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THE UNIVERSITY OF HULL
Institute of Estuarine and Coastal Studies (IECS)

Baseline larval fish assemblages along the Dutch coast, Southern North Sea

Report to Port of Rotterdam.
Project Organization Maasvlakte 2 (PMV2)

Institute of Estuarine and Coastal Studies
University of Hull

19 July 2010

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For and on behalf of the Institute of Estuarine and Coastal Studies	
Approved by:	Prof. Mike Elliott
Signed:	
Position:	Director
Date:	25 October 2010

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EXECUTIVE SUMMARY

European environmental legislation has identified zooplankton as a biological quality element to evaluate the conservation status of coastal waters (EU Water Framework Directive). Disruptive pressure on fish spawning habitat, spawning stock biomass or direct effects on larval fish survival may impair normal demographic mechanisms resulting in altered ichthyoplankton assemblages which could be a sensitive measure of ecological impacts. The goal of this study is to assess a reference condition to scale possible impacts of human activities on larval fish survival and growth. This work (i) presents temporal and spatial dynamics of larval fish assemblages in the near shore (<30km) zone of the Dutch coast along the southern North Sea, and (ii) provides a predictive model of species associations using spatial, temporal and environmental variables to establish the baseline assessment in 2007. Late fish larvae were collected in three separate cruises and included 100 stations sampled in April, July and October 2007 covering an area of 200 km x 30 km. A total of 28 larval fish species were encountered during the surveys with greater larval densities in April and a decreasing trend towards the end of the survey period. Nineteen of these species were collected in April (with 11 unique species to April) grouped in 2 assemblages (cluster analyses & SIMPER), seventeen species in July (4 unique) and 4 assemblages, and finally nine species (1 unique) and 1 assemblage type in October. Herring (*Clupea harengus*), flounder (*Platichthys flesus*) and dab (*Limanda limanda*) dominated the catches in April. Sand goby (*Pomatoschistus minutus*), dragonet (*Callionymus lyra*), and sprat (*Sprattus sprattus*) dominated in July, and in October sand goby (*Pomatoschistus minutus*) was the most abundant species. A significant effect of month of collection on assemblage composition was found (PERMANOVA $p<0.0001$). Seasonal factors explained most of the variance (70%) but total suspended matter (TSM) and Chlorophyll-a were also significantly related to the assemblage composition, although the estimated effects were minor (Redundancy Analysis, RDA). Partial RDA analysis with season and temperature as covariates (to remove seasonal effect) identified TSM and Chlorophyll-a as statistically significant variables although the explained variance was low (5.4%) and presented comparatively small environmental gradients. The study concludes that a seasonal-based model may be a useful baseline reference to describe larval fish assemblages in the area.

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1. INTRODUCTION

Temperate coastal areas experience marked seasonal variability in environmental factors which has resulted in distinct rhythms in the biotic communities. Among the most remarkable of them is the timing of reproduction. There are many examples in ecology of physiological and behavioural mechanisms seeking the best match between favourable conditions and the appearance of offspring. Most fish and the vast majority of marine organisms produce numerous tiny larvae that are dispersed by ocean currents. These early life stages face uncertain survival prospects as they are very vulnerable to predation, require readily available food items and are unable to swim against currents. It is now accepted that variable mortality rates during the relatively short early life stages are the single most important mechanism influencing fish population dynamics. Very small changes in mortality rates in the early life stages of marine fishes can result in large shifts in fish stock biomass (Hjort, 1914; Cowan & Shaw, 2002; Houde, 2002). It is well established that seasonal patterns of primary productivity of coastal seas are followed by a suite of consumer organisms such as fish larvae (Platt *et al.*, 2003), which rely on this transient bounty. Temporal or spatial mismatch with suitable prey, as well as co-occurrence with predators, may result in recruitment failure and weakened year cohorts.

Ichthyoplankton assemblages vary in species identity and abundance in tune with spawning intensity, predation intensity and physicochemical attributes. Above all, physical factors controlling coastal water masses are especially influential in the dispersion of larvae from the spawning grounds into favourable areas such as those where suitable prey items are abundant. However, a number of other processes may interfere with the feeding ability of larval fish. For example, as fish larvae are visual predators, increased turbidity may result in reduced contact rates with suitable prey items and thus impair food ingestion (Rothschild, and Osborn, 1988; MacKenzie and Leggett, 1991). Likewise alteration of the supporting food web and local secondary production may result in indirect effects on fish larvae survival. All of these effects are part of the natural uncertainty to the highly dynamic coastal environment and it could be expected that they play a significant role in structuring coastal fish assemblages.

The southern North Sea along the Eastern English Channel and Dutch coast is dominated by a northward flowing residual current (Otto *et al.,* 1990; Grioche *et al*., 1999; Simpson *et al.*, 1990) that tends to weaken during north easterly wind episodes. The area features a distinct salinity-driven frontal system separating offshore and coastal water masses. Seasonal variability in freshwater runoff controls the location of the front which in turn defines the location and extent of the coastal water mass and currents in the area (Simpson *et al.,* 1990; Otto *et al.,* 1990; Joordens *et al.,* 2001; de Boer *et al.,* 2008). The net result is an area of northward-flowing continental waters maintained to the coast that is likely to exert a structuring role on plankton assemblages, promoting inshore-offshore segregation and retention within the coastal zone (Otto *et al.,* 1990; Dickey-Collas *et al.,* 2009). Furthermore, within the coastal water mass, environmental variables show distinct patterns of variability at different scales. In most years there is a general inshore-offshore seasonal gradient (i.e. salinity, temperature, suspended solids, Chlorophyll-a) but also short term variability in suspended solids, salinity, bottom silt % due to tides or weather episodes especially along, but not restricted to, the coastal fringe. Thus, the hydrological context defines a shallow enclosed coastal area where linkages between physical factors and biological attributes can be studied at a relatively small but relevant scale to larval fish assemblages.

From a conservation viewpoint, coastal zones worldwide are under multiple pressures and it is important to ensure that key processes in the life cycles of marine organisms, such as reproduction, are not disrupted. European environmental legislation has identified zooplankton as a sensitive biological quality element to evaluate the conservation status of coastal waters (EU Water Framework Directive; EU Marine Strategy Directive). Human activities may impose a direct disruptive pressure on the ichthyoplankton assemblage or may indirectly affect the spawning habitat or spawning stock biomass. Potential uses of larval assemblages as a sensitive measure of disruption of normal demographic mechanisms will require both a good understanding of controlling biotic and abiotic factors in coastal environments and the evaluation of the normal variability in spatial and temporal appearance of larval fish assemblages (USEPA, 2000).

As part of the expansion of the Port of Rotterdam, a large deep water terminal, Maasvlakte 2, is to be constructed using sand collected by dredgers from neighbouring areas in the North Sea. Once completed, Maasvlakte 2 will consist of a stretch of reclaimed land on the Dutch coastline sheltering a deep harbour (Berkenbosch *et al.*, 2007) The sand extraction operations during the construction phase of Maasvlakte 2, the resulting change in bathymetry of the nearshore area in its vicinity, increased suspended solids, and the potential changes to the transport of silt, nutrients and fish larvae in the vicinity of the Maasvlakte 2 and along the Dutch coast once the expansion is completed all have the potential to influence the larval fish assemblages. In recognition of the requirement to quantify any possible construction or operational impacts, if any, of Maasvlakte 2 on the larval fish assemblages off the Dutch coast, a baseline study consisting of three separate larval fish surveys was commissioned by the Port of Rotterdam in spring, summer and autumn 2007 before the start of the construction works.

The aim of this report is to describe temporal and spatial dynamics of the larval fish assemblages and estimate the effect of selected environmental factors on the structure of the larval assemblages. In addition, a preliminary assessment of the trophic resource utilised by the more abundant larvae within the seasonal assemblages is presented.

2. METHODS

2.1 Sampling domain and gear

The data are intended to serve as a reference to characterise the nature of environmental impacts caused by sand extraction activities. To ensure the generality of the results, the sampling grid extended well beyond the expected immediate influence of the development, and included 100 stations sampled in April, July and October 2007 covering an area of 6,000 km^2 (200 km x 30 km) along shallow sandy bottoms (Figure 1). Sampling sites were located along transects and extended from the shore (0-5m chart datum) to approx 30km offshore (depth 20-25m). All cruises took between eight to twelve days to complete and positions on transects were visited at random to avoid bias in the dataset. Sampling was conducted exclusively at night from 30 minutes after sunset to 30 minutes before dawn.

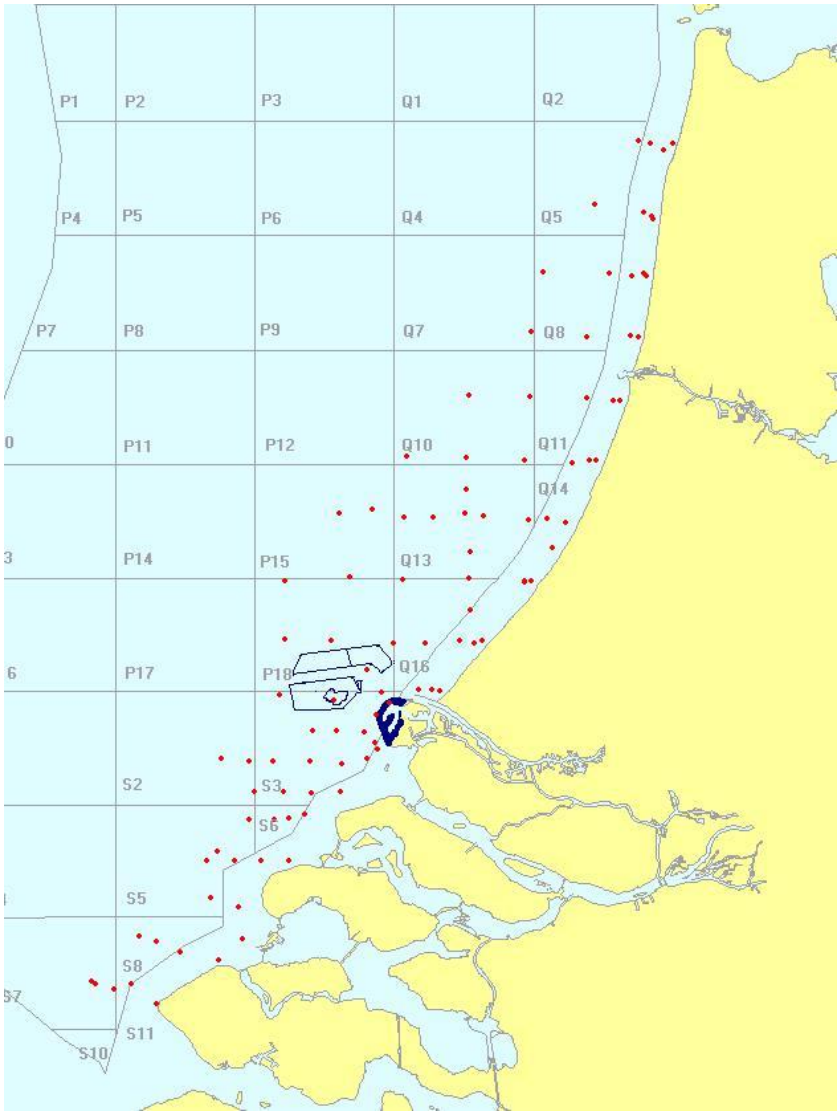


Figure 1. Geographical location of the study area. Sampling stations are illustrated by the red dots. The location of the sand extraction areas is depicted as polygons off the mouth of the Rotterdam port. The area filled in blue is the footprint of Maasvlakte 2.

Late larvae and juvenile fish were collected with a conical MIK net sampler (Munk, 1988). The net had a 2.0 m diameter aperture, was 17 m long with a 15 m main body (1500 µm mesh size), a 1.5 m codend (500µm mesh size) and a final 0.5 m blind solid fabric end to retain the sample. The depth of the sample was maintained at approximately 5m off the bottom by controlling the length and angle of the towing cable. Upon collection the catch was quantitatively sub-sampled if necessary and stored frozen (-40 °C) for analysis (Plate 1). Freezing is considered a humane way to quickly euthanize a large number of larval fish as cold temperature has a sedative effect on cold-blooded organisms. The mouth of the net was equipped with a flow meter and a water quality logger that recorded temperature and salinity at 30 second intervals during the tows. Parallel surveys provided bottom silt percentage (first 10 cm layer), Chlorophyll-a concentration (5m off the bottom), total suspended matter (TSM) (5m off the bottom) and total suspended matter averaged over the water column (TSM avg) for the survey area.

(A). (B)

Plate 1. Photographs of fish larvae during processing. (A) fresh unsorted sample on board previous to freezing and (B) lesser sand-eel (*Ammodytes marinus*) larvae after preservation and sorting in the laboratory (2.5mm-grid graph paper if shown for size reference).

2.2 Sample processing

After each cruise the sample was allowed to thaw overnight in an equal volume of 10% buffered formaldehyde solution in seawater (5% final working concentration). The formaldehyde was then decanted and the sample rinsed in fresh water. Larvae were extracted from the sample and were preserved in 70% methanol for at least four months prior to processing (Plate 1). Finally the larvae were sorted into individual species and photographed along with a reference scale. The standard length of the individual fish was measured on digitised photographs using image analysis software (Image J, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA).

Larval fish were identified to species or lowest taxonomical level possible. The identification was done using morphological larval keys (ICES/CIEM Identification Sheets; Russell, 1976; Munk and Nielsen, 2005) based on external characteristics of the species. Due to overlapping characteristics between the early stages (<7mm standard length) of larval dab

(*Limanda limanda*) and flounder (*Platichthys flesus*) in the April cruise, a subsample of 25 randomly-chosen larvae were cleared in an enzymatic solution and were stained with alizarin blue to obtain vertebrae counts and determine the ratio between these two species. This ratio was assumed constant over the stations where these size ranges were found and computed abundances corrected accordingly.

In April, larval fish collected at all available samples (94 in total) were identified and enumerated. For the July and October cruises a randomly selected subset of 38 and 35 samples from the 100 available were processed. The sub-sample was chosen using a stratified random strategy to ensure good coverage across the entire latitudinal spread (first strata) and depth ranges (nested strata) of the survey. The size of the sub-sample was estimated as the minimum sample size that would provide comparable results in terms of common species coverage based on the April cruise data (Table 1).

Table 1. April ranked list of species with abundance (no/1000m^3) found in the full set of 94 samples and the subset of 33 samples. The species are listed in order of decreasing abundance for the 94 sample set. Differences in rank between the two datasets are highlighted in grey. Species in boldface accounted for 95% of the total abundance of the respective sample set. Asterisks indicate species recorded at less than 5% of the stations analysed.

Species	All 94 sampled sites		Subset of 33 sites	
	no/1000m^3	rank	no/1000m^3	rank
Clupea harengus	180.4	**1**	167.4	**1**
Platichthys flesus	24.7	**2**	14.9	**2**
Limanda limanda	18.1	**3**	14.3	**3**
Arnoglossus laterna	7.3	4	4.7	5
Ammodytes marinus	4.0	5	5.0	4
Solea solea	3.3	6	4.2	6
Pleuronectes platessa	2.1	7	1.5	7
Sprattus sprattus	1.1	8	0.5	9
Merlangius merlangus	0.5 *	9	0.7	8
Liparis liparis	0.3 *	10	0.2	10
Aphia minuta	0.1 *	11	0.1 *	13
Microchirus variegatus	0.1 *	12	0.1	12
Pholis gunnellus	0.1 *	13	0.2	11
Anguilla anguilla	<0.1 *	14	-	-
Pomatoschistus minutus	<0.1 *	15	-	-
Scorpaenidae spp	<0.1 *	16	0.1 *	14
Callionymus lyra	<0.1 *	17		
Dicentrarchus labrax	<0.1 *	18		
Hyperoplus lanceolatus	<0.1 *	19		

2.3 Stomach analysis

The stomach contents of 115 larval fish were examined. The analysis included feeding incidence and identification of stomach contents to the lowest possible taxonomical level. The stomach analysis was conducted on the three most abundant fish species of the April and July cruises and the four most abundant in October. Since most larvae are visual predators that generally feed during the day, the larvae processed for stomach content were randomly collected from those samples taken no less than two hours after sunset.

2.4 Statistical analyses

Raw abundance data were transformed into density estimates using flow meter readings and expressed as number of larvae per 1,000m^3. Expected species accumulation curves were computed using bootstrap analysis as samples are cumulated in permuted orders (999 random permutations) (Primer v6, Clarke and Gorley, 2006).

Species density data were then square-root transformed and only species found in more than 5% of the samples were included in the community analysis. Samples were subjected

to cluster analysis using the Bray-Curtis similarity (cluster average and SIMPROF test, 0.5% significance level, 999 simulation permutations) to identify species assemblages. Statistically significant clusters in the SIMPER analysis were subsequently used to obtain the composition of the assemblages. The species data were further analysed using a permutational MANOVA (PERMANOVA) and to determine whether the larval fish assemblages were different between cruises and to obtain a probability estimate under the null hypothesis (H_0: species were not different between cruises). Similarly, differences across the latitudinal gradient were investigated using a 3- area station assignment (south, central and north area to the Maasvlakte 2 reclamation zone; H_0: species were not different between zones). All these routines were run in the Primer v6 & Permanova + software bundle (Clarke and Gorley, 2006; Anderson *et al.,* 2008). The composition of the assemblages was further explored by comparing the mean length and length frequency distribution of larvae using non-parametric tests, Kruskal-Wallis One-Way Analysis of Variance and Kolmogorov-Smirnov Two Sample Test, respectively (Systat v10, Systat Software, Inc. Chicago). Assemblages with a low number of length observations were excluded from these length analyses.

Finally, we tested the explanatory power of environmental variables on the biotic structure using Redundancy Analysis (RDA) (Brodgad v2.6.4, Zuur *et al.,* 2007). RDA allows for the study of relationships between environmental factors and biotic variables (species) (ter Braak 1986; Leps & Smilauer, 2002). By using the resultant canonical eigenvalues it is possible to assess how well a specific selection of explanatory variables explains the variance in the species data. The RDA graphic output (triplot) positions the samples in a two-dimensional space together with vectors indicating the gradients of the environmental factors and species, which results in a relatively straightforward interpretation of the relationships (see appendix 2 for further explanations details of the interpretation of RDA graphical outputs). Forward selection analysis was used to identify environmental variables which are significantly related to the variation in the distribution of larval fish. Environmental variables were included in the model starting from the variable 'explaining' the most variance and then others added if their sequential addition significantly improved the 'explained' variance (p<0.05 full model permutation test using 999 unique permutations). TSM and Chlorophyll-a (at 5m) were log-transformed and percentage silt (sediment sample) data were square-root transformed before the analysis. All other environmental variables, station depth, temperature, salinity, latitude, degree day (thermal sums starting in January 1st 2007) and cruise month (categorical variable) were left untransformed in these analysis. Before the analysis, Pearson's correlation coefficients were calculated to identify redundant environmental variables. Only one variable for each highly correlated pair was retained (Pearson correlation > 0.9).

3. RESULTS

3.1 Larval fish diversity and effort

A total of 28 larval fish species were encountered during the surveys conducted in April, July and October (Table 2). Nineteen of these species were collected in April (fourteen in the reduced station subset), sixteen in July and eight in October. The larval fish species assemblage collected in April was the most unique in terms of species composition, and contained eleven species (ten in the reduced sample subset) which were not collected during the surveys in July and October (Table 2). Four species were unique to the July survey whilst only one species was unique to the October survey. Two gobies, the transparent goby (*Aphia minuta*) and the sand goby (*Pomatoschistus minutus*), and the clupeid, sprat (*Sprattus sprattus*) were encountered during all three surveys suggesting extended presence in the area.

Table 2. Summary table listing larval fish recorded by survey month. The asterisks indicate total mean abundance, * <1, ** 1-10, * 10-100, and **** >100 larvae 1000m^{-3}. The number of stations processed is given in parenthesis. Underscored asterisks in April indicate absence of the species in the subset of stations analyzed in July and October.**

Latin name	Common name	April (94)	July (38)	October (35)
Sprattus sprattus	Sprat	**	***	*
Aphia minuta	Transparent goby	*	*	*
Pomatoschistus minutus	Sand goby	*	****	***
Limanda limanda	Dab	***		
Platichthys flesus	European flounder	***		
Ammodytes marinus	Lesser sand-eel	**		
Liparis liparis	Common seasnail	*		
Pleuronectes platessa	Plaice	**		
Solea solea	Dover sole	**		
Merlangius merlangus	Whiting	*		
Microchirus variegatus	Thickback sole	*		
Pholis gunnellus	Butterfish	*		
Scorpaenidae sp.	Scorpion fishes	*		
Anguilla anguilla	European eel	*		
Clupea harengus	Herring	****	**	
Arnoglossus laterna	Scaldfish	**	*	
Dicentrarchus labrax	Sea bass	*	*	
Hyperoplus lanceolatus	Grater sand-eel	*	**	
Callionymus lyra	Dragonet	*	***	
Trachurus trachurus	Horse mackerel		***	
Buglossidium luteum	Solenette		**	
Echiichthys vipera	Lesser weaver		*	
Psetta maxima	Turbot		*	
Engraulis encrasicolus	Anchovy		***	*
Sardina pilchardus	Sardine		***	*
Syngnathus rostellatus	Lesser pipefish		**	*
Blennius gattorugine	Tompot Blenny		*	*
Pomatoschistus pictus	Painted goby			*
	No. Spp. in all samples	*19*	*na*	*na*
	No. Spp. per month in subset	**14**	**16**	**8**
	No. unique Spp. per month in subset	**10**	**4**	**1**

The ranks for the species making up 90% of the abundance and the top-10 ranking species was identical in the April subset and in the full April dataset (Table 1). In clear contrast ranks assigned to rare species (defined as those present at less than 5% of the stations) were very different suggesting that larval species not found in the subset would only be fully sampled using larger effort levels. The comparability of the two datasets is further confirmed by the similarity in the K-dominance and predicted species accumulation curves (Figure 2).

(a) **(b)**

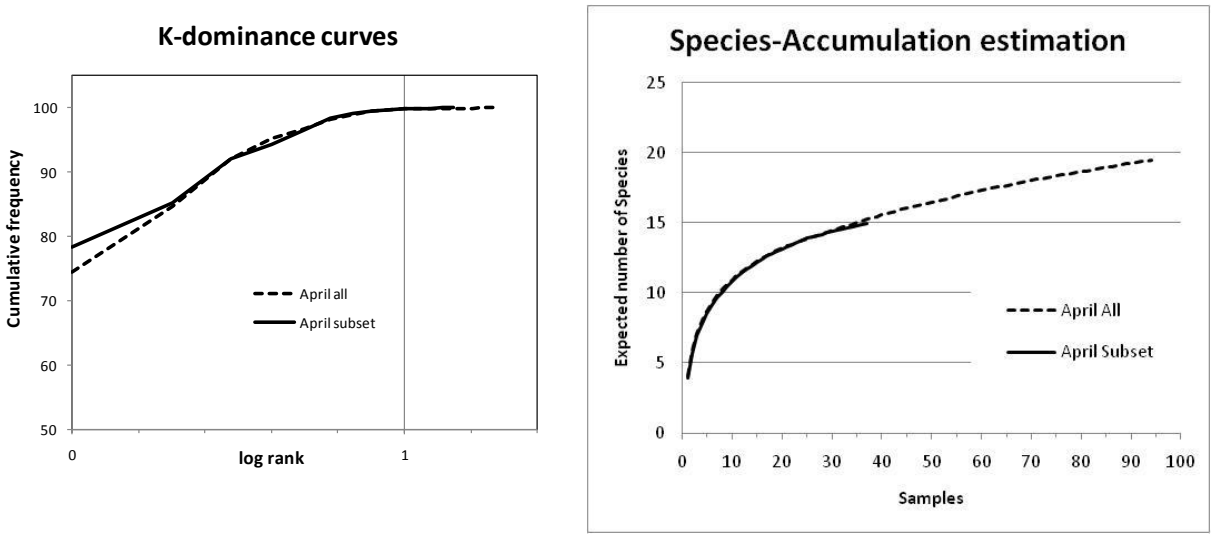


Figure 2. Species dominance (a) and cumulative diversity estimation over samples (effort) (b) between the April sample subset and the whole April sample set. A log rank of 1 indicates 10 species in the species dominance diagram. The expected number of species was estimated from 999 random permutations.

Species accumulation curves by cruise showed an overall increased diversity and lower dominance in July (Figure 3). Similar abundance but greater dominance of common species was found in April, resulting in a higher elevation of the dominance curve. October showed the least diversity in the larval assemblage but showed a similar dominance of common species as in April (Figure 3).

(A) (B)

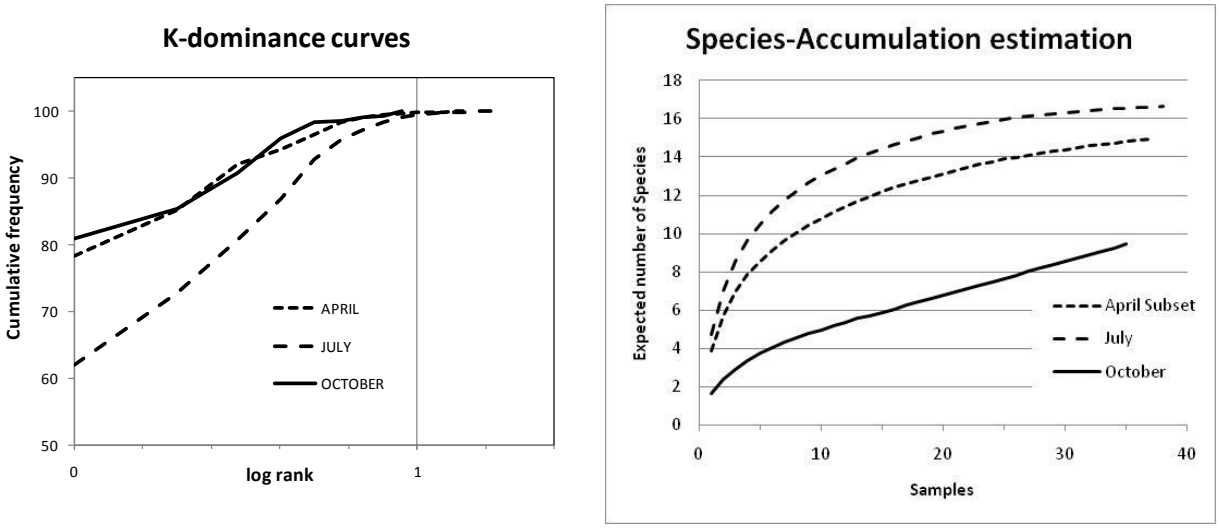


Figure 3. K dominance curves (A). Cumulative species estimation over samples by survey month (B).

3.2 Larval assemblages

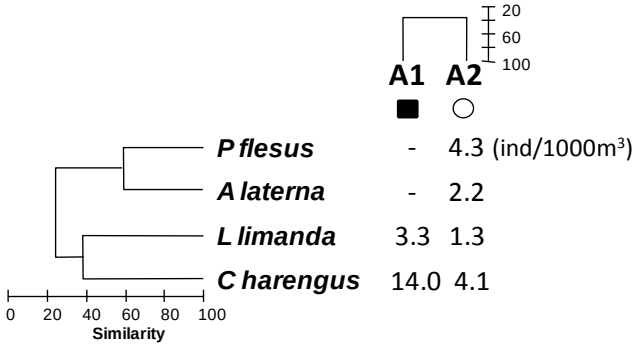
Two and four distinct larval assemblages were found in April and July, respectively. Only one assemblage was found in October (SIMPROF test). The overall similarity of assemblages ranged from 32 to 66% (Figure 4). For the April cruise, the species composition follows a statistically significant north-south spatial distribution (PERMANOVA, p<0.05). The assemblage A2 was clearly associated with shallow nearshore areas to the north of the sample domain (Figure 4A). For the July cruise no particular spatial trend was statistically significant, however assemblages tend to dominate in certain geographical zones (Figure 4B). Notably, J1 and J4 assemblages showed little spatial overlapping. Despite the inconclusive spatial segregation between assemblages in July the differences in average length and length distributions for individuals of the same species assigned to different assemblages were statistically significant for all species where the test could be performed (Table 3). In contrast, in April, only flounder (*P. flesus*) showed mean size difference (K-W test) although no significant differences in length frequency distributions between the two assemblages were found (K-S test) (Table 3).

Additional insight on the composition of the larval assemblages can be gathered directly from the RDA graphical output (Figures 5 and 6). The species vectors pointing and/or superimposing sample clusters indicate the species relevance to the clusters. The association of flatfishes and herring with the April cruise, a more varied assemblage of pelagic species (*P. minutus* and *S. rostellatus*, *T. trachurus*, and clupeioids) in July and a comparatively lack of species for October with only *A. minuta*'s vector associated with this last cruise of the season is therefore evident. Similar species associations can be derived with respect to the environmental variables in the partial RDA graphical output. Deeper more

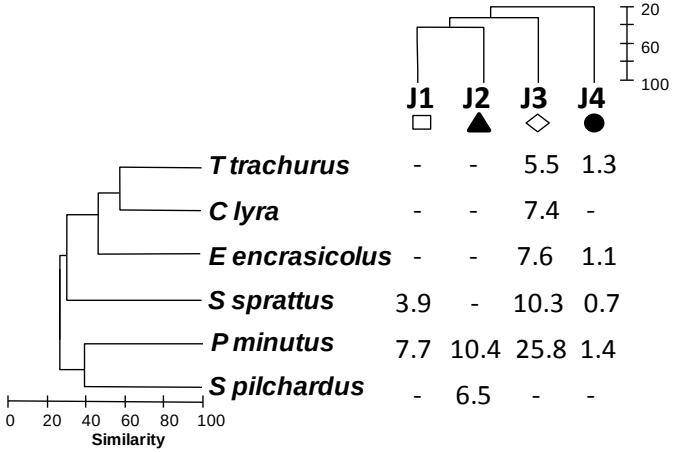
saline and clearer waters are associated with *S. sprattus*, *T. trachurus*, *C. lyra* and *B. luteum*, while inshore, chlorophyll rich and more turbid coastal waters are associated with locally produced flatfishes larvae (*A. laterna*, *P. flesus* and *S. solea*)

Figure 4. (Opposite page) Results for larval assemblage composition and stations clusters by survey month and sampling station. The values given represent abundances (ind.1000m^{-3}). Cluster analysis was done using the Bray-Curtis similarity coefficients and group average linkage. The species contribution to each assemblage was derived from SIMPER analysis. Only species contributing more than 90% to the assemblage identity are given for April and July. Due to the large dominance of *P. minutus* larvae, a larger 99% contribution threshold is presented for the October assemblage.

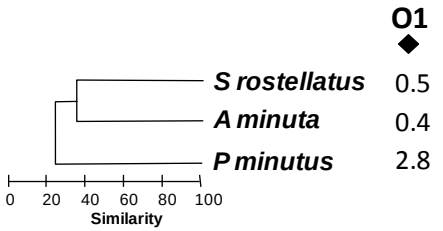
April



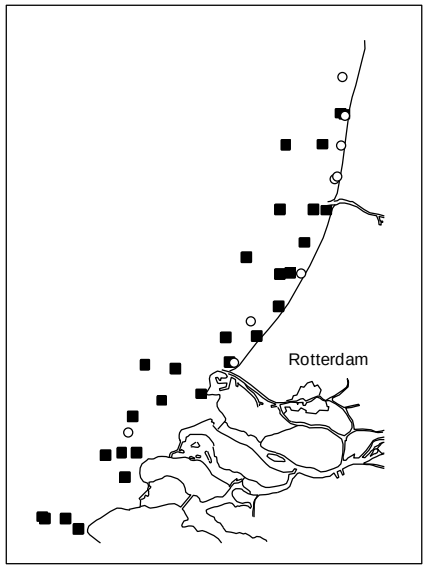
July

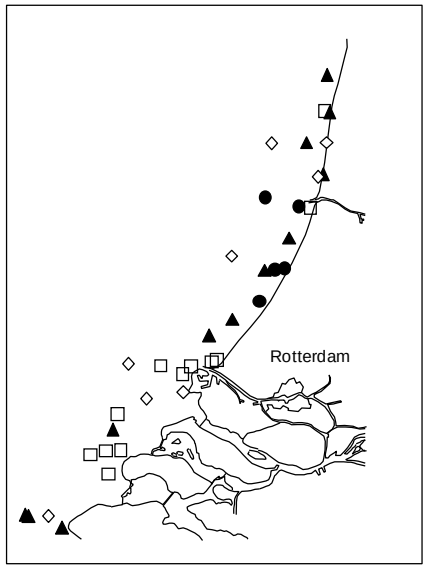


October



Note: X indicate stations where no larvae was found.





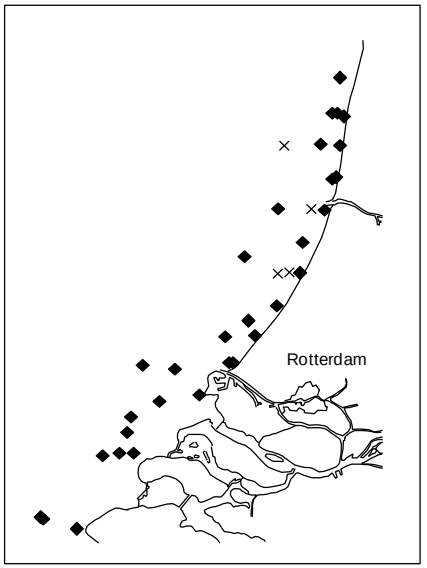


Table 3. Mean lengths ± standard deviation of larvae by species and assemblage identity for each of the three sampling cruises. Sample size is given in parentheses. Statistical differences between mean lengths are given (Kruskal-Wallis One-Way Analysis of Variance), ns, non significant. Superscripts indicate significant differences in length distributions between assemblages (Kolmogorov-Smirnov Two Sample Test), assemblages sharing the same superscript were not significantly different (groups without subscripts were not included in the analysis).

APRIL	Assemblage A1	Assemblage A2	*p*-value
C. harengus	22.5±4.6 (972) [1]	23.9±5.1 (44) [1]	ns
L. limanda	11.3±1.8 (258) [1]	11.7±1.6 (25) [1]	ns
P. flesus	7.0±1.0 (74) [1]	7.3±0.8 (110) [1]	0.03
A. laterna	7.7±0.9 (19) [1]	7.3±0.8 (34) [1]	ns
S. solea	8.4±1.5 (40)	8.4±1.7 (9)	-
A. marinus	20.5±3.75 (39)	22.5±6.0 (2)	-
P. platessa	8.6±1.5 (21)	9.1±1.7 (5)	-
P. gunnellus	30.6±0.5 (3)	29.8	-
S. sprattus	15.5±2.5 (13)	-	-
M. merlangus	13.3±2.2 (11)	-	-
L. liparis	15.4±1.6 (4)	-	-
M. variegatus	7.3±1.0 (2)	-	-
A. minuta	30.5	-	-

OCTOBER	Assemblage O1
P. minutus	13.1±3.2 (222)
S. rostellatus	24.2±10.3 (17)
A. minuta	24.0±2.8 (6)
S. sprattus	27.0±8.1 (6)
B. gattorugine	10.8±6.0 (2)
P. pictus	19.3

JULY	Assemblage J1	Assemblage J2	Assemblage J3	Assemblage J4	*p*-value
P. minutus	14.1±5.0 (237)[1]	13.1±3.4 (265)[1]	10.9±3.4 (220)[2]	11.0±3.4 (19)[1,2]	<0.01
S. sprattus	18.0±5.1 (128)[1]	-	13.9±4.8 (127)[2]	16.1±2.8 (10)	<0.01
S. pilchardus	-	18.0±5.2 (190)[1]	14.7±2.4 (36)[2]	-	<0.01
E. encrasicolus	16.9±2.1 (8)[1]	13.9±3.4 (47)[2]	13.0±4.0 (110)[2]	12.6±2.8 (21)[2]	<0.01
T. trachurus	4.4	4.5±0.8 (5)	5.3±2.1 (92)[1]	8.7±3.0 (29)[2]	<0.01
C. lyra	-	3.6±0.5 (4)	4.1±1.0 (97)	5.0±0.7 (2)	-
S. rostellatus	22.7±7.1 (34)[1]	25.9±9.0 (23)[1,2]	19.4±8.0 (20)[2]	15.0±3.8 (2)	0.02
B. luteum	6.5	4.4	4.8±1.2 (78)	4.3±0.9 (2)	-
A. minuta	12.1±2.4 (51)	-	-	9.7±2.4 (4)	-
H. lanceolatus	39.1	25.2±4.3 (10)[1]	18.7±6.3 (31)[2]	23.5±0.2 (3)	<0.01
C. harengus	14.9±5.0 (29)	-	17.1±1.9 (6)	-	-
E. vipera	-	-	3.9±0.8 (22)	4.7	-
B. gattorugine	14.0±2.6 (2)	18.6	5.3±0.7 (3)	-	-
A. laterna	7.9	-	5.9±2.4 (16)	-	-
P. maxima	12.7	-	-	-	-
D. labrax	11.7	10.7	6.7±5.6 (2)	-	-

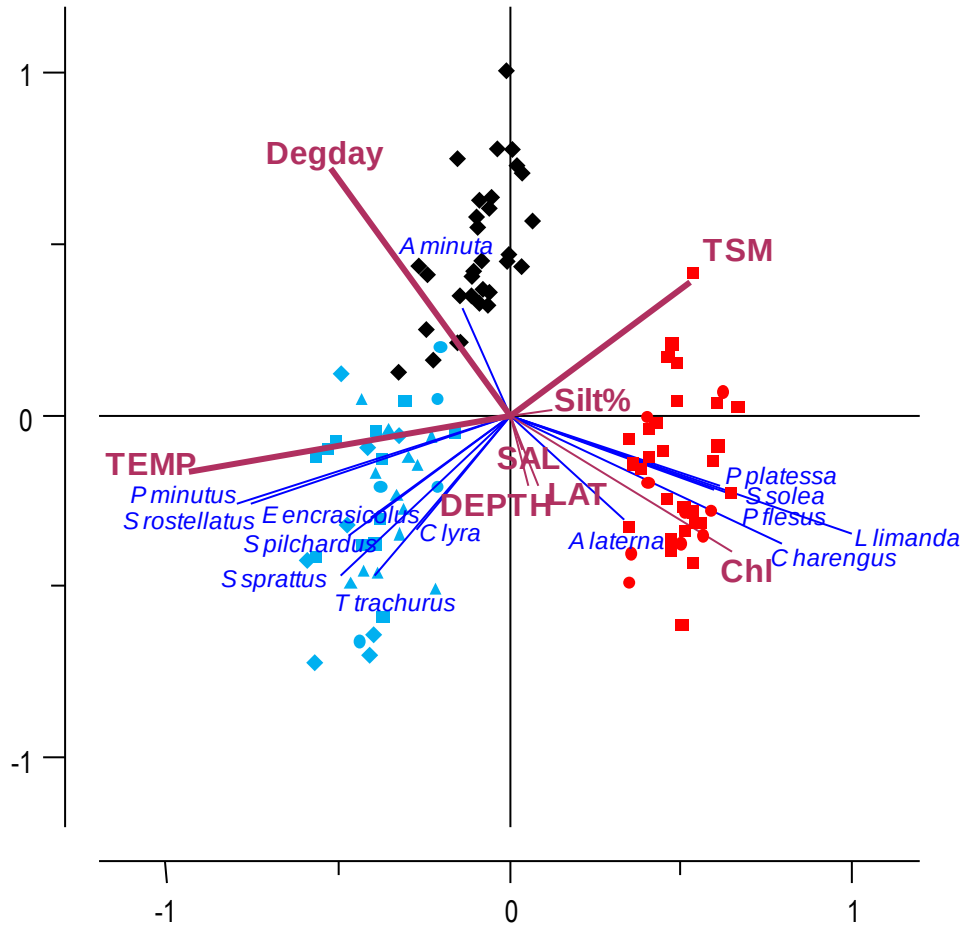


Figure 5. RDA for all cruises. The total sum of eigenvalues is 0.342 and the total variance is 1. The horizontal axis explains 63% of the variability and the vertical axis 18%. Red indicates April, blue July and black October cruises, respectively. The symbols indicate the assemblage identity assigned to the station in the cluster analysis as presented in Figure 4. A brief note on the interpretation on the RDA graphical output is given in appendix 2.

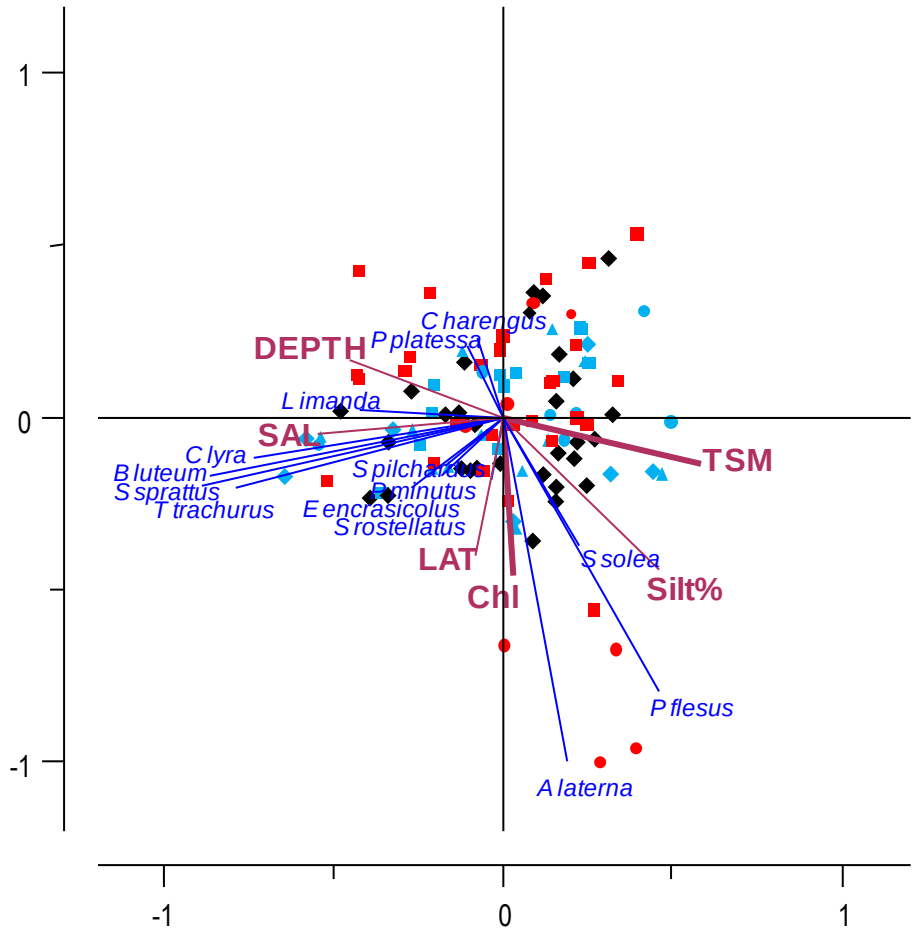


Figure 6. Partial RDA for all cruises. Degree day and temperature were used as covariates. The total sum of eigenvalues is 0.095 and the total variance is 1. The horizontal axis explains 47% of the variability and the vertical axis 30%. Sample labels follow the style given in Figure 5.

3.3 Environmental factors

Clear seasonal differences in larval assemblages (PERMANOVA $p<0.0001$, 9921 unique permutations) were found. Differences in larval assemblages across the area (north-south) were also significant in April, PERMANOVA $p<0.02$ (999 unique permutations), but not in July or October. However, no differences across depth areas were significant at any cruise.

Temperature gradients between cruises were much larger than within cruise variability and were clearly driven by seasonal changes. Since larval fish assemblages are only indirectly affected by temperature through effects on hydrological structural features (i.e. fronts, currents, etc) we included degree-day (thermal sums starting in January 1st 2007) in the RDA analysis to account for mainly seasonal effects. Temperature was the single best explanatory variable, followed by Chlorophyll-a, degree day (or cruise) and TSM (Table 4 and Figure 5). Forward selection using temperature as the first variable (55.4% of the variance is related to temperature) also indicated that degree day, TSM and Chlorophyll-a concentration are significantly related to the composition of the assemblages (total

explained variance 84.5%) (Table 5). The inclusion of degree day as the second largest explanatory variable (14.6% of the variance) in the model together with the functional correlation between temperature and season indicates the overwhelming influence of time of year in the composition of the larval fish assemblage (70% explained variance) (Figure 5). Partial RDA analysis using temperature and degree day as covariates (to remove the seasonal effect) resulted in a very low variance (9.5 %) related to the remaining environmental variables (Table 6 and Figure 6). Forward selection using TSM as the first variable identified Chlorophyll-a concentration as the second significant (p<0.05) variable in the partial model (5.4% of the variance). The analysis indicates that depth and latitude, although not significant (p=0.059), may also be relevant in structuring the larval assemblages (Table 7).

Table 4. Redundancy analysis (RDA) showing marginal effects for the combined cruise dataset showing explanatory variables ranked in order of importance. The total sum of eigenvalues is 0.342 and the total variance (inertia) is 1. The variable Degree day was added to account for the seasonal effect in the assemblage composition.

Explanatory variable		Eigenvalue Using Only One Explanatory Variable	Eigenvalue % using all variables
Temperature	TEMP	0.19	56.69
Chlorophyll-*a*	Chl	0.11	31.35
Total Suspended Matter	TSM	0.09	26.26
Degree day	Degday	0.09	25.72
Station Depth	DEPTH	0.02	5.32
Silt % seabed	Silt%	0.02	4.77
Salinity	SAL	0.01	3.42
Latitude	LAT	0.01	3.02

Table 5. Conditional effects for the combined cruise dataset. The total sum of eigenvalues is 0.342 and the total variance is 1.

Order	Explanatory variable	Increased Total Sum Eigenvalues After Adding New Variable	*F*-statistic	*p*-value
1	**Temperature**	0.19	**24.854**	**0.001**
2	**Degree day**	0.05	**7.32**	**0.001**
3	**Total Suspended Matter**	0.03	**3.934**	**0.001**
4	**Chlorophyll-*a***	0.02	**2.295**	**0.045**
5	Station Depth	0.01	1.987	0.055
6	Latitude	0.01	2.035	0.066
7	Salinity	0.01	1.71	0.106
8	Silt % seabed	0.01	1.516	0.17

Table 6. Partial RDA showing marginal effects for the combined cruise dataset showing explanatory variables ranked in order of importance. The total sum of eigenvalues is 0.095 and the total variance is 1.

Explanatory variable		Eigenvalue Using Only One Explanatory Variable	Eigenvalue % using all variables
Total Suspended Matter	TSM	0.03	29.8
Station Depth	DEPTH	0.02	19.54
Salinity	SAL	0.02	18.21
Chlorophyll-a	Chl	0.02	17.74
Silt % seabed	Silt%	0.02	17.72
Latitude	LAT	0.01	10.41

Table 7. Partial RDA conditional effects for the combined cruise dataset. The total sum of eigenvalues is 0.095 and the total variance is 1.

Order	Explanatory variable	Increased Total Sum Eigenvalues After Adding New Variable	*F*-statistic	*p*-value
1	**Total Suspended Matter**	**0.03**	**3.934**	**0.003**
2	**Chlorophyll-*a***	**0.02**	**2.295**	**0.034**
3	Station Depth	0.01	1.987	0.059
4	Latitude	0.01	2.035	0.059
5	Salinity	0.01	1.71	0.093
6	Silt % seabed	0.01	1.516	0.156

3.4 Stomach analysis

Larvae of ten fish species were processed for stomach contents. The three most abundant species were originally targeted during each survey month. These were lesser sand-eel (*Ammodytes marinus*), herring (*Clupea harengus*) and dab (*Limanda limanda*) in April; dragonet (*Callionymus lyra*), sand goby (*Pomatoschistus minutus*) and sprat (*Sprattus sprattus*) in July; and transparent goby (*Aphia minuta*), sand goby (*Pomatoschistus minutus*) and lesser pipefish (*Syngnathus rostellatus*) in October. Due to preservation artefacts and availability of the transparent goby, a fourth species, sprat (*Sprattus sprattus*), was also analysed in October (Table 8).

Feeding incidence and number of prey items per stomach varied across species. Most species presented food in excess of 50% of the examined larvae. This trend was not shared by sprat (*Sprattus sprattus*) which had consistently low feeding incidence. The gut-fullness, indicated by the number of prey items, also showed variation across species. This may indicate particular feeding behaviour or faster digestion times. Largely, the main prey items for the larval assemblage as a whole were copepods. This prey item was found as adult

forms, nauplii and copepodite larvae. The distribution of copepod families probably reflects seasonal abundance and ecological preferences of the larvae. The second prey item, in order of occurrence, was Cumaceans. Remaining prey items were recorded occasionally indicating only an opportunistic mode of feeding. Neither silt nor sand were found in the stomachs.

Table 8. Feeding incidence and average number of food items by key species and survey month. Larvae with empty stomachs were not included in the calculation of the average values presented. Copepoda, amphipoda and cumacean prey items are given to family level where available. Identifications of remaining prey item were only possible at a higher taxonomical level as only larval stages or incomplete animals were found. Taxonomical ranking follow the classification given in the Integrated Taxonomic Information System (ITIS) (http://www.itis.gov/info.html).

	April			**July**			**October**			
	A. marinus	*C. harengus*	*L. limanda*	*C. lyra*	*P. minutus*	*S. sprattus*	*A. minuta*	*P. minutus*	*S. sprattus*	*S. rostellatus*
Larvae examined	12	12	12	12	12	12	7*	12	12	12
% stomachs with prey	58.3	58.3	58.3	91.7	75.0	25.0	100	91.7	8.3	83.3
Copepoda Shrimp	**3.14**	**32.86**	**10.43**	**6.45**	**2.67**	**5.67**	**3.14**	**7.45**	**2.00**	**22.20**
Unidentified Copepoda	0.86	14.00	0.71	4.09	0.22	2.67	0.43	0.18	1.00	5.80
Order Calanoida	-	-	-	-	-	-	-	-	-	-
Family Centropagidae	-	-	-	-	-	0.33	0.43	0.18	-	0.40
Family Temoridae	1.43	18.86	9.71	1.45	2.44	0.33	2.14	6.55	-	0.30
Family Calanidae	-	-	-	0.18	0.00	0.00	0.14	0.00	-	11.10
Order Harpacticoida	0.86	-	-	0.73	0.00	2.33	0.00	0.55	1.00	4.60
Amphipoda Shrimp	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.09**	**-**	**-**
Family Hyalidae	-	-	-	-	-	-	-	0.09	-	-
Cumacean Shrimp	**-**	**-**	**0.14**	**-**	**0.22**	**-**	**0.14**	**0.09**	**-**	**-**
Family Pseudocumatidae	-	-	0.14	-	0.22	-	0.14	0.09	-	-
Crab larvae	**-**	**-**	**-**	**-**	**0.11**	**-**	**-**	**-**	**-**	**-**
Class Malacostraca	-	-	-	-	0.11	-	-	-	-	-
Barnacle larvae	**-**	**-**	**-**	**-**	**0.22**	**-**	**-**	**-**	**-**	**-**
Superfamily Balanoidea	-	-	-	-	0.22	-	-	-	-	-
Clam larvae	**0.29**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Class Bivalvia	0.29	-	-	-	-	-	-	-	-	-
Squid larvae	**-**	**-**	**-**	**0.09**	**-**	**-**	**-**	**-**	**-**	**-**
Class Cephalopoda	-	-	-	0.09	-	-	-	-	-	-
Unidentified	**-**	**-**	**0.14**	**-**	**0.11**	**-**	**-**	**-**	**-**	**-**

4. DISCUSSION

The larval assemblages were found to contain mainly large larvae. The MIK sampling net used in this study is very efficient on middle to late larval stages but less so on early, therefore smaller, larva (Munk, 1988). By the time of their capture these late larval stages have been exposed to, and therefore able to integrate, environmental forcing factors affecting larval abundance (i.e. dispersion routes, predation, food availability, etc). This potential ability to integrate physical and biological quality aspects is fundamental to ecological monitoring (USEPA, 2000). Moreover, larger larval size gives more confidence in larval identifications (Russell, 1976). The results of our study show that the majority of larvae sampled are common coastal fish species and similar in composition to assemblages previously described in the area (Russell, 1976; Beyst *et al.,* 1999; Grioche *et al.*, 1999; Grioche *et al.,* 2001; Munk and Nielsen, 2005). This consistency suggests that larval fish assemblages are suitable to evaluate impacts if we assume that they present a reliable integrator of overall habitat quality from food to the physicochemical environment (USEPA, 2000). The task is to identify relevant and sensitive metrics within a realistic degree of sampling effort.

In almost all practical situations monitoring programs are bound by time and budgetary constraints but at the same time need to deliver a precise and robust evaluation, which is only achieved when there is enough replication (which raises statistical power) in the sampling design (USEPA, 2000). We estimated the minimum sample size to ensure a consistent pattern in the larval fish assemblage collected in April at approximately 33 samples. The three most abundant species (*C. harengus*, *P. flesus* and *L. limanda*) were identical in both the subset of 33 samples and the full complement of 94 available samples. Moreover there was little difference in the ranking of the rest of the species found in terms of abundance (Table 1). However, there are important differences between the two datasets in regard to rare species. Importantly the five least abundant species in the 94-sample April dataset were not found in the subset of 33 samples and the proportion of species found at less than 5% of the stations is much larger (10 species out of 18) in the 94-sample dataset than in the reduced subset (1 species out of 13). The direct effect of effort on diversity estimates is well known in ecology (Magurran, 2004; Clarke and Gorley, 2006; Zuur *et al.*, 2010). The inclusion of more samples increases the likelihood of sampling rare species leading to increased diversity estimates, but results in a relatively minor contribution to determinations of overall abundance and large variability (i.e. noise) in the abundance estimates of these rare species. Robust indicators are essential in ecological assessments and must represent an unbiased view of the ecological component under assessment (USEPA, 2000). Our analysis disregards rare species and further transforms raw abundances to downweight the contribution of dominant species, practices that are common in multivariate community analysis (Leps and Smilauer, 2003; Clarke and Gorley, 2006; Zuur *et al.,* 2010). The intention is to: (1) concentrate on meaningful signals that can be reliably assessed at the level of effort used in this study and (2) devise a baseline that will be less sensitive to large interannual variability in larval fish abundance. The dominance and species accumulation curves indicate that the level of effort together with the analysis focusing on species well represented in the dataset were adequate to derive meaningful conclusions about the seasonal and spatial patterns of fish assemblages in the area of study. It is of note that rarity does not imply a lack of importance since rare species could be very important in the identification of assemblages (Miller, 2002) but the information they provide is only robust at a larger effort level, and therefore not considered here.

A seasonal pattern in assemblage composition was clearly detected in the community analysis. This seasonal structure is probably only indirectly related to recorded environmental gradients (i.e. temperature, salinity, Chlorophyll-a, total suspended matter etc) as the effect is more likely to be linked to differences in spawning season, location of spawning grounds and hydrological processes (i.e. fronts, water masses, currents, etc.) (Miller, 2002; Grioche *et al.*, 1999; Lee *et al.*, 2005; Brodeur *et al.,* 2008; Erftemeijer *et al.* 2009; Olivar *et al.*, 2010). Temperature and photoperiod are powerful seasonal cues used extensively to control spawning season in captive fish (Shimizu, 2003) and explain seasonal effects in field studies (Gillet and Quetin, 2006; Genner *et al.*, 2010). Within the context of this baseline, degree day may be a potential good reference to account for the effects of temperature on spawning seasonality. The low correlation between Chlorophyll-a and TSM and species gradients in the partial analysis (i.e. after removing the seasonal effect by including degree day and temperature as covariates) may be a reflection of the overall homogeneous environmental conditions within the survey cruises. Alternatively, the low correlations may reflect that there is hardly any effect of Chlorophyll-a and TSM on the species abundances in the assemblages. Larval fish have limited capacity to control their dispersion pathways unless aided by currents. Therefore, their abundance and distribution are more likely influenced by the location of spawning grounds than small scale favourable environmental conditions as apparent in dispersion models for the area (Erftemeijer *et al.* 2009; Dickey-Collas *et al.*, 2009) and mortality patterns (Houde, 1987). It is of note that larval fish mortality rates are influenced by a number of factors, particularly the timing of spawning in relation to the abundance of their prey and predators (Cushing, 1990; Platt *et al.*, 2003). It is likely that any causal relationship is then somehow affected by a number of biotic relationships affecting the predictive value of the environmental variables (Houde, 1987). In any case, the explained variance is low, and hence the relevance and utility of the partial RDA model as a predictive tool for ecological evaluation is limited.

In general, the hydrological patterns known for the area and the appearance and distribution of larval assemblages can be linked together. The April assemblage is dominated by *C. harengus* larvae probably originating from spawning grounds to the south and offshore to the sampling area (Munk and Christensen, 1990; Dickey-Collas *et al.*, 2009) drifting into the area aided by the northerly flowing residual current (Dickey-Collas *et al.*, 2009). The rest of the April assemblages are notably restricted to flatfishes (*L. limanda, P. platessa, P. flesus, A. laterna and S. solea*) probably spawned locally and retained within coastal waters (Beyst *et al.*, 1999; Grioche *et al.*, 2001). The low contribution of *C. harengus* and relative dominance of *P. flesus and A. laterna* in assemblage A2, together with a direct association with increased Chlorophyll-a content and TSM, suggests a role for nearshore hydrological features in the retention of flatfish larvae. This effect may be also partially due to directed vertical movements in phase with favourable tidal currents (Grioche *et al.*, 2000). These effects may ultimately be the cause of the assemblage A2 preferential occurrence in very shallow coastal locations to the north of the sampling area while more offshore and southern locations are restricted to assemblage A1 which is dominated by *C. harengus* influx across the density-driven frontal system separating offshore and coastal water masses.

By the summer months (July cruise) a much more complex structure was apparent with larger diversity and four identifiable assemblages. The sand goby (*P. minutus*), an abundant estuarine and coastal species, was a significant component of all 4 assemblages. These larvae probably originated from local sand goby stocks that migrate to the shallow subtidal fringe of estuaries to spawn (Russell, 1976; Munk and Nielsen, 2005). Interestingly,

significant size differences between assemblages were found, suggesting larvae of different source or age. Significant size differences were also found in clupeids (*S. sprattus, E. encrasicolus and S. pilchardus*), Atlantic horse mackerel (*T. trachurus*), dragonet (*C. lyra*), and sandeel (*H. lanceolatus*) further confirming a spatially segregated complex pool of larvae (i.e. source or age). The source of these larvae is probably a mixture of local spawned (younger) and older larvae that have drifted into the survey area (ICES, 2001). This implies the existence of active coastal transport processes, larval behavioural adaptations, differential larval mortality or a combination of factors leading to the segregation of assemblages. In July however, the spatial distribution of the assemblages did not show any statistically significant pattern which hampers associating assemblages to environmental factors or oceanographic features. Further studies focusing on regional conditions will be necessary to relate the sources of larvae with assemblage formation and explanatory variables in the summer months. In clear contrast with the peak complexity of the summer assemblages, by October, fish larvae have largely completed metamorphosis and disappeared from the plankton with only sand goby (*P. minutus*) remaining in the samples (Russell, 1976).

Gray (1997) used larval fish field data in impact assessment but failed to detect effects suggesting that natural variability was larger than any possible signal resulting from the sewage discharge under study. Background inherent natural variability in larval assemblages has been linked to weather patterns (Brodeur *et al.*, 2008) and despite the efforts is still largely unpredictable. Even a seasonal baseline may not be effective to define a reference condition to estimate deviation scores for impact assessment if the natural variability is too large or the scale of impacts is small in comparison to the size and range of the larval assemblages (USEPA, 2000). In the context of the proposed Maasvlakte-2 reclamation project, computer simulations of the distribution of larval fish with or without the reclaimed land clearly suggest order-of-magnitude mismatch between larval fish distribution range (hundreds to thousand of kilometres) and extent of direct effects on the hydrodynamic field (tens of kilometres) suggesting that no overall impacts are likely (PMR, 2006; Erftemeijer *et al.*, 2009; Dickey-Collas *et al.*, 2009). The present baseline assessment together with historical data may prove valuable for follow up assessments. Cluster analysis and ordination based on similarity estimates can potentially detect whole assemblage responses to alterations in the larval transport routes particularly if the extent of the area under disturbance is known *a priori* and the larval assemblage extends beyond the boundaries of the impacted area. If the assemblages change or lose coherence it is likely that larval survival and recruitment will be affected. Future work could be devoted to the formulation of logistic regression models, using best explanatory variables to derive probability estimates for the larval assemblage baseline conditions.

An alternative approach to correlation with abiotic forcing variables which may prove effective would be to determine feeding incidence and the main prey types by key larval fish in a representative sub-sample. Larval fish are subjected to extremely large mortality rates mainly linked to predation and starvation (Houde, 1987; Cushing, 1990; Houde, 2002). Inappropriate levels of nutrition quickly cause starvation and death either directly (starvation) or indirectly by increased predation pressure on weakened individuals (Cushing, 1990). Therefore, stomach-fullness could be a sensitive indicator to gauge possible impacts on the larval population. Assuming that the prey field would be similar for all larval fish we found indication of directed feeding behaviours with definitive preferential prey types and feeding incidence. Copepoda especially from the order Calanoida, family Temoridae were the

dominant prey items found in the larvae examined with six other taxa, mostly meroplankton larvae, making up the rest of the food items. It should be noted that prey spectrum may be influenced by the composition of the zooplankton community available and as such feeding incidence alone may be a more reliable and easy to compute index. The limited sample size examined and variation in the sample source for the analysed larvae precludes a formal statistical analysis but there is indication of species-specific variability. Given that prey availability may fluctuate widely from year to year, it is suggested that the necessary reference for impact assessment using feeding data could be best gathered from larval fish sampled at control sites at the same time as areas under evaluation. Stomach-fullness could be used at higher spatial and temporal resolution than assemblage-based analysis which will be more likely to be affected by large scale hydrodynamic processes. Future research is needed to assess the validity and usefulness of the approach before this or any feeding or prey-related parameter could be developed into a useful index of environmental quality for environmental impact assessment.

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APPENDIX 1. RAW ABUNDANCE DATA BY CRUISE

April abundance data (No. 1000m^{-3})

Sample Number	Included in Subsample	*Ammodytes marinus*	*Anguilla anguilla*	*Aphia minuta*	*Arnoglossus laterna*	*Callionymus lyra*	*Clupea harengus*	*Dicentrarchus labrax*	*Hyperoplus lanceolatus*	*Limanda limanda*	*Liparis liparis*	*Merlangius merlangus*	*Microchirus variegatus*	*Pholis gunnellus*	*Platichthys flesus*	*Pleuronectes platessa*	*Pomatoschistus minutus*	*Scorpaenidae spp*	*Solea solea*	*Sprattus sprattus*
1	NO	0	0	0	0	0	105.75	0	0	2.1	0	0.54	0	0	0.5	0	0	0	0	0
2	YES	0	0	0	4.56	0	10.86	0	0	0.4	0	0	0	0	4.9	0	0	0	0	0
3	NO	0	0	0	134	0	98.62	0	0	0	0	0	0	0	690	9	0	0	0	0
4	NO	0	0	0	381	0	586.66	0	0	31	0	0	0	0	803	41	0	0	31	0
5	YES	0	0	0	47.1	0	37.71	0	0	0	0	0	0	0	113	0	0	0	9.4	0
6	YES	0	0	0	56.3	0	15.37	0	0	0	0	0	0	0	133	0	0	0	15	0
7	NO	0	0	0	0	0	72.97	0	0	6.6	0	0	0	0	23	7	0	0	0	0
8	NO	0	0	0	0	0	11.79	0	0	5.9	0	0	0	0	3.9	0	0	1.97	0	0
9	NO	22.6	0	0	0	0	113.10	0	0	5	0	0	0	0	0	0	0	0	0	0
10	NO	5.77	0	0	0	0	80.82	0	0	0	0	0	0	0	0	0	0	0	0	0
11	NO	1.88	0	0	0	0	26.35	0	0	0	0	0	0	0	0	2	0	0	0	0
12	NO	0	0	0	0	0	33.10	0	0	3.5	0	0	0	0	0	1	0	0	0	0
13	YES	1.09	0	0	1.09	0	259.92	0	0	3.3	0	1.09	0	1.1	0	0	0	0	1.1	0
14	NO	0	0.77	3.84	0.77	0	99.87	0	0	3.1	0	0	0	0	1.5	1	0	0	0.8	0
15	YES	2.19	0	0	2.19	0	28.52	0	0	2.2	0	0	0	2.2	15	7	0	0	0	0
16	YES	0	0	0	1.8	0	91.95	0	0	0	0	0	0	0	1.8	0	0	0	0	0
17	NO	0	0	0	0	0	36.10	0	0	0	0	4.51	0	0	9	2	0	0	0	0
18	NO	0	0	0	0	0	96.14	0	0	11	0	0	0	0	0	0	0	0	0	0
19	YES	0	0	0	0	0	458.71	0	0	19	0	0	0	0	0	9	0	0	14	0
20	YES	164	0	0	0	0	237.65	0	0	16	0	11.7	3.9	0	0	0	0	0	3.9	5.844
21	NO	71.5	0	0	0	0	387.27	0	1.49	0	0	0	0	0	0	0	0	0	1.5	0
22	YES	7.46	0	0	0	0	467.43	0	0	2.5	0	4.97	0	0	0	0	0	0	2.5	7.459
23	NO	0	0	0	0	0	28.56	0	0	0	0	3.57	0	0	0	0	0	0	3.6	0
24	NO	0	0	0	0	0	82.62	0	0	2.2	0	0	0	0	0	0	0	0	0	0
25	NO	0	0	0	0	0	319.69	0	0	3.3	0	0	0	0	0	0	0	0	6.6	0
28	YES	0	0	1.86	0	0	96.93	0	0	0	1.9	0	0	3.7	0	0	0	0	3.7	0
29	NO	0	0	0	0	0	74.61	0	0	0	0	0	0	0	2.9	1	0	0	0	0
30	NO	0	0	0	0	0	97.69	0	0	0	0	0	0	0	1.3	1	0	0	2.6	0
31	YES	0	0	0	0	0	90.52	0	0	0	0	0	0	0	0	0	0	0	0	0
32	YES	0	0	0	0	0	386.39	0	0	0	0	0	0	0	0	0	0	0	0	0
33	NO	1.96	0	0	0	0	58.66	0	0	0	0	0	0	0	0	0	0	0	0	13.69
34	YES	0	0	0	0	0	150.00	0	0	3.7	0	0	0	0	0	0	0	0	3.7	0

April abundance data

Sample Number	Included in Subsample	*Ammodytes marinus*	*Anguilla anguilla*	*Aphia minuta*	*Arnoglossus laterna*	*Callionymus lyra*	*Clupea harengus*	*Dicentrarchus labrax*	*Hyperoplus lanceolatus*	*Limanda limanda*	*Liparis liparis*	*Merlangius merlangus*	*Microchirus variegatus*	*Pholis gunnellus*	*Platichthys flesus*	*Pleuronectes platessa*	*Pomatoschistus minutus*	*Scorpaenidae spp*	*Solea solea*	*Sprattus sprattus*
35	YES	4.79	0	0	0	0	210.73	0	0	14	0	0	0	0	0	0	0	0	0	0
36	NO	0	0	0	0	0	309.92	0	0	3.5	0	0	0	0	0	0	0	0	0	0
39	NO	0	0	0	0	0	100.84	0	0	2.8	0	0	0	0	0	0	0	0	0	0
40	NO	0	0	0	0	3.09	730.12	0	0	3.1	0	0	0	0	0	0	0	0	3.1	0
41	YES	0	0	0	0	0	85.20	0	0	0	0	0	0	0	0	0	0	0	0	0
42	NO	3.63	0	0	0	0	338.47	0	0	6	0	1.21	0	0	0	1	0	0	0	0
43	NO	2.9	0	0	0	0	168.29	0	0	1.9	0	0	0	0	1	0	0	0	1	2.902
44	NO	70.9	0	0	0	0	498.77	0	0	52	0	2.73	0	0	2.7	3	0	0	0	0
45	NO	8.41	0	0	0	0	291.08	0	0	34	0	5.05	0	0	0	0	0	0	0	1.683
47	YES	0	0	0	0	0	411.17	0	0	29	0	0	0	0	2.2	0	0	0	3.2	0
48	NO	0	0	0	0	0	280.50	0	0	40	3.6	0	0	0	5.4	4	0	0	0	0
49	NO	0	3.91	0	0	0	429.84	0	0	27	3.9	0	0	0	47	0	0	0	20	0
50	YES	0	0	0	44.6	0	554.07	0	0	22	0	0	0	0	134	3	0	0	22	0
51	YES	1.07	0	0	4.28	0	13.93	0	0	2.1	0	0	0	0	65	2	0	0	4.3	0
52	NO	0	0	0	0	0	159.45	2.1	0	27	2.1	0	0	0	101	10	0	0	17	0
53	NO	0	0	0	0	0	215.15	0	0	43	0	0	0	0	11	4	0	0	3.6	0
54	YES	0	0	0	0	0	125.95	0	0	64	2	0	0	0	4	6	0	0	0	0
55	YES	0	0	0	0	0	13.71	0	0	0	0	0	0	0	5.9	0	0	0	0	0
56	NO	0	0	3.13	0	0	191.23	0	0	22	0	0	0	0	0	0	0	0	6.3	0
57	NO	0	0	0	0	0	483.82	0	0	38	4.2	0	0	0	13	0	4.21	0	8.4	0
58	YES	0	0	0	0	0	94.96	0	0	47	0	3.72	0	0	20	4	0	1.86	0	0
59	NO	0	0	0	0	0	687.42	0	0	137	0	0	0	0	16	0	0	0	0	0
60	NO	1.85	0	0	0	0	450.89	0	0	9.2	1.8	3.7	0	0	3.7	0	0	0	0	3.696
61	NO	2.47	0	0	0	0	297.94	0	0	11	0	0	0	0	1.2	0	0	0	1.2	1.236
62	YES	0	0	0	0	0	11.74	0	0	0	0	0	0	0	0	0	0	0	0	0
63	NO	0	0	0	0	0	74.71	0	0	34	0	0	0	0	15	11	0	0	0	0
64	NO	0	0	0	0	0	410.06	0	0	24	1.5	0	0	0	4.5	3	0	0	1.5	1.508
65	YES	0	0	0	0	0	227.96	0	0	31	1.6	0	0	0	1.6	0	0	0	0	0
66	YES	0	0	0	0	0	106.49	0	0	32	0	0	0	0	0	3	0	0	0	0
67	NO	0	0	0	0	0	539.35	0	0	45	0	0	0	0	0	0	0	0	0	0
68	YES	0	0	0	0	0	108.37	0	0	38	0	0	0	0	0	3	0	0	0	0
69	NO	0	0	0	6.92	0	484.14	0	0	21	0	0	0	0	14	0	0	0	28	0

April abundance data

Sample Number	Included in Subsample	*Ammodytes marinus*	*Anguilla anguilla*	*Aphia minuta*	*Arnoglossus laterna*	*Callionymus lyra*	*Clupea harengus*	*Dicentrarchus labrax*	*Hyperoplus lanceolatus*	*Limanda limanda*	*Liparis liparis*	*Merlangius merlangus*	*Microchirus variegatus*	*Pholis gunnellus*	*Platichthys flesus*	*Pleuronectes platessa*	*Pomatoschistus minutus*	*Scorpaenidae spp*	*Solea solea*	*Sprattus sprattus*
70	YES	0	0	0	0	0	9.70	0	0	9.7	0	0	0	0	0	0	0	0	4.9	0
71	NO	0	0	0	0	0	21.16	0	0	11	0	1.11	0	0	0	1	0	0	0	0
72	YES	0	0	0	0	0	122.77	0	0	75	0	3.07	0	0	6.1	5	0	0	0	0
73	YES	0	0	0	0	0	128.90	0	0	35	0	0	0	0	0	0	0	0	0	0
74	YES	0	0	0	3.51	0	149.12	0	0	0	0	0	0	0	14	5	0	0	12	0
75	NO	0	0	0	0	0	87.48	0	0	0	0	0	0	0	7.1	0	0	0	5.4	0
76	YES	0	0	0	0	0	89.98	0	0	13	0	0	1.34	0	13	0	0	0	11	0
77	NO	0	0	0	0	0	78.03	0	0	80	0	0	0	0	5.3	0	0	0	0	0
78	NO	0	0	0	0	0	0.00	0	0	22	0	0	0	0	0	0	0	0	0	0
79	NO	0	0	0	0	0	2.69	0	0	17	1.3	0	0	0	1.3	1	0	0	0	0
80	YES	0	0	0	0	0	196.95	0	0	0	0	0	0	0	0	0	0	0	0	0
82	NO	0	0	0	0	0	166.56	0	0	18	0	0	1.75	0	1.8	0	0	0	0	0
83	NO	0	0	0	0	0	317.65	0	0	131	0	0	0	0	0	5	0	0	0	0
84	NO	0	0	0	0	0	145.24	0	0	23	0	0	0	0	0	12	0	0	17	0
85	NO	0	0	0	0	0	304.71	0	0	25	0	0	0	0	0	5	0	0	15	0
86	YES	0	0	0	0	0	15.08	0	0	21	0	0	0	0	0	2	0	0	0	0
87	NO	0	0	0	0	0	17.41	0	0	9.1	0	0.83	0	0	0	0	0	0	0.8	0
88	NO	0	0	0	0	0	20.03	0	0	2.6	0	0.87	0	0	0	1	0	0	2.6	0
89	NO	0	0	0	0	0	15.82	0	0	5.3	0	0	0	0	0	0	0	0	0.9	0
90	NO	0	0	0	0	0	43.44	0	0	3	0	0	0	0	0.8	1	0	0	0	6.097
91	NO	0	0	0	0	0	5.94	0	0	4.2	0	0	0	0	1.2	1	0	0	0.6	1.781
92	NO	0	0	0	0	0	88.69	0	0	6.5	0	0	0	0	0	0	0	0	0	5.047
93	NO	0	0	0	0	0	41.68	0	0	13	0	0	0.83	0	0	0	0	0	0	10
94	NO	3.39	0	0	0	0	192.18	0	0	15	0	0	0	0	0.8	3	0	0	0	0
95	NO	0.86	0	0	0	0	70.55	0	0	22	0	0.86	0	0	2.6	5	0	0	0.9	2.581
96	NO	0	0	0	0	0	39.50	0	0	46	0	0	0	0	0	0	0	0	0	24.21
97	NO	0	0	0	0	0	72.49	0	0	99	3.1	0	0	0	0	5	0	0	3.1	13.88
98	NO	0	0	0	0	0	314.86	0	0	10	0	0	0	0	0	3	0	0	0	0
99	YES	0	0	0	0	0	343.82	0	0	5.8	1.9	0	0	0	0	2	0	0	17	0
100	NO	0	0	0	0	0	3.18	0	0	0	0	0	0	0	0	0	0	0	0	0

July abundance data

Sample Number	Aphia minuta	Arnoglossus laterna	Blennius gattorugine	Buglossidium luteum	Callionymus lyra	Callionymus sp.	Clupea harengus	Dicentrarchus labrax	Echiichthys vipera	Engraulis encrasicolus	Gobidae	Hyperoplus lanceolatu	Pomatoschistus minut	Psetta maxima	Sardina pilchardus	Sprattus sprattus	Syngnathus rostellatus	Trachurus trachurus
2	0	0	0	0	1.566	0	0	0	0	0	0	1.57	244	0	269	0	12.5	0
5	0	0	0	0	0	0	0	0	0	0	0	0	250	0	131	0	1.7	0
6	0	0	0	0	2.067	0	0	0	0	107	0	0	533	0	0	72.35	8.27	6.2
13	0	0	0	0.91	0	0	0	0	0.9	10.9	0	0	9.11	0	0	0	0.91	10
15	0	0	0	0	0	0	0	0	0	0	0	0	89.2	0	0	12.31	2.05	0
16	0	0	0	0	0	0	0	0	0	0	0	0	60.8	0	0	6.40	3.2	0
19	0	0	3.47	0	0	0	1.73	0	0	0	0	0	79.7	0	0	12.13	3.47	0
20	0	20.66	0	99.4	1012	0	0	2.58	27	114	0	6.45	258	0	0	157.50	3.87	106
22	0	0	0	0	0	0	0	0	0	4.38	0	1.46	58.4	0	0	8.76	0	0
26	0	0	0	0	0	0	0	1.49	0	0	0	0	234	0	0	26.87	5.97	0
27	0	0.519	0	0	0	0	7.26	0	0	0	0	0	50.8	0	0	4.67	4.67	0.52
28	0	0	0	0	0	0	0	0	0	0	0	0	210	0	8.18	0	13.6	0
29	0	0	0	0	0	0	0	0	0	0	0	0	1753	0	0	0	0	0
31	0	0	0.93	0	0.934	0	0	0	0	0	0	0	160	0	13.1	0	3.74	0
32	0	0	0	0	0	0	0	0	0	0	0	0	155	0	12.6	0	0	0
34	4.1725	0	0	0	0	0	0	0	0	0	0	0	33.4	0	0	64.67	6.26	0
35	0	0	0	0	0	0	0	0	0	3.16	0	0	18.9	0	0	7.57	0.63	0
41	0	0	0	17.8	62.31	0	0	0	3	475	0	0	819	0	142	0	14.8	29.7
47	0	0	0	0	0	0	0	0	0	3.92	0	0	20.2	0	12.1	0	0	0.6
51	0	0	0	0	0	0	0	0	0	20.5	0	0	28.9	0	15.8	0	1.86	0
54	0	0	0	0	0	0	0	0	0	4.07	0	1.36	9.49	0	8.13	0	0	2.71
55	0	0	0	0.42	0.831	0	0	0	0	0.42	0	0	1.66	0	0	0.42	0.83	0
58	0	0	0	0	0	0	0	0	0	0	0	0	35.4	0	21.2	0	0	0
62	0	0	0	0	0	11.4	0	0	0	85.4	0	0	188	0	0	11.39	22.8	11.4
65	0	0	0	0	0	0	0	0	0	0	0	19.1	380	0	65.9	0	4.25	2.12
66	0	5.59	0	81.1	58.74	0	0	0	2.8	75.5	0	92.3	280	0	0	548.22	5.59	81.1
68	0	0	0	0	0	0	0	0	0	0	0	0	75.1	0.87	0	0.87	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	119	0	55.2	0	4.25	0
70	0	0	0	1.89	0	0	0	0	0	20.8	1.89	0	0	0	60.6	0	5.68	0
72	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2.33	0	5.66
73	0	0	0	0	0	0	0	0	0	0	0	0	1.13	0	0	1.13	0	1.13
74	0	0	0	1.09	0	0	15.2	0	0	0	0	0	29.4	0	0	5.44	7.61	0
76	0	0	3.51	22.8	45.69	0	21.1	0	0.9	0	0	1.76	165	0	45.7	397.12	1.76	182
80	0	0	0	6.48	0	0	0	0	0	0	0	0	2826	0	0	207.39	6.48	0
86	0	0	0	0	1.202	0	0	0.6	0	0	0	0	171	0	66.1	0	0.6	0
92	0	0	0	0	0	0	0	0	0	0	0	0	25.8	0	0	11.71	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0	48.5	0	0	90.10	10.4	0
99	0.32	0	0	0	0	0	0	0	0	2.9	0	0	2.58	0	0	0	0	0

October abundance data

Sample Number	Aphia minuta	Blennius gattorugine	Engraulis encrasicolus	Gobidae	Pomatoschistus minutus	Pomatoschistus pictus	Sardina pilchardus	Sprattus sprattus	Syngnathus rostellatus
2	0	0	0	0.832	0	0	0	0	0
5	0	0	0	0	9.58	0	0	0	0
6	0	0	0	0	3.38	0	0	0	0
13	0	0	0	0	0	0	0	0	0
15	1.57	0	0	0	47.09	0	0	0	0
16	0	0	0	0	5.86	0	0	0	0
19	0	0	0	0	4.01	0	0	0	1.337
20	1.364	0	0	0	4.09	0	0	0.682	1.364
22	1.602	0	0	0	10.41	0	0	0	0.801
28	0	0	0	0	1.51	0	0	0	0
31	0	0	0	0	5.15	0	0	0	0
32	0	0	0	2.344	2.34	0.781	0	0	0
34	0	0	0	0	8.37	0	0	1.57	1.047
35	0	0	0	0.487	3.89	0	0	0	0.487
41	0	0	0	0	2.97	0	0	0	3.708
47	0	0	0	0	2.13	0	0	0	0
50	5.487	0	2.743	0	137.17	0	2.743	6.859	6.859
51	0	0	0	0	0.48	0	0	0	0
54	0	0	0	0	0	0	0	0	0
55	0	0	0	12.92	3.04	0	0	0	0
58	0	0	0	3.93	0.00	0	0	0	0.786
62	5.436	0	0	0	4.66	0	0	0	0
65	0	0	0	0	1.44	0	0	0	0.721
66	0	0	0	0	0	0	0	0	0
68	0.627	0	0	0	1.25	0	0	0	0
69	7.926	0	0	0.61	1.22	0	0	0	0
70	0	0	0	0	0.00	0	0	0	0
72	0	0	0	0	1.38	0	0	0.691	0
73	0	0	0	0	0	0	0	0	0
74	0	0	0	0	27.99	0	0	0	0
76	0	1.476	0	1.476	0.00	0	0	0	0.738
80	0	0	0	0.677	5.41	0	0	0	0.677
86	0	0	0	0	12.74	0	0	0.849	0
94	0	0	0	1.172	63.27	0	0	0	1.172
99	0	0	0	0	0.80	0	0	0	0.797

APPENDIX 2. RDA GRAPHICAL OUTPUT INTERPRETATION

This appendix in intended to aid in the interpretation of the RDA ordination diagrams (i.e. triplots) presented in this report (Figures 5 and 6). Further and more detailed explanation for this or related linear ordination methods can be found in Leps and Smilauer (2003) and Zuur *et al.* (2007). The contents of an RDA ordination diagram provide information on the relationships between sample scores, species and environmental variables. The samples are represented by symbols in a 2D space, and the species and the environmental variables by vectors. It is important to note that the interpretation of the RDA ordination diagrams must be done in relative terms since the absolute values associated to a particular object do not usually have any real meaning in the ordination space.

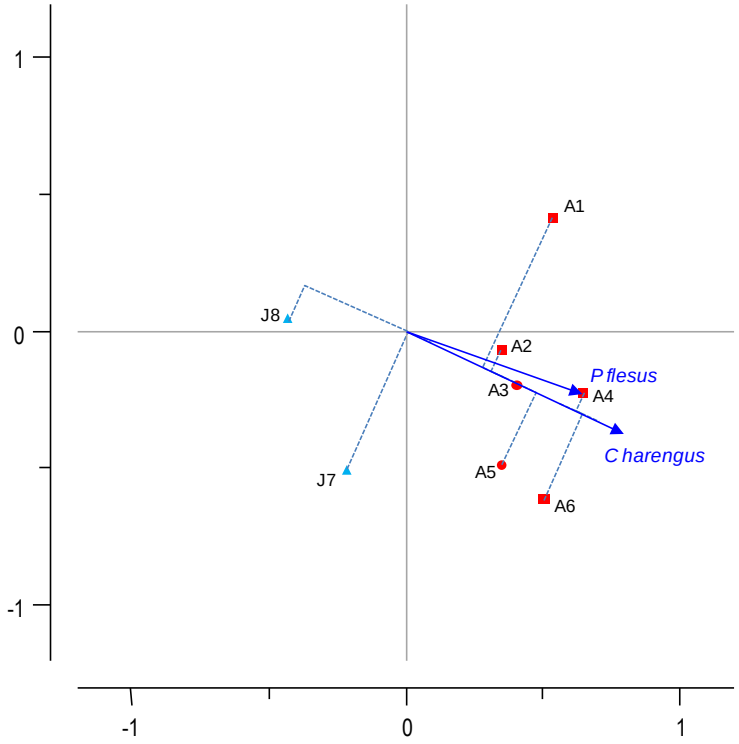

Figure A2-1. Prediction of the species abundances in samples

Projecting the sample points perpendicular to a species' arrow produces a prediction of the species contribution to the sample. Again this must be used in relative terms as the diagram used the fitted, not the observed, values of the abundances. The largest abundance of *C harengus* in April (red symbols) is predicted in samples A6 and A4 and then in decreasing order A5, A3, A2 and A1 (note that absolute distance to the vector is not relevant). With respect to *P flesus*, the order from largest to smaller contribution for April would be A4, A6, A5, A3, A2 and A1. A sample point whose projection reaches the origin of the coordinate system is predicted to have an average value of the corresponding species (i.e. J7) or those in the opposite direction a less than average, often zero, value (i.e. J8).

Similar interpretation is valid if the sample points are projected perpendicular to environmental variable vectors. In this case the order realized on the vector provides a

ranking of samples from largest (close to the vector tip) to smaller values of the variable.

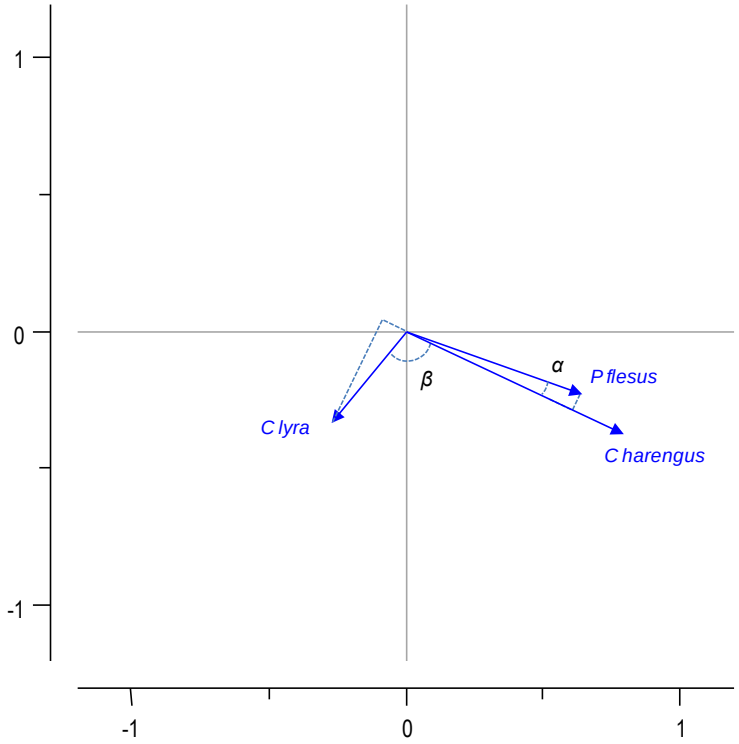

Figure A2-2. Prediction of level of correlation among species

Arrows pointing in the same direction correspond to species that are predicted to be positively correlated and therefore found together in a particular sample. Species vectors that meet at approximately right angles are predicted to have a low correlation. Therefore smaller angles, approaching zero, indicate species associations or assemblages. The correlations can also be estimated by projecting the arrow tips of the other species onto a particular species arrow. Following this interpretation we can predict that *C. harengus* and *P. flesus* are usually found together while *C. lyra* and *C. harengus* are almost, probably never, found in the same sample.

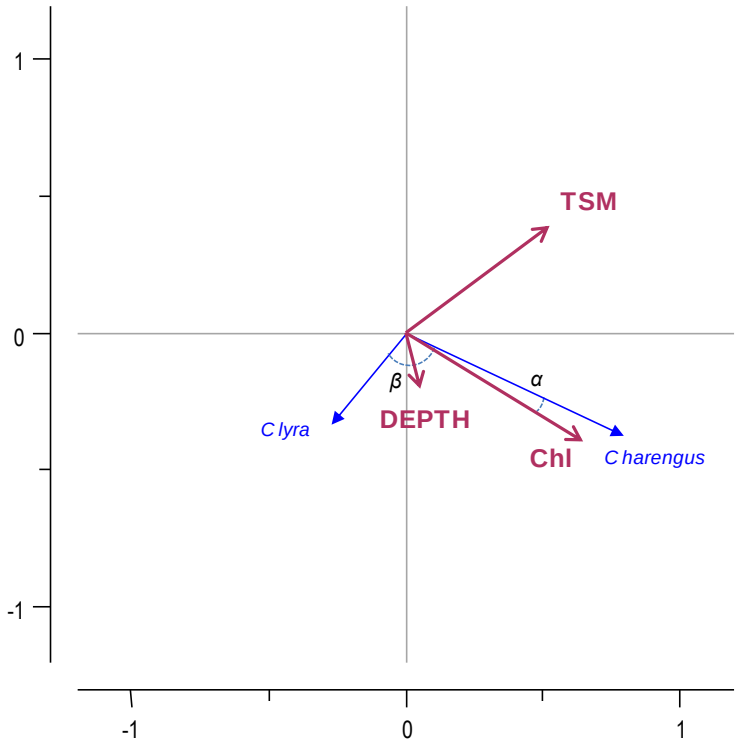

Figure A2-3. Prediction of level of correlation coefficients between species and environmental variables

We can use the same rules as described in Figure A2-2 to estimate relationships between species vectors – this can be used to visualize relationships between species and environmental variable vectors (or environmental vectors alone). While comparing the correlation of chlorophyll-a concentration (Chl) with the two species vectors presented in the figure above, we could use the angles to predict that Chl is not a relevant factor to explain *C. lyra* abundances (angle approximating 90º) while the same factor has a large positive correlation with *C. harengus* (the higher the Chl value, the higher the expected abundance of *C. harengus*). Many other relationships can be derived, for example *C. lyra* is negatively correlated with TSM and positively correlated with depth indication of the expected higher abundance of this species in deeper, less turbid waters.

The projection of the species arrow tips onto the vector of the environmental factor provides a similar yet more accurate approximation of the relationships (projections are not shown for clarity in Figure A2-3). Finally the length of the vector of environmental variables indicates the size of the effect and allows visualization of the more influential environmental factors. In the example provided the relative larger size of Chl and TSM vectors suggest that they have larger effect than DEPTH which is represented by a smaller vector.