Effect of water quality on blackflies (Diptera: Simuliidae) in Flanders (Belgium)

Koen Lock [a,*], Tim Adriaens [b], Peter Goethals [c]

[a] eCOAST Marine Research, Esplanadestraat 1, 8400 Ostend, Belgium
[b] Research Institute for Nature and Forest, Kliniekstraat 25, 1070 Brussels, Belgium
[c] Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, J. Plateaustraat 22, 9000 Ghent, Belgium

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ABSTRACT

To assess the ecological water quality in Flanders (northern part of Belgium), macroinvertebrates have been collected by the Flemish Environment Agency. During the present study, the blackflies collected between 1997 and 2009 were identified to species level. In total, more than 44,000 specimens were identified, belonging to 12 different species. Sensitive species were restricted to small brooks, while species tolerating lower oxygen concentrations and higher nutrient concentrations were also present in larger watercourses. Several species were either restricted to watercourses in the Campine region (northeast Flanders) or the loamy region (southern Flanders), while the other regions only contained eurytopic species. The prevalence of blackflies increased from less than 5% to almost 30% in the nineties, but did not further increase during the next decade. Habitat suitability models (logistic regressions, artificial neural networks, support vector machines and classification trees) could accurately predict the presence or absence of blackflies. An ensemble forecast, based on predicted oxygen and nutrient concentrations due to planned water quality improvement strategies, predicted that blackflies prevalence will rise to 42% in 2015 and 64% in 2027. Since blackflies only possess a moderate sensitivity, they could occur in all types of running waters with a good water quality. As a good ecological status is required by the European Union Water Framework Directive for all surface waters, it is thus apparent that more efforts will be needed to improve the water quality in Flanders.

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Introduction

Habitat destruction and degradation, pollution, flow modification and invasion by alien species reduced biodiversity in fresh waters much more than most affected terrestrial ecosystems (Sala et al., 2000; Dudgeon, 2010). Some industrialized countries have made considerable progress in reducing water pollution from domestic and industrial point sources, however, threats from excessive nutrient enrichment from intensive agriculture are still growing (Smith, 2003) and the number of alien species keeps rising (Gherardi, 2007; Messiaen et al., 2010). Until present, river management in Flanders has mainly been conducted at the river basin level by installing wastewater treatment plants and imposing standards for effluent concentrations. Although these measures already resulted in a significant improvement of the chemical and ecological water quality since the eighties (VMM, 2010), most Flemish water bodies still lack the good ecological status which is required by 2015 by the European Union Water Framework Directive (WFD) (European Council, 2000).

Regional governments (Flanders, Brussels and Wallonia) are responsible for the nature conservation policy in Belgium and this regional scale is thus appropriate to perform faunistic studies. Multiple threats affect surface waters in Flanders, the Dutch speaking northern part of Belgium. Flanders has a very high population density of 456 citizens per km^2, about 87% of the households is connected to a sewage system, but only 70.3% is actually treated (VMM, 2009a). Because rainwater is often not collected separately, untreated water is regularly discharged after heavy rains, resulting in problematic drops in dissolved oxygen concentration and peak levels of substances such as ammonium. Flanders is also heavily industrialized and exhibits (mainly intensive) agriculture on 53% of the land (VMM, 2009a). In addition, structural integrity of surface waters is threatened by thousands of weirs that have been built for flood control, hundreds of kilometers of artificial banks that have been installed and because the majority of the river channels has been straightened (VMM, 2009a).

To assess the ecological water quality, the use of biotic indicators (macrophytes, phytoplankton, phytobenthos, fish fauna and macrobenthic fauna) is required by the WFD. The Multimetric Macroinvertebrate Index Flanders (MMIF) was recently developed to meet the requirements of the WFD (Gabriels et al., 2010). A lot of common macroinvertebrate taxa in Flanders, including

* Corresponding author.
E-mail address: Koen_Lock@hotmail.com (K. Lock).

Simuliidae, extended their distribution area over the last two decades (Lock et al., unpublished data), which could be linked to the general improvement of the chemical water quality (VMM, 2010).

Blackfly larvae are often the dominant suspension feeders in lotic ecosystems and are efficient and opportunistic colonizers (Zhang et al., 1998). Therefore, they provide an important link between suspended particles and predators (Malmqvist et al., 1999). The ecology of the European blackfly species has been well studied and characteristics such as their longitudinal distribution and saprobic values have been documented (Lechthaler and Car, 2005). Despite their ecological importance, blackflies hardly have been studied in Flanders. Blackflies suck blood from mammals, including humans, and from birds, with negative effects ranging from nuisance to occasional death. They also transmit bacteria, nematodes, protozoa and viruses, however, they are poorly studied as vectors of these agents, relative to other biting flies (Malmqvist et al., 2004).

During the present study, blackflies captured by the Flemish Environment Agency were identified to species level and their presence was linked to the measured environmental parameters. In addition, habitat suitability models were used to predict the presence or absence of blackflies in surface waters with four modeling techniques: logistic regressions, artificial neural networks, support vector machines and classification trees, which all have been frequently used for habitat suitability modeling. Subsequently, these four modeling techniques were used to make an ensemble forecast of the blackfly prevalence in 2006 and two future scenarios in 2015 and 2027, where oxygen and nutrient concentrations were predicted based on planned water quality improvement measures.

Materials and methods

Flanders is situated between the Netherlands in the north and France and Wallonia (southern part of Belgium) in the south. In the context of water quality monitoring, the Flemish Environment Agency sampled macroinvertebrates at several thousand sampling sites since 1989. Sites were usually sampled every three years and sampling took place throughout the year except during winter. Macroinvertebrates were sampled using a standard handnet as described by Gabriels et al. (2010) and this method has been used since 1989. A stretch of 10–20 m was sampled for approximately 5 min. Sampling effort was proportionally distributed over all accessible aquatic habitats, including bed substrates (stones, sand or mud), macrophytes (floating, submerging, emerging), immersed roots of overhanging trees and all other natural or artificial substrates, floating or submerged in the water. Each aquatic habitat was explored in order to collect the highest possible richness of macroinvertebrates. For this purpose, kick sampling was performed. In addition to handnet sampling, animals were manually picked from stones, leaves and branches.

Conductivity, dissolved oxygen and pH were measured in the field during each sampling event. Other chemical variables (content of ammonium, nitrite, nitrate, Kjeldahl nitrogen, orthophosphate, total phosphorus, chemical and biological oxygen demand) were retrieved from monitoring data of the chemical water quality, which is also performed by the Flemish Environment Agency. As the chemical monitoring, which is usually performed on a monthly basis, was not performed simultaneously with macroinvertebrate sampling, measurements from the last date before macroinvertebrate sampling were used. Additionally, the slope of a watercourse was determined based on the difference in height between two points 1000 m apart using GIS-software applied on the Flemish Hydrographic Atlas (AGIV, 2006). The same database was used to determine the sinuosity on a stretch of 100 m. A map of Flanders with the indication of the different regions is presented in Fig. 1. The highest point in the study area has an altitude of only 288 m ASL, the
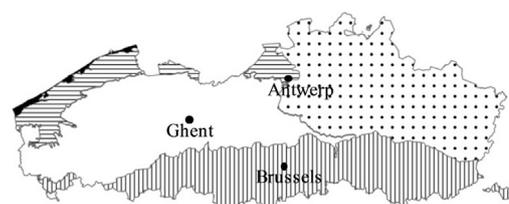

Fig. 1. Map of Flanders with indication of the different regions: dune region (black), polder region (horizontal stripes), sandy region (white), Campine region (dots) and loamy region (vertical stripes).

whole region can thus be considered as lowland. Flanders almost completely consists of quaternary and tertiary depositions and natural mineral substrates hardly occur: only a few rivers with gravel and some small streams with travertine depositions are present.

Diptera are only identified to family level by the Flemish Environment Agency. During the present study, all last-instar larvae and pupae of blackflies sampled since 1997 were identified to species level by using the identification keys developed by Jensen (1997), Bass (1998) and Seitz (2008). A direct gradient analysis was applied to determine which environmental parameters might be responsible for the differences in species composition, since environmental variables were explicitly incorporated in the analysis. To test whether a linear or unimodal method was needed, a detrended correspondence analysis (DCA) was performed. Since the length of gradient was greater than four, a unimodal method was needed and therefore, the Canonical Correspondence Analysis (CCA) option from the program package CANOCO (Ter Braak, 1988) was applied. A log-transformation ($\log(x+1)$) was applied prior to the CCA to normalize the data. The concentration of phosphorus, orthophosphate and Kjelldahl nitrogen were not used in the CCA because these variables were strongly correlated with the ammonium concentration (Pearson $R > 0.70$). Differences in environmental parameters between sites where blackflies were present or absent were analyzed using Kruskal–Wallis ANOVA using Statistica software (StatSoft, 2004).

To model habitat suitability for blackflies, all records of Simuliidae since 1990 were used (i.e. also records from before 1997 and records from early instars, which were not identified to species level). In total, blackflies were present in 2463 samples and as absence data, 2463 samples were randomly selected from sites where blackflies had never been observed. The dataset was randomly split into two thirds for training and one third for validation. To avoid overfitting during calibration, a tenfold cross-validation was performed. Four modeling techniques were applied to model the presence/absence of blackflies: logistic regressions, classification trees, artificial neural networks and support vector machines. Logistic regressions (LR) were used to predict the probability of occurrence of an event by fitting data to a logit function logistic curve. Here, a multinomial logistic regression with ridge estimator was used as a generalized linear model. Classification trees (CT) summarize the relationships between explanatory variables and the response variable (i.e. presence/absence of blackflies) in a dichotomously branching tree. Each bifurcation is defined by a certain value of one of the explanatory variables dividing the dataset in two more homogenous subsets. CT were grown automatically using the J48 algorithm, which minimizes the impurity of the subsets (Witten and Frank, 2005). A fully grown tree would explain the training data with a high accuracy, but it would fail for unseen data due to overfitting. CT generality was increased by pruning, which yielded simpler trees that usually result in better classification of unseen data. Artificial neural networks (ANN) are non-linear statistical data modeling tools, which are based on the architecture of biological neural networks and consist of a group of interconnected
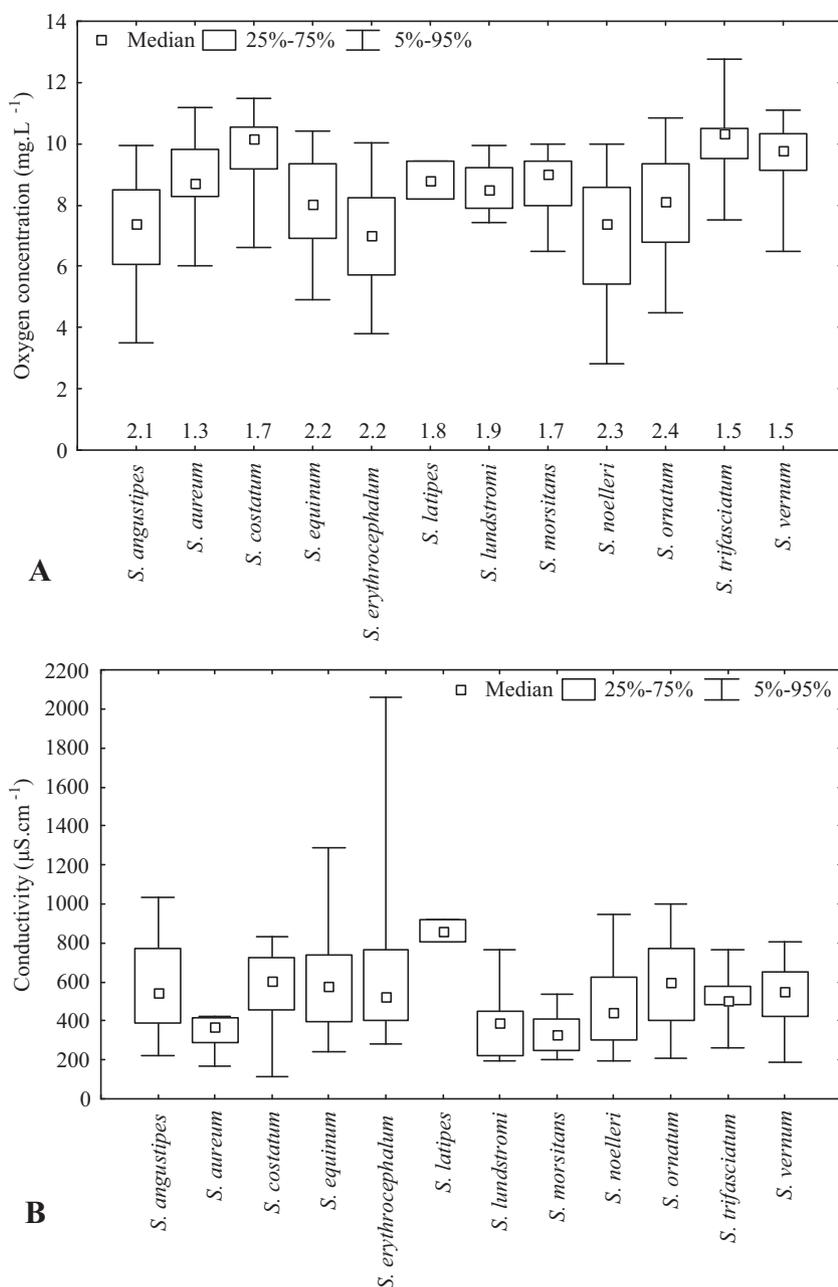
Fig. 2. Box and Whisker plots of the oxygen concentration with indication of the saprobic index for each species according to Lechthaler and Car (2005) (A) and the conductivity (B) for the encountered blackflies.

computing units or neurons (Lek and Guégan, 1999). During a learning phase, connection weights among the neurons are adapted by backpropagating training data through the net. Support vector machines (SVM), which only recently became popular for modeling ecological data (Ambelu et al., 2010; Hoang et al., 2010; Pino-Mejias et al., 2010), are developed from a linear classifier using a maximum hyperplane to separate two classes. In a non-linear case, the central idea of classification with SVM is to map training data into a higher-dimensional feature space and to compute separating hyperplanes that achieve maximum separation between classes. The maximum separation hyperplane is only a function of the training data that lie on the margin and are called support vectors. Platt's sequential minimal optimization algorithm (Keerthi et al., 2001) was used for training a support-vector classifier, which generally replaces all missing values, transforms nominal attributes into binary ones and multi-class problems are solved using pairwise classification.

All modeling techniques were performed using WEKA software (Witten and Frank, 2005). To evaluate the performance of each technique, the percentage of correctly classified instances (% CCI) and Cohen's kappa statistics (K) were calculated (Witten and Frank, 2005).

The PEGASE water quality model (Deliège et al., 2009) was used to simulate the improvement of the water quality in Flanders by the years 2015 and 2027 (Ronse and D'heygere, 2007). The PEGASE model is a detailed hydrodynamic, deterministic water quality model that consists of three submodels: a hydrological and hydrodynamic submodel, a thermal submodel and a biological submodel. In the first scenario (2015), the standard policy as well as the proposed measures in the first period of the district plans are implemented. In the second scenario (2027), all proposed restoration measures are implemented and the water received from the neighboring countries is expected to be of a good quality.
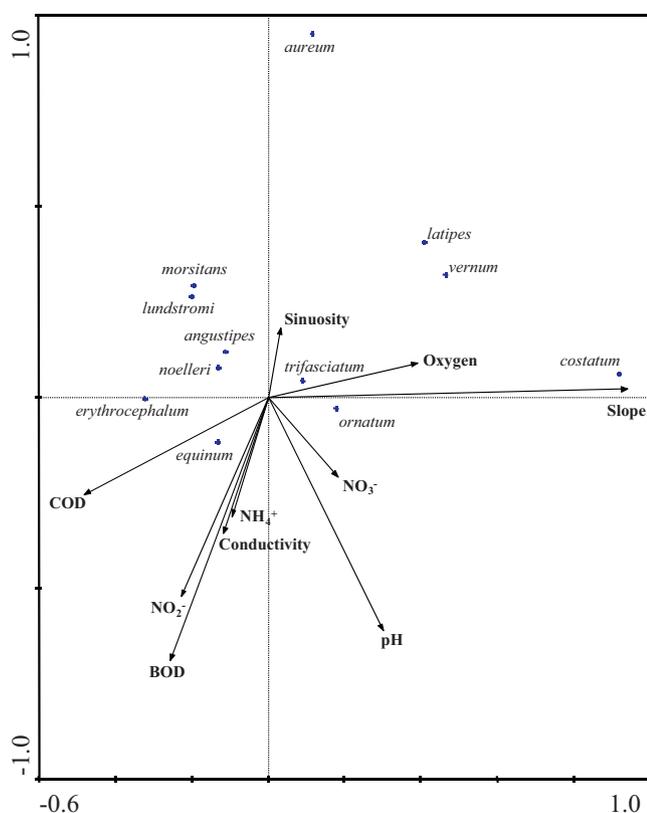
Fig. 3. Canonical Correspondence Analysis biplot of the *Simulium* species scores and the environmental variables sinuosity, oxygen, slope, nitrate (NO_3), pH, ammonium (NH_4), conductivity, biological oxygen demand (BOD), nitrite (NO_2) and chemical oxygen demand (COD).

Based on the planned measures, especially collecting and treating a higher fraction of the domestic waste water, oxygen and nutrient concentrations were modeled using the water quality model PEGASE (VMM, 2009b). More specifically, models were developed using the four mentioned techniques based on the concentration of oxygen, biological oxygen demand, nitrate, ammonium, Kjeldal nitrogen, phosphorus and orthophosphate. River morphology was not included in these models because this is not likely to change substantially by 2027, especially because there are no large scale plans to change river morphology. An ensemble forecast based on the four techniques was used to model blackfly prevalence in a 'reference' situation in 2006 and the two future scenarios in 2015 and 2027.

Results

During the present study, more than 44,000 blackflies were identified, representing almost 2500 records. In total, 12 different species were encountered (Table 1). *Simulium aureum*, *Simulium latipes*, *Simulium costatum*, *Simulium trifasciatum* and *Simulium vernum* were restricted to small brooks, while other species also occurred in larger watercourses (Table 1). The abovementioned species as well as *Simulium lundstromi* and *Simulium morsitans*, which are both restricted to the Campine region (characterized by acid sandy soils), only occurred in well oxygenated waters (Fig. 2A). The Campine species *S. aureum*, *S. lundstromi* and *S. morsitans* were only observed at low conductivities, while other species also tolerated higher values (Fig. 2B).

In the Canonical Correspondence Analysis, the first axis (Eigenvalue of 0.30) coincided mainly with a strong slope (Fig. 3). The second axis (Eigenvalue 0.06) coincided mainly with a low biological oxygen demand and a low pH (Fig. 3). Species restricted to small brooks were plotted in the upper right quadrant, with a high slope and high oxygen concentrations, whereas species that also occurred in larger watercourses were plotted more to the left. Species characteristic for the Campine region were plotted in the upper left, while species from the loamy region, which is characterized by a higher pH, were plotted to the right. Distribution maps of the 12 species encountered in Flanders are presented in Fig. 4.

Since the start of the monitoring, blackfly prevalence increased significantly (Spearman's $R = 0.82$, $P < 0.001$) from less than 5% to almost 30%, however, since 2000 the prevalence fluctuated around 30% and no further increase was observed (Fig. 5). Kruskal–Wallis ANOVA indicated that pH, conductivity, biological oxygen demand, chemical oxygen demand, ammonium, nitrite, nitrate, Kjeldahl nitrogen, phosphorus and orthophosphate were significantly lower (all $P < 0.001$) and dissolved oxygen, slope and sinuosity were significantly higher (all $P < 0.001$) in samples with blackflies compared to samples without blackflies (Table 2).

Presence and absence of blackflies could be accurately modeled based on the measured variables with the four applied modeling techniques, which all performed with a similar accuracy (Table 3). An example of a strongly pruned classification tree is presented in Fig. 6; according to this tree, blackflies only occurred when biological oxygen demand and ammonium content were not too high. Based on modeled oxygen and nutrient concentrations, the four applied modeling techniques were used to make an ensemble forecast of the blackfly prevalence. The modeled prevalence increased from 34% in the reference year 2006 to 42% and in 2015 and 64% in 2027 (Fig. 7).

Discussion

Species in Flanders

During the present study, 12 species of blackflies were encountered in Flanders. One of these, *S. costatum*, was previously not reported for Belgium (Van Den Neucker, 1991). *S. costatum* is a species occurring from the crenal to the metarhithral zone (Feld et al., 2002; Lechthaler and Car, 2005) and in Flanders, it was frequently observed in springs and spring brooks in the loamy region. Besides the species recorded during the present study, no additional species have yet been found in Flanders.

Blackfly species assemblages are usually mainly determined by stream size (Malmqvist et al., 1999; Feld et al., 2002; Ofenböck et al., 2002; Lautenschläger and Kiel, 2005; Illésová et al., 2008) and assemblages are also known to differ between regions (Feld et al., 2002; Ofenböck et al., 2002; McCreadie and Adler, 2006). Also in Flanders, species assemblages differed between regions and several species were restricted to small streams, while other species, were also observed in larger watercourses. The reported longitudinal zonation and saprobic valence for the different species (Lechthaler and Car, 2005) closely corresponded with the catchment area and the range of oxygen concentrations and conductivities at which the different species were found during the present study (Fig. 2). *Simulium ornatum* is for example a tolerant species, which occurs in all types of running waters (Zhang et al., 1998; Malmqvist et al., 1999; Feld et al., 2002; Ofenböck et al., 2002; Lautenschläger and Kiel, 2005; Lechthaler and Car, 2005) and in Flanders, this is the most common blackfly. *S. ornatum* is also the natural vector for the cattle filarial nematode *Onchocerca lienalis* Stiles, which induces host fecundity depletion (Renshaw and Hurd, 1995), which indicates that the occurrence of blackflies can also be of economic importance.

Table 1
Checklist of the Flemish blackflies (Diptera: Simuliidae), with indication of the number of samples per water type where each species was found.

| River type	Very large river	Large river	Small river	Large brook	Large Campine brook	Small brook	Small Campine brook	Polder watercourse	Total
Catchment area	>10,000 km²	600–10,000 km²	300–600 km²	50–300 km²	50–300 km²	<50 km²	<50 km²	Not applicable	
Simulium (Boophthora) erythrocephalum (De Geer 1776)	1	61	52	111	200	73	237	1	736
Simulium (Eusimulium) angustipes Edwards 1915		1		6	30	81	90	4	212
Simulium (Eusimulium) aureum Fries 1824							19		19
Simulium (Hellichiella) latipes (Meigen 1804)						2			2
Simulium (Nevermannia) costatum Friederichs 1920						34			34
Simulium (Nevermannia) lundstromi (Enderlein 1921)					9		5		14
Simulium (Nevermannia) vernum Macquart 1826						24	6		30
Simulium (Simulium) morsitans Edwards 1915				2	8		6		16
Simulium (Simulium) noelleri Friederichs 1921				1	14	40	59	2	116
Simulium (Simulium) ornatum Meigen 1818	2	52	4	159	109	559	268		1153
Simulium (Simulium) trifasciatum Curtis 1839						18			18
Simulium (Wilhelmia) equinum (Linnaeus 1758)		30	9	29	41	6	14		129
Number of species	2	4	4	5	7	9	9	3	12
Number of samples	41	1920	355	821	859	2969	2217	1037	10,179
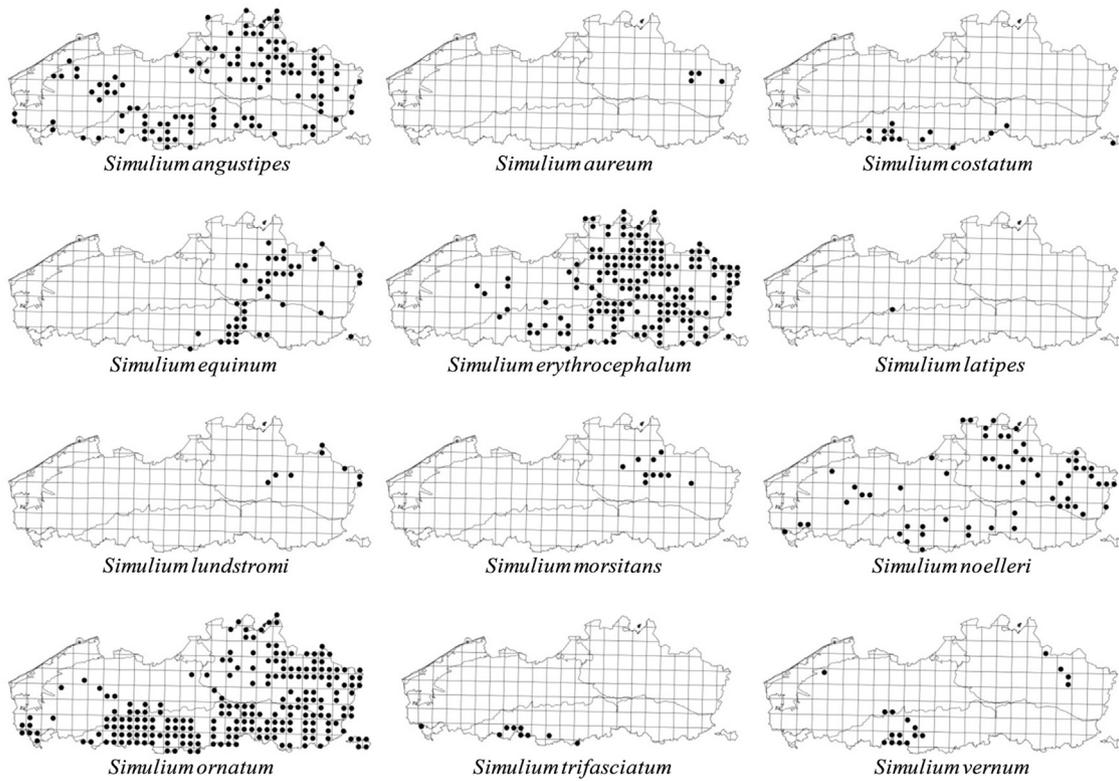
Fig. 4. Distribution of the blackfly species in Flanders, with indication of the regions and a grid of 10 km × 10 km UTM-squares.

Habitat suitability modeling

Although some blackflies are very sensitive to organic pollution, the most tolerant species, which are also the most widespread species in Flanders, possess a moderate sensitivity to organic pollution (Lechthaler and Car, 2005; Gabriels et al., 2010). Feld et al. (2002) observed blackflies in 93% of the samples and they were only absent when current velocities dropped below 6 cm s^{-1}, nitrate was higher than 140 mg L^{-1}, biological oxygen demand raised above 12 mg L^{-1} or conductivity surpassed 1740 μS cm^{-1}. In several studies, blackflies were even captured in all samples (Zhang et al., 1998;

Table 2

Median values (with 10 and 90 percentiles) of the assessed environmental parameters in samples where blackflies were present or absent (BOD: biological oxygen demand; COD: chemical oxygen demand).

	Present	Absent
pH	7.6 (7.0–8.1)	7.7 (7.3–8.3)
Conductivity (μS cm^{-1})	558 (286–930)	914 (491–2110)
Oxygen (mg L^{-1})	7.7 (5.0–10)	6.7 (2.5–11)
BOD (mg L^{-1})	3 (2–6)	5 (2.5–15)
COD (mg L^{-1})	21 (9.2–46)	41 (17–92)
Ammonium (mg L^{-1})	0.45 (0.11–2.7)	1.9 (0.33–8.6)
Nitrite (mg L^{-1})	0.10 (0.022–0.33)	0.15 (0.030–0.46)
Nitrate (mg L^{-1})	3.0 (0.77–7.8)	4.0 (0.40–11)
Kjeldal nitrogen (mg L^{-1})	2.0 (1.0–4.9)	3.5 (1.4–12)
Phosphorus (mg L^{-1})	0.50 (0.15–1.0)	0.90 (0.28–2.9)
Orthophosphate (mg L^{-1})	0.15 (0.060–0.52)	0.41 (0.10–1.7)
Slope (m 1000 m^{-1})	1.4 (0.20–7.9)	0.54 (0.078–2.6)
Sinuosity	1.03 (1.00–1.23)	1.01 (1.00–1.12)

Fig. 5. Evolution of the prevalence of blackflies in Flanders from 1990 to 2010.

Table 3

Correctly classified instances (CCI) and Cohen's kappa statistics (K) for calibration and validation with four different modeling techniques that were used to predict the presence/absence of blackflies based on the measured variables.

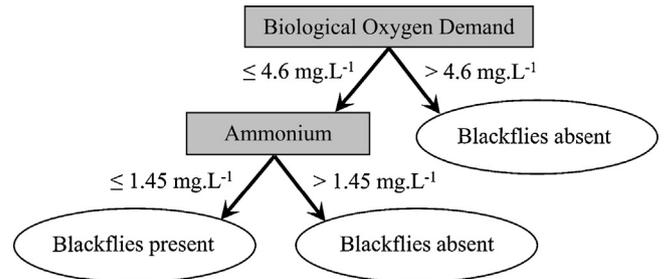|	Calibration		Validation	
	CCI (%)	K	CCI (%)	K
Logistic regressions	86.8	0.74	84.7	0.69
Support vector machines	81.5	0.63	78.7	0.59
Artificial neural networks	85.5	0.71	84.3	0.69
Classification trees	81.5	0.63	79.0	0.58

Fig. 6. Strongly pruned classification tree that predicts the presence or absence of blackflies in Flemish surface waters (correctly predicted instances 79%, Cohen's kappa 0.58).
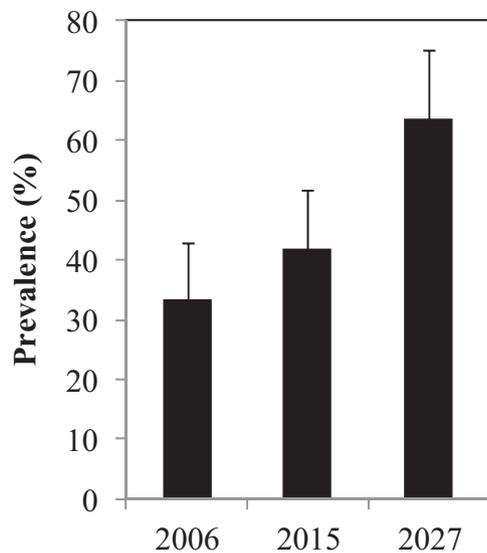
Fig. 7. Ensemble forecast of blackfly prevalence (with standard deviation) in 2006, 2015 and 2027 based on water characteristics modeled with PEGASE.

Malmqvist et al., 1999; Lautenschläger and Kiel, 2005; Illésová et al., 2008), indicating that Simuliidae are usually present in running waters if the organic pollution is not too severe. With the exception of brackish waters and waters with a very low current velocity (both largely restricted to the polder region), all types of running waters in Flanders could potentially contain blackflies and their current absence in most waters is caused by human disturbances. Due to the general improvement of the chemical water quality in Flanders, the prevalence of blackflies increased from less than 5% to almost 30% during the nineties (Fig. 5). Since 2000, however, their prevalence remained around 30%. This is in accordance with the reduction of for example ammonium and orthophosphate concentrations, which decreased during the nineties but stagnated afterwards (VMM, 2010).

River managers and stakeholders could use ecological models to select between different restoration options and management strategies in order to efficiently allocate restoration efforts (Mouton et al., 2009). In Fig. 6, an example of a strongly pruned classification tree is presented, which indicates that blackflies are only present when biological oxygen demand and ammonium content are not too high. This example shows that classification trees could be easily understood and communicated. This technique is thus suitable to convince river managers, decision makers or even the public (Boets et al., 2010; Dominguez-Granda et al., 2011). A modeling technique that is efficient for a certain dataset might fail for another, it is thus impossible to identify a generally applicable best modeling technique. Thuiller et al. (2009) therefore proposed that ensemble forecasts with several modeling techniques should be made and that the resulting range of predictions should be analyzed rather than relying on the results of a single model. The models developed in the present study were all able to accurately distinguish suitable from unsuitable habitats for blackflies based on physical–chemical variables (Table 3). Although blackflies can already occur in running waters with a moderate quality, most surface waters in Flanders are still not good enough to allow their occurrence, since their prevalence still fluctuates around 30%. An ensemble forecast with the four used modeling techniques indicated that blackfly prevalence will increase from 34% in the reference year 2006 to 42% in 2015 and 64% in 2027, if the planned measures are carried out (Fig. 7). The modeled prevalence in 2006 (34%) was slightly higher than the observed prevalence in 2006 (25%). This difference can be explained by the fact that the prevalence was not only modeled for the sites that were effectively monitored in 2006, but for the whole modeled catchments. In fact, the modeled prevalence in 2006 was well within the range of the values observed during the last decade (23–37%) (Fig. 5), which reflects that the ensemble forecast accurately predicted blackfly prevalence. The scenarios for 2015 and 2027 are mainly based on the planned installation of waste water treatment plants, however, since the Flemish government tends to plan more than is actually carried out in the field, it can be expected that the water quality will not even improve as much as predicted and additional measures should be taken. To obtain a good ecological water quality in all Flemish surface waters, which should be the case by 2015 (or at the very latest by 2027 if this is not feasible) according to the WFD (European Council, 2000), there is thus still a lot of work to be done.

Environmental management

According to De Cooman et al. (2007), the goals of the WFD could only be achieved by implementing small-scale efforts such as natural bank restoration, fish passage construction or river channel re-meandering, which affect physical and chemical conditions both locally and at basin scale. This kind of measures are undoubtedly beneficial, however, they are also very expensive. Especially re-meandering is costly and our data indicated that sinuosity has only a minor effect on blackfly occurrence (Table 2 and Fig. 3). Habitat restoration within a small stretch is usually not sufficient to realize changes in benthic invertebrate community composition and restoring habitats on a larger scale, using more comprehensive measures and tackling catchment-wide problems are required for a recovery of the invertebrate community (Jähnig et al., 2010). The results from the present study indicate that the chemical water quality is still the limiting factor and more cost-effective measures might therefore be the creation of buffer zones and the installation of constructed wetlands for small-scale waste water treatment. In Flanders, agricultural land usually extends up to the river banks and buffer zones are rarely present, although these are known to decrease runoff of nutrients and pesticides (Sahu and Gu, 2009; Tran et al., 2010). Riparian corridors are manageable areas and their creation along European watercourses should receive priority in order to achieve a good ecological status (Wasson et al., 2010). Constructed wetlands are considered as a cost-effective alternative for point-source treatment of effluents that cannot be connected easily to a sewage treatment plant (Boets et al., 2011).

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