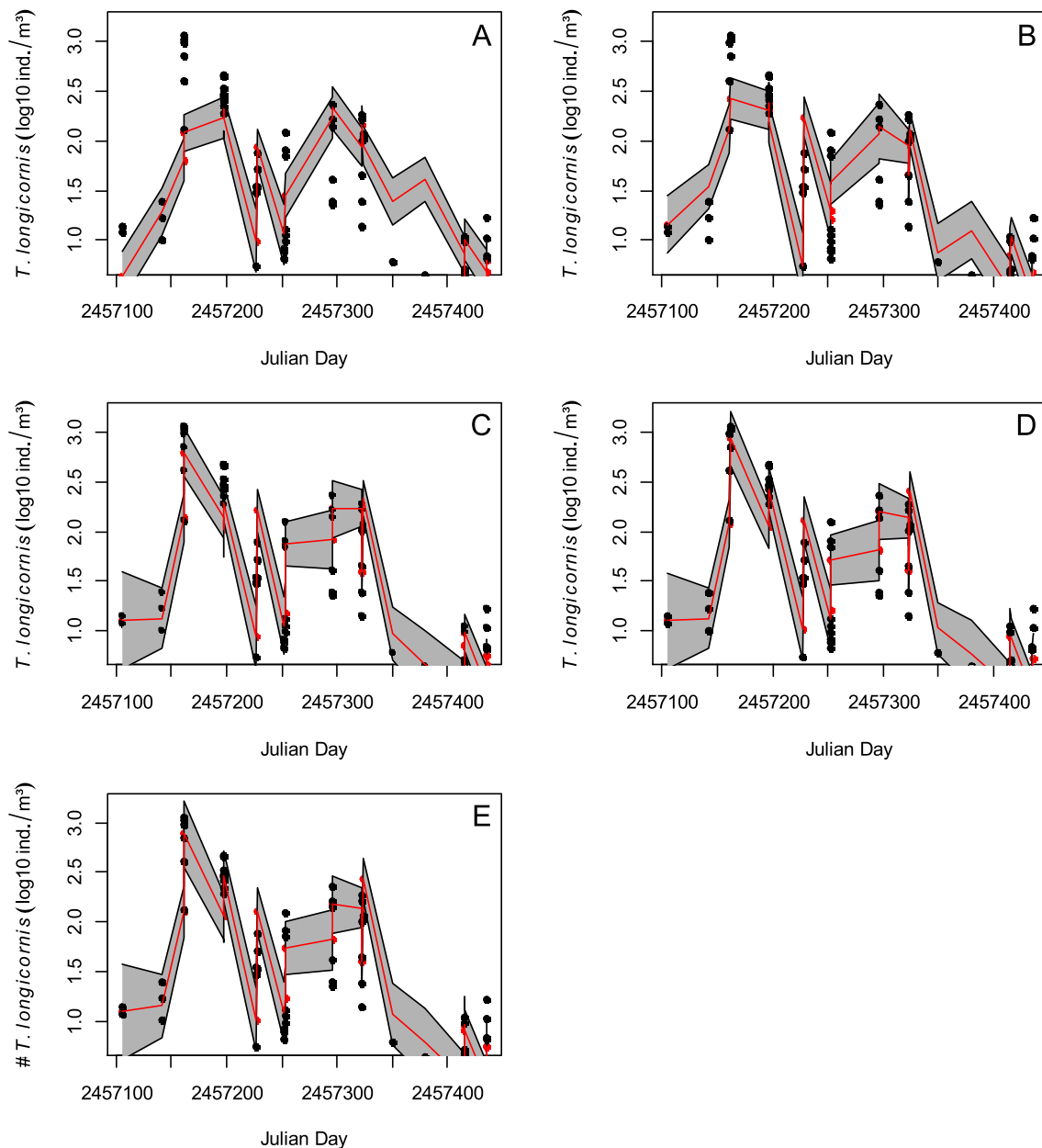
### 4.2. Relative contribution of multiple stressors to copepod diversity and density dynamics

By means of MMI on generalized additive models we were able to unravel the relative contribution of chlorophyll *a*, temperature, nutrients, salinity and pollutants to the dynamics of the most abundant calanoid copepod species in the BPNS. This technique is promising to facilitate future risk characterization of chemical toxicants, as it will allow prioritizing future monitoring needs and as it will increase our understanding on the effect of multiple drivers on a complex ecosystem. In spite of its clear advantages, the models that were generated in the present research did not take into account interactions between variables and indirect effects through species interactions such as competition and predation. Hence, knowledge on the system you are working with remains an important exigence in order to interpret the results of MMI. In the future, the addition of mechanistic models (e.g. Everaert et al., 2015b) in combination with targeted experiments (e.g. Everaert et al., 2016) can enhance our understanding of the ecosystem and potential underlying interactions.

The relative importance of the different predictors were confirmed for most species by displaying the predicted versus observed values of



**Fig. 4.** Average relative importance ( $\pm$  SE) of different stressors for the most common calanoid copepod species in the BPNS, averaged over models with  $ER < 2$ . Exact values of the average relative importance can be found in supportive information (Table S1).



**Fig. 5.** Predicted (red line) versus observed (black dots) abundances of *Temora longicornis*. A: *T. longicornis* densities modeled with only temperature, B: *T. longicornis* densities modeled by temp + sumtox, C: *T. longicornis* densities modeled by temp + sumtox + chl *a*, D: *T. longicornis* densities modeled by temp + sumtox + chl *a* + sal, E: *T. longicornis* densities modeled by temp + sumtox + chl *a* + sal + DIN/SiO<sub>4</sub>. Deviance explained is 63.7%, 75.3%, 82.4%, 83.4% and 83.5% for panel A, B, C, D, and E respectively. The grey polygon delimits the 95% confidence interval around the predictions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stepwise constructed models, with predictors being added to the model in decreasing order of relative importance (Fig. 5, Figs. S13 to S16). For *P. marinus*, the relative importance of the MMI results did not coincide with the results from this second method (Fig. S17). This species might be less suitable for this type of ecological modelling as adult copepods were absent in wintertime but reached high abundances from July till October (Fig. 2), leading to large discrepancies between the estimates of the three basic functions composing the smoother for this species (Eq. (4)). Until further investigation, results for this species should be interpreted with extra care.

Temperature was an important predictor of copepod density and diversity. This is consistent with the fact that temperature is a crucial determinant for hatching success, size, development rate and/or fecundity of several copepod species such as *T. longicornis*, *Acartia* sp., *C.*

*hamatus*, *Pseudocalanus* sp. and *P. marinus* (Chinnery and Williams, 2004; Halsband et al., 2002; McLaren, 1965; Uye et al., 1982; Zervoudaki et al., 2013) explaining the high relative importance of this predictor for the densities of the copepod species (Fig. 4). For *Pseudocalanus* sp. and *P.s marinus* the importance of temperature was less prominent. It is likely that the regulating role of temperature for these species was partially outweighed by the other variables in the models (Fig. 4). The species-specific optimal temperatures predicted by the smoothers (Fig. S3) were consistent with previous findings (Chinnery and Williams, 2004; Halsband et al., 2002; McLaren, 1965; Uye et al., 1982; Zervoudaki et al., 2013).

In contrast to our expectations, chlorophyll *a* had, compared to other covariates, a relatively low contribution in most models, and was not even included in the models predicting the densities of *C. hamatus*,

*P. parvus* and *P. elongatus* (Fig. 4). We had expected chlorophyll *a* to be an important predictor for all copepod species, as the cycling in the concentration of this pigment marks the cycle of increasing and decreasing concentrations of phytoplankton cells, which are an important food source for most copepod species. In the BPNS however, the presence of harmful blooms of the inedible algae *Phaeocystis globosa* is known to disturb the relation between chlorophyll *a* concentration and the amount of edible algae present for the copepods (Muyllaert et al., 2006). Thus, even though chlorophyll *a* can give an indication of the start of the algal bloom, the chlorophyll *a* marker might not be the best marker for food availability in this area. In order to account for the different effects of the presence of edible and non-edible algal blooms on copepod densities, we included the DIN/SiO<sub>4</sub> ratio in our models. In the North Sea, changes in the nutrient composition determine the transition from the spring diatom bloom to the bloom of the harmful, inedible algae *P. globosa*. When the spring bloom of diatoms diminishes due to Si limitation, *P. globosa* starts to bloom until the concentration of N and P starts to diminish as well (Muyllaert et al., 2006). The DIN/SiO<sub>4</sub> ratio reached a high relative importance in the models of several species, especially for *P. elongatus* and *P. parvus* (Fig. 4). However, due to the complexity of interactions between planktonic organisms, the link between nutrients and zooplankton abundances is very intricate, making a more thorough investigation with mechanistic models as in Lancelot et al. (2005) and Everaert et al. (2015b) advisable to confirm these findings. A potential solution to identify the presence of *P. globosa* is the use of additional pigments, such as chlorophyll *c3*, which is a marker pigment for *P. globosa* in the BPNS (Muyllaert et al., 2006). On top of this, it should be considered that not all copepod species are purely herbivorous. For example, *T. longicornis*, *Acartia* sp. and *C. hamatus* are able to change their diet according to the circumstances, as they also feed on microzooplankton, copepod eggs, copepod nauplii and even fish larvae (Dam and Lopes, 2003; Kleppel, 1993; Saage et al., 2009; Wiadnyana and Rassoulzadegan, 1989). This feeding behavior might partially buffer the effect of changes in the algal concentrations.

*Acartia* sp., *T. longicornis*, *C. hamatus*, *Calanus* sp. and *P. elongatus* are known to prefer more saline waters (Chinnery and Williams, 2004; Collins and Williams, 1981; Holste et al., 2009; Möllmann et al., 2003), while *P. parvus* and *P. marinus* are meso- to -polyhaline species (Suzuki et al., 2013). The water in the BPNS has a rather high salinity (30.29 to 34.84 - in our study), which is within the preferred range for all modelled species. For most species, salinity only contributed a little to explain the dynamics in their densities. Only for *C. hamatus* and *P. elongatus*, the relative contribution of salinity to the models was quite large (i.e. > 31%) (Table 3, Fig. 4). As salinities stayed within the preferred range for these species, other factors that were not considered in the model might have a confounding role here. The salinity range encompasses a spatial gradient, as coastal water is less saline than the water further offshore. Other factors along this gradient may reinforce the effect of the rather small range in salinity.

The effect of chemical mixtures on marine organisms is still poorly understood. Everaert et al. (2015b) found indications of effects of complex mixtures of pollutants at low concentrations on phytoplankton growth. Experimental research on phytoplankton by Echeveste et al. (2010) has shown toxic effects of complex mixtures of pollutants at concentrations 20–40 folds those found in the North-East Atlantic Ocean. Several other studies investigated the effect of chemical mixtures on phytoplankton species (e.g. Arrhenius et al., 2004; Claessens et al., 2013; Wang et al., 2008), but studies on the effects of chemical mixtures on zooplankton are still scarce and often focus on acute toxicity effects (e.g. Barata et al., 2005; Verslycke et al., 2003). Our study aimed at bridging this knowledge gap by unraveling the relative contribution of a mixture of PCBs and PAHs to calanoid copepod densities. *T. longicornis*, *Acartia* sp. and *P. marinus* models suggested a high relative importance of the PCB and PAH mixture occurring in the BPNS. The left part of the smoother for the toxicants contains the majority of the data points and shows increased densities at intermediate toxicant

concentrations. The right part is only based on 3 data points, so cautiousness is advised when interpreting the smoother, where the emphasis should be made on the zone containing the majority of the data points (Fig. S6). As the 'extreme' data points can force the shape of the smoother and hence the predicted effect of the toxicants on the densities of the species, the effects of mixed toxicants on copepod species should be examined more thoroughly with dedicated experiments. The PAH concentrations measured remained below the EQS set in the context of the Water Framework Directive (WFD; 2000/60/EC) by the European commission (EC, 2008) and the Flemish government (Vlaamse regering, 2010) (Table 1), while PCB concentrations often exceeded the EQS values during our study (Table 1). By multiplying the PCB and PAH concentrations with the K<sub>ow</sub> values, we assumed non-polar narcosis to be the main mode of action (McCarty and Mackay, 1993). Summing the PAH and PCB concentrations is justified as the high number of components in the mixture will lead to additivity of the effects of these components, according to the funnel hypothesis (Barata et al., 2005; Warne and Hawker, 1995). This is a simplification of reality, as there will be other modes of action and antagonistic or synergistic effects that are not completely cancelled out by the large number of components in the mixture. Additionally, we have to keep in mind that other toxicants are also present in the environment, potentially influencing the effect of the PCBs and PAHs on the densities of the copepods. Even though it would be preferred to include all toxicants occurring in the environment, the cost-benefit of monitoring all these toxicants might be low. Future research should focus on unraveling the combined effects of chemical mixtures, in order to lower the uncertainty in this field.

## 5. Conclusions

We applied MMI on generalized additive models to analyze the relative contribution of different stressors to the dynamics of the most abundant calanoid copepod species in the BPNS. The importance of temperature as a predictor for trends in diversity indices and for the densities of the copepod species was the most consistent pattern in the MMI outcome. The relative contribution of other variables was species-dependent. Toxicants were important predictors for three out of six species indicating that chemical mixtures at low concentrations should not be left unattended when performing risk assessments in a natural environment.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2017.09.038>.

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