Spatial spring distribution of the copepod *Eurytemora affinis* (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium)

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A B S T R A C T

The spatial spring distribution of *Eurytemora affinis* (adults and C5) in the Scheldt estuary (Belgium) brackish and freshwater reaches was studied in between 1996 and 2007. The bulk of the *E. affinis* population being generally situated in the brackish water reach (salinity > 0.5); we studied which environmental factors are responsible for its recent sporadic occurrence in the freshwater estuarine reach. Using PLS analysis, it is shown that its presence upstream is limited by a sufficient oxygen concentration (>4 mg l^{-1}) that is associated with temperature. Not only are the environmental conditions in the upstream zone important, but also the frequent presence of an O_2 minimum zone in the mid-estuary (O_2 min < 1.3 mg l^{-1}) seems to block the movement of the downstream *E. affinis* population in an upstream direction. Occasionally, the bulk of the population is however situated upstream. During these periods, high *E. affinis* abundance was also observed in the Durme tributary. Our findings suggest the possibility to use *E. affinis* as an "indicator" species of water quality, but also lead us to stress the necessity to consider conditions over the entire estuary when studying restoration effects, not exclusively in the zone of interest.

1. Introduction

The Scheldt is one of the few remaining extensive salt – brackish – freshwater tidal river/estuarine systems in Europe. In particular its freshwater tidal upstream (<0.5 salinity) reach is a rare habitat in Europe (Meire et al., 2005). Having a drainage basin which is heavily impacted by anthropogenic activity, the Scheldt was considered as one of the most polluted systems in Europe during the second half of the 20th century (Baeyens et al., 1998; Heip, 1988). The most polluted zone of the estuary, the downstream freshwater area, situated between Rupelmonde (km 103 from the mouth) and Antwerpen (km 90), was heavily impacted by several sources of disturbance and pollution, because of port infrastructure and industrial activities surrounding Antwerpen, but also by organic pollution coming from untreated wastewater of the Brussels agglomeration arriving in the Scheldt through the Rupel tributary (km 103). This area also coincides with the downstream part of the maximum turbidity zone (MTZ) of the estuary, and hence concentration of organic matter in the region is very high. In the Seventies, this situation led to – among other pollution characteristics – very low oxygen concentrations in this part of the estuary (Van Damme et al., 1995).

However, as a result of substantial emission reduction efforts throughout the watershed and the construction of water purification plants in the Brussels area, an improvement of the water quality is observed since the Nineties. Oxygen concentration improved considerably in the freshwater stretch from 1996 to 2006, associated with a decrease in N concentrations, mainly of NH_4 (Van Damme et al., 2005). As such, oxygen concentration can be considered as a proxy for the global water quality in the Scheldt estuary. At present, some stretches of the estuary still present indications of poor water quality. In the zone between 82 and 110 km from the mouth, oxygen concentrations below 0.5 mg l^{-1} are still regularly encountered.

Because of its key position as a link between primary producers and higher trophic levels, the zooplankton community has been studied since 1996 within the context of a multi disciplinary follow-up of the evolution of this restoring estuary (OMES project) (Tackx et al., 2003, 2004). As in most temperate estuaries, the Scheldt

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zooplankton spring community in the brackish – freshwater fringe is dominated by the calanoid copepod *Eurytemora affinis* (Castel and Feurtet, 1986; Soetaert and Van Rijswijk, 1993; Peitsch et al., 2000; Devreker et al., 2008). This paper considers the spatial distribution of this species in the context of the improving water quality of the Scheldt estuary, from 1996 till 2007.

The euryhaline calanoid copepod species complex *E. affinis* is generally known to inhabit brackish systems such as estuaries and salt marches in the Northern hemisphere. It is also able to invade freshwater reservoirs and lakes (Lee, 1999). Lee et al. (2003) demonstrates true installations of freshwater populations in various systems. The spatial distribution of *E. affinis* in the Scheldt estuary at first received attention in the frame of a comparative study of the spring zooplankton communities in European estuaries carried out during spring 1992. This study showed that, in the Ems (The Netherlands) and the Gironde (France) estuary, *E. affinis* had its peak abundance at salinity around 2 (Sautour and Castel, 1995). In the Scheldt however, peak abundance of *E. affinis* during the same period was observed further downstream, at salinities between 10 and 12. This difference in spatial distribution was explained by the very low water quality around the brackish water – freshwater fringe in the Scheldt estuary, which – as explained earlier – at that time characterised the highly polluted maximum turbidity zone around the harbour of Antwerpen (Soetaert and Van Rijswijk, 1993; Sautour and Castel, 1995) (Fig. 1).

Apparently, *E. affinis* in the Scheldt could not survive at its supposed salinity optimum of a few units (as deduced from the positioning of the bulk of its populations in the other estuaries). Following the improvement of water quality, Appeltans et al. (2003) demonstrated a tenfold increase in *E. affinis* abundance at Antwerpen between the periods 1989–1991 and 1996–1998. This shift in positioning was correlated to an increase in oxygen concentration around Antwerpen. Appeltans et al. (2003) suggest that below a threshold oxygen concentration between 0.6 and 1.6 mg l^{-1}, *E. affinis* could not remain in the Antwerp region and oxygen deficiency could act as a "barrier effect" to the upstream or downstream expansion of the copepod. Since the observations of (Appeltans et al., 2003), we sporadically observe *E. affinis* upstream of Antwerpen, sometimes in considerable abundance (cf. results).

So, the first aim of the present paper is to understand which environmental factors influence the spatial distribution of *E. affinis* in the Scheldt, and particularly its presence and abundance upstream in the freshwater reach. Following the hypothesis of Appeltans et al. (2003), we also test if the persistent presence of a low oxygen concentration zone in the Scheldt acts as a barrier for upstream or downstream expansion of *E. affinis*.

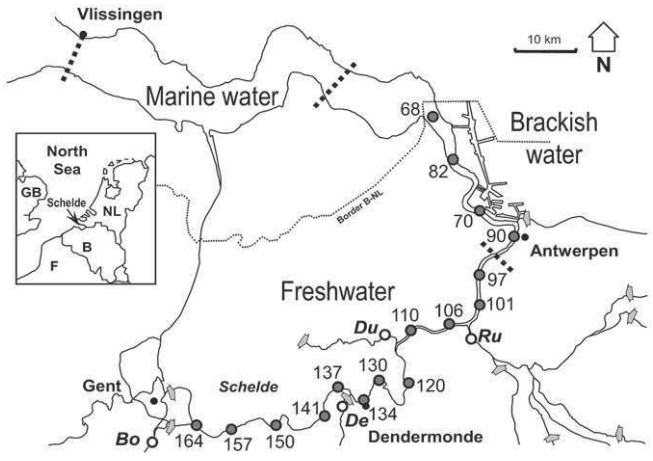

Fig. 1. Map of the Scheldt estuary with OMES sampling stations, designated by their distance in km upstream from Vlissingen. Arrows indicate the end of tidal influence on the tributaries (Bo: Bovenschelde, De: Dender, Du: Durme, Ru: Rupel).

2. Material and methods

2.1. Study site

The Scheldt estuary has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands (Fig. 1). Contrarily to most of the other temperate estuaries, the Scheldt estuary is characterised by vertically well mixed water flows (Baeyens et al., 1998), inducing most of the time no salinity or current stratification (Heip, 1988). Within the framework of the OMES project, samples are taken monthly at 16 stations (Fig. 1) since 1996 until present, with an interruption between 2000 and 2002. All stations are situated in the brackish and freshwater part of the estuary (Fig. 3). This paper considers only the months of February–May of this dataset, because of the occurrence of *E. affinis* in the Scheldt mainly in this period of the year.

2.2. Sampling and analysis

2643 water samples where collected in the middle of the river throughout the entire studied period, by means of bucket hauls from the ship. At each station, a set of environmental variables were measured. Temperature (T) and dissolved oxygen (O_2) were measured *in situ* with a 'WTW OXI 91' oxygen meter, salinity was measured with a 'WTW LF 91' conductivity-meter using the Practical Salinity Scale, Kjeldahl nitrogen (Kj-N) and total phosphorus (P) were measured by colorimetry using an SKALAR SA 5100 segmented flow analyser. Dissolved organic carbon samples where filtered on Whatman GF/C glass fibre filters, then treated with H_2SO_4 acidification and flushing with nitrogen, then set free by UV-irradiation. Suspended particular matter (SPM) samples were filtered on pre-combusted Whatman GF/C filters. From 1995 to 2001, Chlorophyll *a* (Chl *a*) samples were filtered on pre-combusted 45 μm Sartorius filters, extracted in 90% acetone and analyzed using reversed phase HPLC. The reader is referred to Van Damme et al.

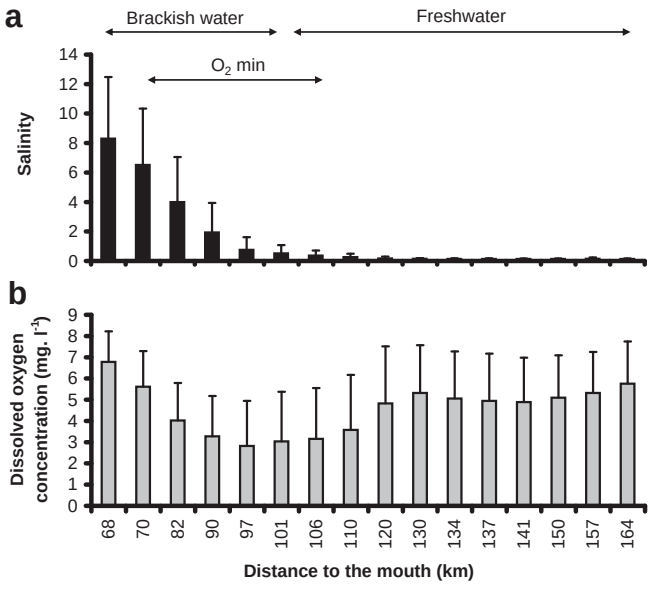

Fig. 2. Spring spatial distribution of mean salinity (a) and mean oxygen concentration (b), with their standard deviation, in the Scheldt estuary over the period 1996–2007. The locations of the minimal oxygen concentrations observed for all samplings are situated in the "O_2 min" range.

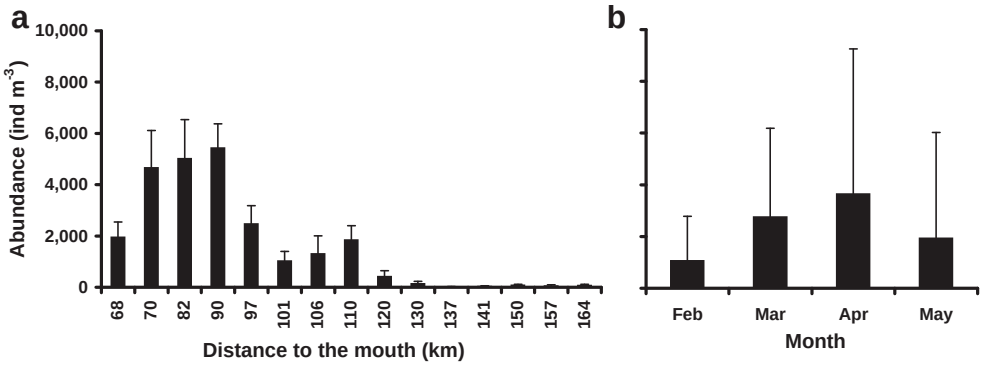

Fig. 3. Mean spring (February–May) abundance of *Eurytemora affinis* adults and C5 measured in the Scheldt estuary from 1996 to 2007, sorted by stations (a) or by months (b). Black lines show standard deviation.

(1997, 2005), for more details on the methodologies used. From 2002 to present, Chlorophyll *a* samples were filtered over a 25-mm diameter Whatman GF/F glass fibre filter. Pigments were then extracted and analysed by HPLC according to the method of Wright and Jeffrey (1997). More details on the methodologies used are presented in Lionard et al. (2008).

Since 1996, at each station, a volume of 50 litter of surface water was collected by means of bucket hauls and filtered through a 50 μm net. The collected zooplankton was anaesthetised with carbohydrated water and subsequently fixed in a formaldehyde solution (4% final concentration). Samples were analysed by binocular microscope (90× magnification) for zooplankton species composition and abundance. For some years (1996, 1997, 1998, 2002) data on the abundance of *E. affinis* are available for all 16 stations. For the other years, zooplankton sampling was limited to 6 stations (km 68, 90, 110, 134, 150 and 164) and hence *E. affinis* abundance data are only available for these stations. In the tributaries, environmental variables and zooplankton samples were taken from the shore, 10 km upstream of the mouth, within 24 h of the estuarine sampling. The same methods were used as in the estuary.

The Administration of Waterways and Sea (AWZ) provides daily discharge measures of the Bovenschelde, the Dender and the Rupel. The upstream discharge data at these stations were used to estimate downstream discharge, taking into account all the physical features of the Scheldt estuary. Daily average discharges for km 68 are used in our dataset. This station is located at the end of the study area and integrates discharge values of upstream stations.

2.3. Data analysis

A strong linkage exists between the abundance distribution of *E. affinis* and the brackish water – freshwater gradient observed for almost all environmental variables in the estuary (Soetaert and Van Rijswijk, 1993; Tackx et al., 2003; Van Damme et al., 2005). Thus, the relatively small changes in the upstream abundance, compared to the abundance of the bulk of the population, cannot be studied using general linear models (GLM) or principal component analysis (PCA) on data over the entire zone studied. Moreover, there are some missing values in the dataset. We therefore choose to carry out partial least square (PLS) regressions to identify the environmental variables that best explain the upstream distribution of *E. affinis*, based on predictors importance and regressions coefficients (Höskuldsson, 1988). Indeed, this method provides a mean to solve the problem of co-linearity between tested variables, thanks to the variables importance index. It shows which predictors are significantly more influent on the dependant variables than others. The R^2Y index is the proportion of the total variability of the dependant variables explained by the regression. A PLS regression is significant when its Q^2 index is equal or superior to 0.05. All variables, except temperature, were log-transformed to improve normality.

Simple regressions, equality of variances, k-mean clustering, parametrical and non parametrical tests were performed with Statistica 6 (version 6.0; Statsoft Inc., Tulsa, USA). SIMCA-P (version 9.0; Umetrics AB, Umeå, Sweden) was used to perform PLS regression. All graphs and statistical tests, including PLS analysis, were based on the same dataset, including 38 month of samplings.

Spring abundance data of adult and C5 *E. affinis* from 1996 till 2007 were used in this study. In the following, the term "*E. affinis*" refers *to E. affinis* adults and C5. We considered two ways to characterise the distribution of *E. affinis* in the estuary. Firstly, we quantified it simply by its upstream (cf. below) abundance. Secondly, we characterised its relative abundance in the upstream part using an "Upstream/Downstream Homogeneity index" for every sampling date, using the following formula:

$$UDH = 1 - (|D - U|)/(D + U)$$

D: Downstream mean *E. affinis* abundance
U: Upstream mean *E. affinis* abundance

In order to test a potential barrier effect of the low oxygen zone, we considered O_2 min, the lowest dissolved oxygen concentration measured in the estuary, as a spatial fringe between upstream and downstream abundances. The distance to the mouth of O_2 min is strongly correlated to the distance to the mouth of 0.5 salinity (Spearman rank test $p = 0.000005$), so the station corresponding to the O_2 minimum can effectively be considered as a spatial fringe between upstream and downstream reaches. Mean *E. affinis* abundance downstream to the O_2 min station was calculated considering the stations which distance to mouth is inferior to the distance where the O_2 min was measured. Upstream mean abundance was calculated considering the stations which distance to mouth is superior or equal to it.

This UDH varies from 0 (total heterogeneity) to 1 (total homogeneity), whether maximal abundance is located upstream or downstream.

3. Results

3.1. Spring distribution of salinity, dissolved oxygen and *Eurytemora affinis* abundances in the studied area

As shown in Fig. 2, the mean spring oxygen concentration as measured during 1996–2007 decreases from km 68 in upstream

direction to reach a minimum in between km 82 and 110 and increases further upstream. In the low concentration zone, values can be as low as 0.1 mg l^{-1} at some stations. Oxygen concentrations are therefore still generally low in the middle zone covering some 40 km of the Scheldt estuary.

The spatial distribution of *E. affinis* as observed in between 1996 and 2007 practically always peaks in the zone between km 70 and Antwerpen (km 90), at salinities between 4 and 8 (Fig. 3a). At the same time, *E. affinis* adults are also occasionally observed upstream of Antwerpen in the freshwater reach of the Scheldt, even as far upstream as Melle (km 164) (Figs. 1, 3a and 4). The *E. affinis* population in the Scheldt now seems to have its peak abundance at similar salinity reaches as earlier observed in the Ems and the Gironde (Sautour and Castel, 1995). Mean maximal abundances are found during April (Fig. 3b). It even penetrates in the freshwater (<0.5 salinity) reach. Its presence in the upstream part of the estuary seems however very variable.

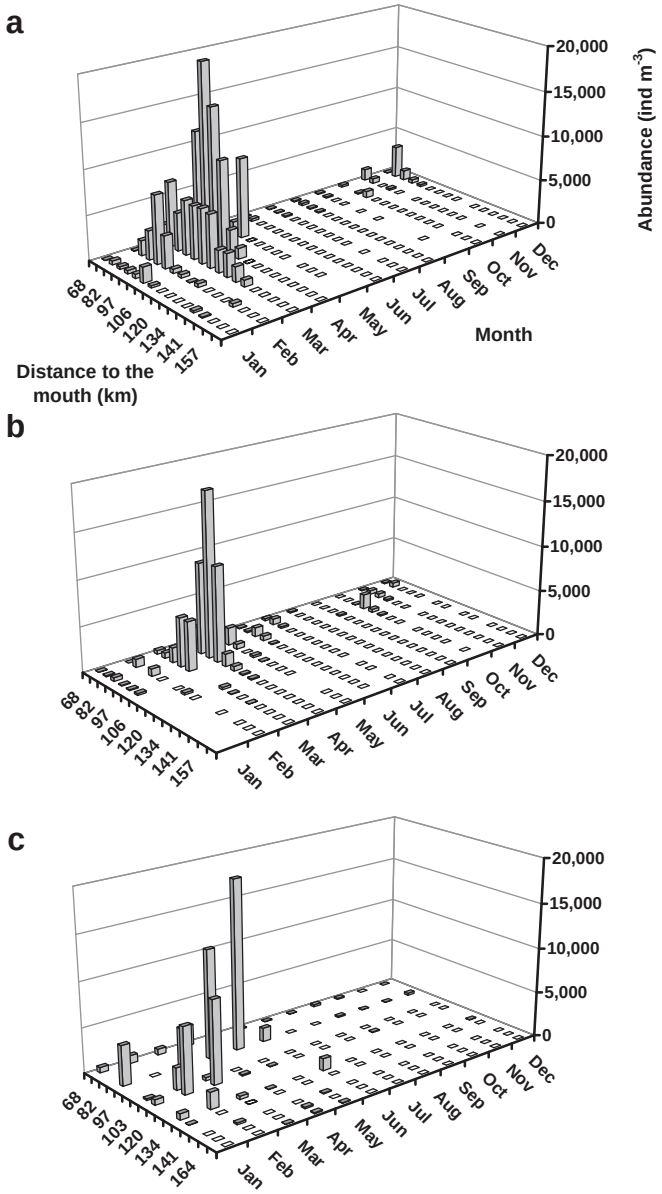

Fig. 4. Examples of various spatial distributions of *Eurytemora affinis* adults in the Scheldt estuary, during 1998 (a), 1997 (b) and 2004 (c). White squares mean null values.

An example of this variability is given in Fig. 4. During some months, such as March 1998, *E. affinis* is present and abundant as far upstream as km 134 (Fig. 4a) whereas during other months, such as April 1997, *E. affinis* remains downstream km 106 and is quasi absent upstream from this station (Fig. 4b). Inversely, it sometimes happens that the bulk of the population is located in the freshwater reaches, upstream km 103, such as for example during February and March 2004 (Fig. 4c).

3.2. Influence of environmental factors on the distribution of *Eurytemora affinis*

A PLS analysis was carried out to determine which environmental variables influence the upstream mean abundance of *E. affinis* and its UDH. In addition, we tested relations between the significant predictors to surface any possible correlations between these. The results are shown in Table 1.

R^2Y indexes are rather good in all analyses. O_2 min and O_2 are the most important and significant factors explaining upstream abundance and UDH, with a positive influence. SPM is significant in explaining UDH and upstream mean abundances, with a positive influence. Kj-N negatively influences upstream mean abundance. T and Q respectively negatively and positively influence UDH. Contrarily to upstream mean abundance, UDH is better explained by O_2 min than by O_2. O_2 is negatively influenced by Kj-N, tot P and T, but is strongly and positively influenced by Q (Table 1). Kj-N is strongly related to tot P but not to T, which therefore has an impact on O_2 which is independent from Kj-N. Given that the Kj-N importance coefficient is superior to that for the tot P in explaining upstream abundance of *E. affinis*, we can consider Kj-N concentration to represent both the Kj-N and tot P effect on *E. affinis* upstream − downstream distribution. O_2 or O_2 min importance are higher than that of Kj-N, tot P and T in explaining UDH and upstream abundance. Therefore, oxygen concentration has its own independent effect on the distribution of *E. affinis*.

To summarise, O_2 min, O_2, Kj-N, SPM, T and Q seem to be the most likely factors governing the upstream-downstream distribution of *E. affinis* in the Scheldt estuary. To visualise the combined

Table 1
Partial least squares regression results, using the station where the lowest dissolved oxygen concentration was measured as upstream/downstream fringe. Significant results are marked with an asterisk. O_2 min: lowest dissolved oxygen concentration measured in the estuary, O_2: upstream mean dissolved oxygen concentration, Kj-N: upstream Kj-N mean concentration, tot P: upstream tot P mean concentration, CHL *a*: upstream mean Chl *a* concentration, SPM: upstream mean SPM concentration, T: upstream mean temperature, Q: Mean runoff at km 68 (from day −7 to sampling day). See text for explanation.

Dependant variables		*E. affinis* Upstr.	UDH	O_2	Kj-N
Predictors importances	O_2 min	1.37*	1.41*	–	1.12
	O_2	1.49 *	1.37*	–	1.06
	Kj-N	0.97*	0.48	1.08*	–
	tot P	0.94	0.42	1.36*	2.09*
	CHL *a*	0.08	0.01	0.12	0.37
	SPM	1.28	1.30*	0.83	0.05
	T	0.62	1.38*	1.38*	0.27
	Q	0.14	0.37	0.64	0.20
Predictors coefficients	O_2 min	+0.18*	+0.18*	–	−0.19
	O_2	+0.19*	+0.18*	–	−0.18
	Kj-N	−0.13*	−0.06	−0.23*	–
	tot P	−0.12	−0.06	−0.30*	+0.36*
	CHL *a*	−0.01	0	−0.03	−0.06
	SPM	+0.17	+0.17*	+0.18	−0.01
	T	−0.08	−0.18*	−0.30*	−0.05
	Q	−0.02	−0.05	+0.14	−0.03
n		38	38	38	38
R^2Y		0.38	0.39	0.41	0.47
Q^2		0.33	0.32	0.24	0.33

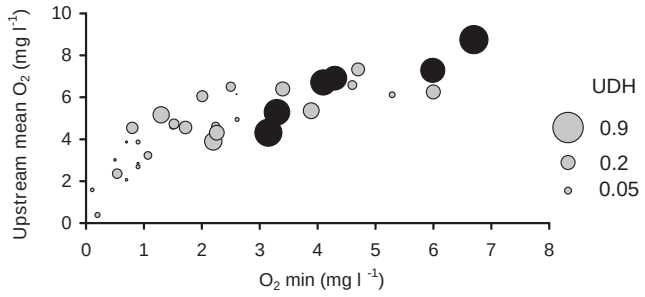

Fig. 5. Relation between UDH, O_2 min and O_2. Two UDH groups (high values in black, lower values in grey) were divided by a k-mean analysis.

effect of upstream O_2 concentration and the potential O_2 min barrier, we plotted UDH values in an O_2 min and O_2 biplot (Fig. 5). We divided UDH values in 2 groups with a k-mean analysis to separate higher and lower values.

A set of very high UDH values (Fig. 5) are observed when O_2 min is superior to 3 mg l^{-1}. If we consider upstream O_2, we find these high UDH values above 4 mg l^{-1}. Nevertheless, at these oxygen concentrations, several low homogeneity values are observed as well, mainly (8 cases out of 14) in the area corresponding to O_2 min $<$ 3 mg l^{-1}. In the zone corresponding to upstream $O_2 < 4$ mg l^{-1} and O_2 min < 1.3 mg l^{-1}, only very low UDH values are observed. This figure also illustrates that there is a very clear relation ($p < 10^{-13}$) between upstream mean O_2 and O_2 min.

In addition, considering the influence of environmental variables, we also considered the possibility that the abundance of the *E. affinis* population itself influences its spatial distribution. In other words, the population spreads out (in upstream or downstream direction, depending on where the population maximum abundance is situated), when its abundance becomes too high. Considering the previous results, we have therefore tested the relation between the maximal *E. affinis* abundance and the mean upstream abundances under several conditions (Fig. 6).

When O_2 min is superior to 3 mg l^{-1}, a clear relation exists between maximal *E. affinis* abundance observed and mean upstream or downstream abundance (Fig. 6a, c), depending on whether maximal abundance is found downstream or upstream. At lower O_2 min values, no correlation exists (Fig. 6b, d).

4. Discussion

This study aims to get a better understanding of the factors which control the spatial distribution and more specifically the recent expansion of *E. affinis* upstream the Scheldt estuary.

Tidal phase at sampling cannot be controlled for logistic reasons, but a recent study (Toumi, unpublished data) has shown that, in the Scheldt estuary, *E. affinis* surface abundance is representative of the entire water column abundance when considering mean values over 15 sampling occasions.

4.1. Influence of environmental factors on the distribution of Eurytemora affinis

A possible explanation for the sporadic occurrence of *E. affinis* upstream could be the importance of runoff. The upstream migration of *E. affinis* could be possible only during low runoff periods, and hampered by high runoff. However, runoff shows a significant but positive influence on the UDH values. So this hypothesis can be ruled out. This is in contradiction to major changes in the positioning of the *Eurytemora hirundoides* (synonym of *E. affinis*; Busch and Brenning, 1992) population at high and low runoff periods, observed in the Gironde estuary (Castel and Feurtet, 1986). However, these authors considered the March to October period, while our study considers only the spring bloom of *E. affinis*, during which runoff variations are smaller (200–600 m^3 s^{-1}) than those considered in the Gironde study over an entire year (200–2000 m^3 s^{-1}; Gasparini, pers. comm.).

Feeding conditions such as phytoplankton abundance could also influence the spatial distribution of *E. affinis*. Chlorophyll *a* concentrations in the study area are higher upstream than downstream and increasing with time over the 1996–2007 period (unpublished results). Chl *a* concentration did not appear as significantly influencing *E. affinis* upstream abundance or UDH values in the PLS analysis. This can be explained by the fact that, already during 1997, grazing experiment using natural Scheldt water showed that the ratio phytoplankton/suspended matter was sufficiently high for *E. affinis* to select phytoplankton at maximum rate (Gasparini et al., 1999; Tackx et al., 2003). So the subsequent increase in phytoplankton concentration probably did not improve feeding conditions for *E. affinis*. However, the fact that SPM has a significant and positive effect on UDH and upstream mean abundance can be explained by the fact that SPM concentration is higher in the freshwater region than in the downstream, brackish water zone. As, most of the time, the bulk of the *E. affinis* population is situated downstream, high UDH values correspond to a spreading upstream, towards these higher SPM concentrations. As explained above an effect of SPM concentration on the feeding conditions for *E. affinis* is unlikely (Gasparini et al., 1999; Tackx et al., 2003).

The PLS regression performed demonstrated that, O_2 min, O_2, Kj-N, SPM, T and Q significantly influenced the upstream-downstream distribution of *E. affinis* in the Scheldt estuary. Moreover, an independent impact of the oxygen concentration was distinguished from the seasonal influence. O_2 min, O_2 and Kj-N can be considered as representing "water quality". The fact that O_2 is more influent than O_2 min in explaining upstream abundances is quite logic considering that O_2 and upstream abundance are both based on an upstream mean. The fact that O_2 min is slightly more influent than O_2 on UDH suggests an independent effect of O_2 min and so a potential barrier effect on *E. affinis* expansion. So our results confirm the earlier suggestion by Appeltans et al. (2003) that oxygen concentration has an important impact on the distribution of *E. affinis* in the Scheldt estuary. According to our results, there is always a strong heterogeneity between upstream and downstream abundance when upstream mean oxygen concentration is inferior to 4 mg l^{-1} or when the O_2 min threshold value is less than 1.3 mg l^{-1} (Fig. 5). The oxygen threshold values found in this study are in the range of the 0.6–1.6 mg l^{-1} range reported by Appeltans et al. (2003). In semi-enclosed coastal waters (Turkey Point, Florida, USA), Stalder and Marcus (1997) also reported shifts in populations of three calanoids species (*Labidocera aestiva*, *Acartia tonsa* and *Centropages hamatus*) below 2 mg l^{-1} and experimentally observed declines in survival at oxygen concentrations below 0.9 mg l^{-1}. We demonstrated that a good relationship exists between the O_2 min and the upstream mean oxygen concentration, indicating that the water quality of these two zones is linked (Fig. 5) and that O_2 min can be used as an indicator of water quality upstream of the O_2 minimum. O_2 min can as such represent conditions for *E. affinis* presence upstream. Some relative high values of upstream oxygen concentrations (>4 mg l^{-1}) are associated with low UDH values (Fig. 5), representing situations when *E. affinis* is scarce or absent upstream, even when O_2 concentration seems to be sufficiently high in the area. Verification showed that these values correspond to situations where the bulk of the abundance is located downstream and O_2 min values are below 3 mg l^{-1} (Fig. 5). Therefore, these are situations in which upstream oxygen conditions are

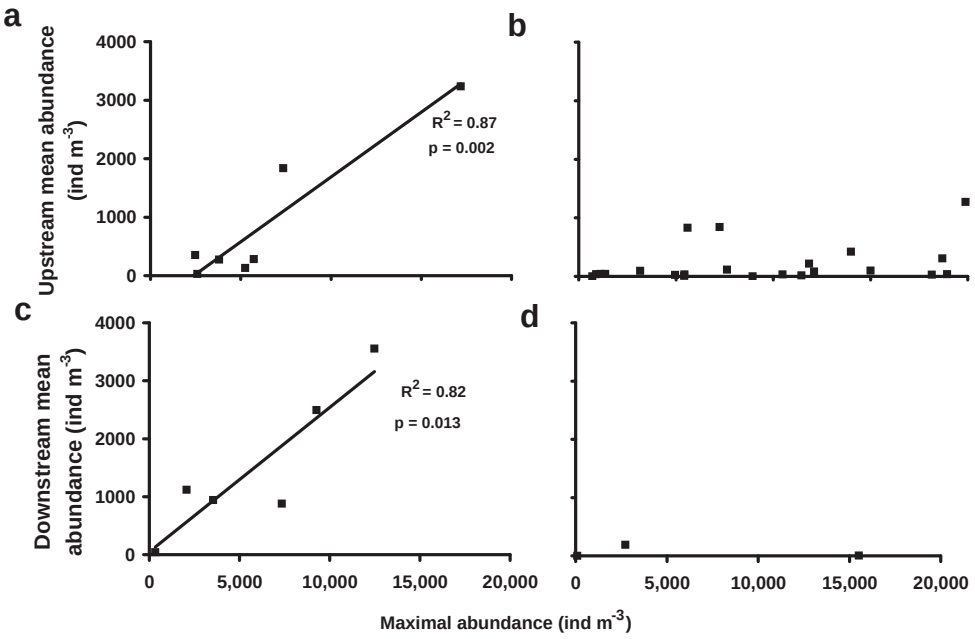

Fig. 6. Relation between maximal *E. affinis* abundance and upstream or downstream mean abundance of the copepod *Eurytemora affinis* in the study area, when O_2 min > 3 mg l^{-1} (a, c) and when O_2 min < 3 mg l^{-1} (b, d). O_2 min was used as upstream/downstream fringe value. When maximal abundances are located downstream the O_2 min location, the mean upstream abundance is considered (a, b). When maximum abundance is situated upstream the O_2 min location, the mean downstream abundance is considered (c, d).

permissive to the expansion of *E. affinis*, but the O_2 min value is not. So occasionally, *E. affinis* seems to be effectively blocked by a low oxygen barrier in its expansion upstream. This also explains the superior importance of O_2 min to the upstream mean oxygen concentration in influencing UDH in PLS regression (Table 1).

As to the influence of Kj-N on the *E. affinis* distribution, it should be reminded that we have considered Kjeldahl nitrogen as representing the associated phosphorous concentrations as well. As shown from Table 1, high Kj-N concentrations generally co-occur with low O_2 minima and hence low upstream O_2 concentrations. High concentrations of nitrogen and phosphorous manifest a high eutrophication level, which induces a strong consumption of oxygen in the water column. Indeed, in the Scheldt estuary, more than the third of the oxygen consumption is due to nitrification, inducing a strong impact on the N-load (Ouboter et al., 1998).

The respectively negative and positive effect of T and Q on UDH is obviously explained by seasonality. UDH values are indeed higher in early spring (February and March), when temperatures are colder and discharge values stronger, than later in the study period (not shown). Moreover, oxygen concentration, which itself greatly explains UDH, is also linked to these two factors.

The absence of relation between T and *E. affinis* upstream abundances is not surprising in our analysis, because this relationship is probably not linear. Indeed, as in the Seine estuary (Mouny and Dauvin, 2002), maximal abundances are found when temperatures varies between 10 and 15 °C in the Scheldt estuary, during April, and not when they are warmer. Devreker et al. (2004, 2009) also found optimal temperature for naupliar survival and hatching time around 15 °C.

It should be born in mind that oxygen concentration is related to Kj-N but that Kj-N is not related to T or Q. As such, low oxygen concentrations upstream or in the O_2 min area can be explained by a blended effect of seasonality and water quality.

In conclusion, the UDH of the distribution of *E. affinis* seems to be first limited by low oxygen concentration, itself limited independently by a high eutrophication level and/or by the natural seasonal influence.

Water quality seems adequate to explain most of the *E. affinis* upstream expansions. However, the potential influence of biotic interactions, which are not taken into account in our study, could also influence the abundance and the distribution of *E. affinis*. Predation pressure, for example, could block the upstream expansion of the copepod, or reduce its abundance. It has been shown for the spring 1993 period that, in the brackish part of the Scheldt, the diet of the mysid *Neomysis integer* consisted practically solely of *E. affinis* (Fockedey and Mees, 1999). As suggested by Verslycke et al. (2004), it is possible that *N. integer* populations shifted upstream the estuary since the improvement of the water quality in the maximum turbidity zone, but the distribution of this species in the upstream part of the Scheldt estuary since this period has not been studied yet. In addition, in the low salinity zone of the Scheldt, *E. affinis* and various hyper benthic species form an important food resource for the diet of juveniles of dominant fish species such as sprat and herring (Maes et al., 2005). In our study, the upstream expansion of *E. affinis* decreased in late spring (during higher temperatures and lower discharge values). This period also corresponds to the bloom of cyclopids in the freshwater part of the estuary (Tackx et al., 2004). Thus, competition could also hamper *E. affinis* upstream expansion in late spring.

4.2. Influence of the bulk of the population size on the distribution of Eurytemora affinis

The relation between observed maximal abundance and mean upstream or downstream abundance (Fig. 5) is significant when O_2 min is superior to 3 mg l^{-1}, and totally absent when O_2 min is inferior to 3 mg l^{-1}. This result suggests that there is indeed a spreading out of the *E. affinis* population with increasing size of its population, but this spreading out is hampered when oxygen concentrations are low. This enforces the concept of the oxygen as an ecological barrier for zooplankton. When this barrier is absent (O_2 min > 3 mg l^{-1}), expansion of populations towards upstream or downstream is possible, otherwise it becomes limited.

Further verification of the data revealed that very high UDH values (>0.7) are only found when upstream mean abundances are

slightly superior to downstream ones, and/or when maximal abundances are located upstream to the O_2 min value. When these maximal abundances were located downstream, UDH values remain lower. This suggests that the expansion of the copepod is easier in downstream than in upstream direction. Nevertheless, it is also possible that the upstream population receives individuals from another source than the downstream population and/or develops independently from the downstream population, at least during conditions which result in high UDH values.

4.3. Origin of the populations

The most evident sources of the upstream population – apart from the downstream one – are potential populations harboured in the tributaries Dender, Durme and Rupel (Fig. 1). Only the Durme regularly shows a considerable abundance of *E. affinis* (up to 3800 ind. m^{-3}). In order to test if this tributary could play a reservoir role, we considered all cases when bulk of the *E. affinis* abundance is located upstream the Scheldt estuary, and compared it to *E. affinis* abundance in the Durme. In these specific cases, there is a significant correlation between Durme *E. affinis* abundance and mean upstream abundances (Fig. 7).

The fact that, in these cases, *E. affinis* abundance in the Durme is higher than in the upstream Scheldt (Fig. 7), suggests that the Durme population is either fed by an inland source or that it has previously been imported from the downstream Scheldt population and developed well in this tidal inlet, which has environmental conditions similar to the upstream Scheldt area (unpublished data). The first possibility seems unlikely, as the Durme drainage is reduced to a few local polders.

Considering the cases when the bulk of the population is located downstream (Fig. 2), which represents the majority of our samplings, it seems logic to suppose this downstream population is the source of the upstream individuals. We support this statement by several arguments. When there is a rather good upstream/downstream homogeneity (UDH > 0.2), the abundance of *E. affinis* at the station where the O_2 min was observed is never null and always varies between 1000 and 17 000 ind. m^{-3}. So there seems to be a persistent linkage between upstream and downstream reaches under permissive water quality conditions, which is also represented by the correlations between maximum abundance and upstream or downstream mean abundance (Fig. 6a, c). We checked *E. affinis* abundance in the Durme in all cases when bulk of the abundance is located downstream the Scheldt estuary. In these cases, Durme abundances are never superior to 700 ind. m^{-3}. So it seems unlikely that, when the bulk of the population is located downstream, the origin of upstream individuals lies in the Durme.

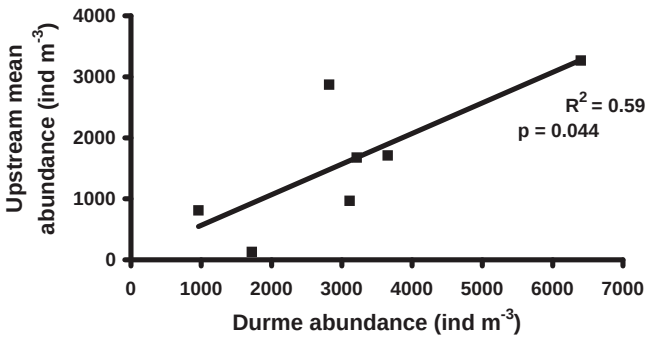

Fig. 7. Comparison between *Eurytemora affinis* abundance in the Durme tributary and its mean abundance in the upstream part of the Scheldt estuary, under conditions when the bulk of the population is located in the upstream reach.

As we saw previously, when upstream oxygen conditions are permissive and O_2 min values are not, a real ecological barrier exists. In our database, this situation corresponds to 11 cases. If there were two independent populations, we would sometimes record considerable abundances simultaneously in the upstream and downstream reaches under permissive conditions. There would indeed be no reason for the upstream population to be hampered in its development because of a downstream low O_2 min value. Actually, this situation never happens. The bulk of the population is always situated upstream or downstream and associated with low UDH values (<0.2). Moreover, *E. affinis* abundance in the Dender tributary, which is closed to estuarine input by locks, always remains below 80 ind. m^{-3}. There is no obvious reason why local populations should develop in the Durme and not in the Dender. In the Rupel tributary, which does receive freshwater input from the Zenne, Dijle and the Nete rivers, *E. affinis* abundance is generally low (<200 ind. m^{-3}) except at occasions when abundance in the upstream Scheldt is also high.

Another argument in favour of a "one population" hypothesis is the fact that Lee (1999), in a study on *E. affinis* of North America, Europe and Asia, shows that genetic variance among *E. affinis* within drainages is only 5%. We have little historic information on the presence of *E. affinis* in the Scheldt drainage. De Pauw (1973), reports the *E. affinis* population to be present from the mouth to Schijn (km 78) and to consistently peak around Zandvliet (km 65), as was the case during 1989–1991 (Soetaert and Van Rijswijk, 1993; Sautour and Castel, 1995). It should be mentioned that during the period studied by De Pauw (1967–1969), the Scheldt was already heavily polluted (Van Damme et al., 1995; Heip, 1988), which explains the absence of *E. affinis* upstream of km 78 (Schijn). Verraes (1968) at the time also reports a paucity of copepods in the Scheldt estuary, and its absence from the Rupel during the Nineteen Sixties. During our study period, the mean upstream abundance of *E. affinis* shows no pattern with time by simple regression ($p = 0.38$). This also suggest that *E. affinis* is not stably installed in the upstream area. So while at present, we cannot definitively exclude the existence of an upstream population which would be totally independent of the downstream one, this seems very unlikely.

We conclude that the recent upstream occurrence of *E. affinis* in the Scheldt is clearly linked to water quality, as represented by oxygen, Kjeldahl nitrogen (and associated total phosphorous). These factors have a direct influence, but are themselves seasonally influenced by temperature. Our results also show the importance that environmental conditions in one zone can have on living conditions for a species in another area of the system. Hence the necessity to take into account the water quality of the entire estuary to understand expansion of living populations. Fig. 8 represents a synthesis of the various environmental situations occurring in the Scheldt with regard to the spatial distribution of *E. affinis*.

UDH analysis could also be used to study the influence of hypoxia (or other limiting conditions) on the distribution of other pelagic organisms than *E. affinis*, in estuaries or river systems under two conditions. First, the system must include a local and periodic hypoxic area, which is located somewhere in the organism's expansion area. Secondly, the system must transit by this particularly area: the organisms should not be able to expand towards upstream or downstream reaches by any other way. More generally, the UDH analysis presented here can be applied to any ecosystem which spatial scale can be studied in one dimension (e.g. rivers or estuaries) and which includes a potential abiotic or biotic barrier.

The occurrence of *E. affinis* in the upstream Scheldt area can to some extent be considered as a tracer of the success of the restoration process in the Scheldt. As the conditions for its existence seem to be clearly related to threshold values of environmental

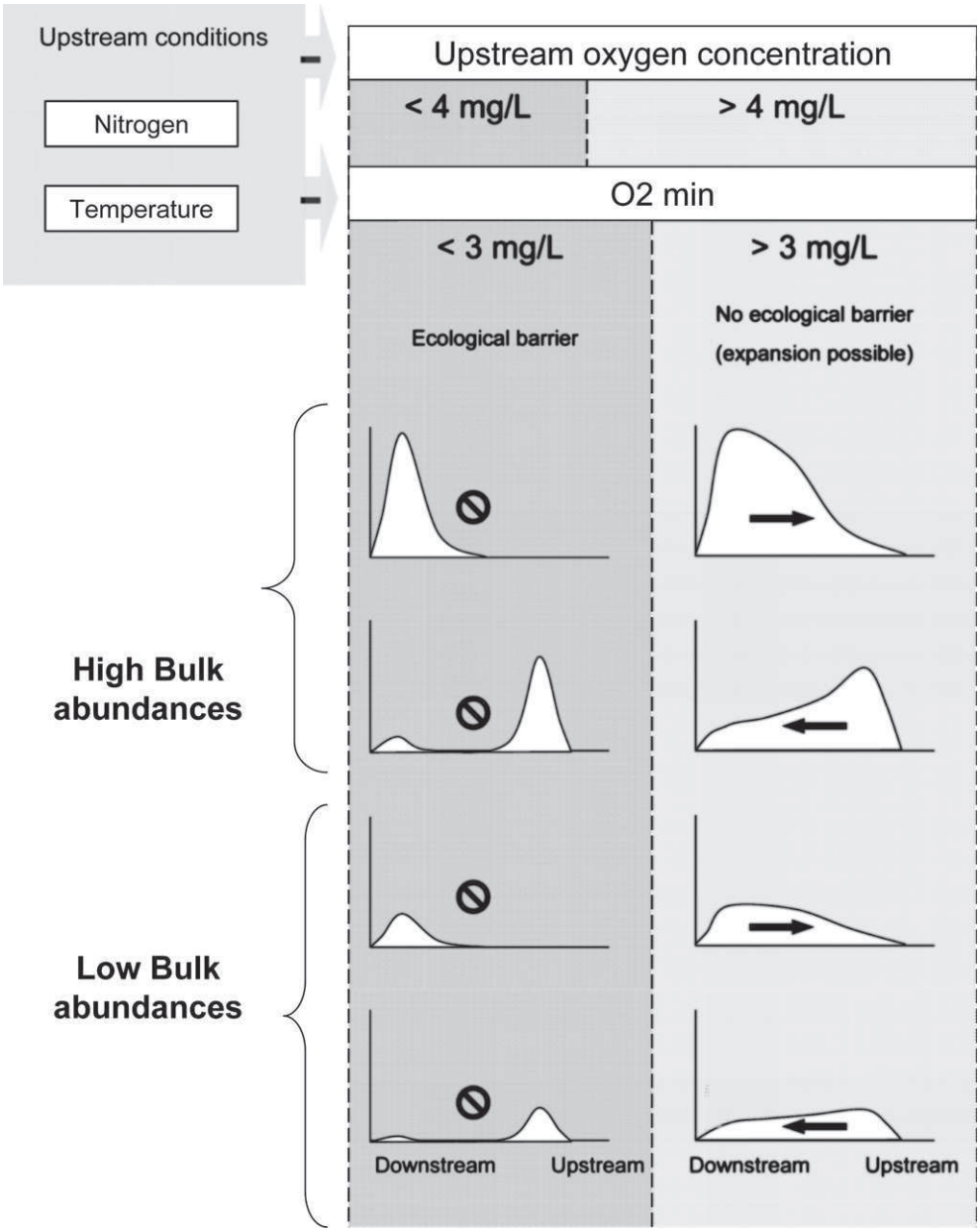

Fig. 8. Conceptual scheme representing the environmental conditions for the spatial distribution of *Eurytemora affinis* adults in the Scheldt estuary. Distance to the mouth is represented on the *X*-axis, *E. affinis* abundance on the *Y*-axis.

variables, this opens perspectives for modelling its occurrence. *E. affinis* being an easily recognisable species, and considering its importance as prey for mysids and fish in the Scheldt (Fockedey and Mees, 1999; Maes et al., 2005) and temperate estuaries in general (Knutson and Orsi, 1983; Mouny and Dauvin, 2002; Winkler et al., 2003, Winkler and Greve, 2004) it seems to be a good "indicator" candidate.

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