Historic developments in macrozoobenthos of the Rhine-Meuse estuary: From a tidal inlet to a freshwater lake

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Abstract

Water works during the 1960s and 70s changed the northern part of the Rhine-Meuse estuary in the south-west of the Netherlands into a freshwater lake, from west to east divided into three basins called the Haringvliet, the Hollands Diep and the Biesbosch. Concurrently water quality parameters (e.g. nutrients and pollutants) changed drastically during the last 50 years. This study combines macrozoobenthic monitoring data from the region from 1960 to 2001 with trends in abiotic parameters to evaluate historic developments of the communities, including densities, species numbers and diversity, and assess future developments as a first step to a rehabilitation of the estuary as planned for January 01, 2008. During the 1960s, the macrozoobenthic densities of Oligochaeta and/or Polychaeta dominated communities increased with a gradual decrease of salt water intrusion and salinity variability. The first years after the basins became stagnant, the species numbers per sample and the Shannon diversity were high due to coexistence of salt and freshwater species. An increase in nutrient and pollutant loads led to a decrease in the macrozoobenthos densities. As water and sediment quality gradually improved, nowadays the former estuary contains high diversity high density macrozoobenthic communities, whereas Oligochaeta and/or Polychaeta were dominant in the 1960s, and Bivalvia and Gastropoda were more abundant during the 70s. Macrozoobenthic communities moved from the east to west with a time-lag, which may primarily be attributed to changing salinities, salinity variances and oxygen levels. Therefore, the current communities of the Haringvliet show similarities with the communities that occurred already during the 1960s in the Biesbosch. This study shows the value of macrozoobenthos monitoring data over longer periods. The possible impact of a new salt water inlet in the west of the Haringvliet, allowing in the near future salt water to enter 11.5 km eastward, yet alternated by frequent flushing with freshwater to ascertain that the salt intrusion does not reach further, on the development of the macrozoobentic communities is discussed.

Keywords: benthic macrofauna, density, diversity, long-term, abiotic changes, estuary closure, SW-Netherlands

1. Introduction

Following the flood disaster of 1953 in the delta of the rivers Rhine and Meuse in the Netherlands, drastic measures have been taken (Nienhuis and Smaal, 1994). Constructions were made to meet a certain safety level for the inhabitants of the delta, and to ascertain freshwater availability (Ferguson and Wolff, 1984). They comprised amongst others the construction of dams and weirs changing several of the Dutch estuarine inlets into fresh- and salt water lakes (Saeijs and Stortelder, 1982). The continuous salt-to-fresh-water gradient of the Rhine-Meuse estuary consisting of the basins Haringvliet, Hollands Diep and Biesbosch, between the North Sea (Haringvliet pre-delta) and the rivers, gradually changed into a freshwater lake, as construction works took several years. With the complete closure, salinity dropped rather sharply in large parts of the estuary (Peelen, 1970), but a further decrease went slowly (Tönis et al., 2002). From 1970 on the water movements in the Rhine-Meuse estuary were fully controlled with the completion of the 'Haringvliet dam' (Nienhuis et al., 2002). Simultaneously the tidal currents almost completely disappeared, resulting in increased sedimentation and altered water quality (Ferguson and Wolff, 1984; Smit et al., 1995). The composition of the incoming river water in terms of nutrient and pollutant loads did also drastically change during and after the completion of these infrastructures. Water quality firstly decreased until the eighties due to the inflow of polluted river water and subsequently improved again from the eighties as a result of enforced sanitation measures (Nienhuis et al., 2002; Bij de Vaate et al., 2006).

Long-term monitoring series of macrozoobenthos development in estuaries, and in particularly of communities in water bodies subject to drastic changes in nature, are scarce (Tapp et al., 1993; Mees et al., 1995). Especially for the Dutch delta, monitoring projects on macrobenthic communities were mostly of short-term as they were restricted to the period during and just after the water works (Lambeck, 1982; Meire et al., 1994; Seys et al., 1994)). Later studies were even more limited in time and space (e.g. Soetaert et al., 1994; Smit et al., 1995) and/or restricted to certain groups of organisms (e.g. Coosen et al., 1994; Bruyndoncx et al., 2002). However, transitional waters are widely recognized as regions with high productivity that perform several vital functions (e.g. nursery - and foraging areas, migration routes and habitats) for a wide range of species (Mees et al., 1995; Meire et al., 2005). Several of the species depending on these habitats are at threat (Masero, 2003; Meire et al., 2005; De Nooij et al., 2006). Macrozoobenthic species that are mostly not mentioned in the lists of endangered species, should not be overlooked in the monitoring of these areas as they represent important food sources for target species (Wolff and De Wolf, 1977; Lambeck et al., 1989), might be used as valuable indicators for the status of the environment (Warwick and Clarke, 1993, 1995; Occhipinti-Ambrogi et al., 2005) and many of them are of high economical value (Rueda et al., 2005).

The drastic changes in the Rhine-Meuse estuary must have had a huge impact on the macrozoobenthos communities, densities and diversity. The combination of macrozoobenthos data covering the spatio-temporal extent of this full scale management manipulation of a water system together with abiotic data might be valuable for determining the effects of the management, understanding the underlying processes, and predicting future developments for this area and other transitional waters. Getting more insight in the effects of the past management on the macrozoobenthic communities, has a high relevancy in relation to the restoration of the estuarine character of this water system by means of the regulated input of salt water through the inlet in the western part of the Haringvliet as planned for January 01, 2008 (Peijs, 2004). This regulated salt water inlet, meant to increase the nature values of the Haringvliet basin (Jacobs et al., 2003), is known as the installation of the 'Chink-regime' in the 'Haringvliet dam' (Burgers et al., 2004; Van Leeuwen et al., 2004).

The aims of this study were (1) to clarify historic changes in macrozoobenthos densities and diversity between 1959 and 2001 in the Rhine-Meuse estuary, in relation to the infrastructure works and environmental conditions; and (2) to discuss possible effects of future management on the ecosystem and more particularly on the macrozoobenthos in the area.

2. Material & methods

2.1. Study area

The study area is the main outlet of the rivers Rhine and Meuse in the Netherlands known as the Haringvliet, Hollands Diep and Biesbosch that are the remains of the historically brackish seaward part, the transitional mainly freshwater part, and the freshwater tidal area of the estuary, respectively (Fig. 1). Although 54 to 60 % of the Rhine water reaches the sea by a more northern route due to the digging of a shortcut from Rotterdam to the sea from the second half of the 19th century on (Peelen, 1974; Ferguson and Wolff, 1984), we will call the study area the Rhine-Meuse estuary further on. After the flood disaster of 1953, it was decided to turn the Rhine-Meuse estuary into a more or less stagnant freshwater lake. The historic overview given in Fig. 1, sums up the major changes in the area since the start of building the 'Haringvliet dam' from 1958 onwards. From this period on, the salt water intrusion in the Rhine-Meuse estuary was gradually blocked by infrastructure works. Between 1965 and 1970 the free connection between the Haringvliet and the North Sea was closed step by step. Already in 1969 the salt water intrusion from the southern delta was blocked by the 'Volkerak dam' leading to the nearly totally fresh becoming of half of the basin Haringvliet (Peelen, 1970).

From the 1990s on, visions on integral water management changed. Ideas were raised to restore natural estuarine conditions in the man-made freshwater bodies, however under regulated conditions to ensure freshwater intake possibilities for agriculture and drinking water. The plan was called 'tamed tide' (Van Leeuwen et al., 2004). As a first step, the establishment of the 'Chink-regime' for the sluices in the 'Haringvliet dam' is planned for January 01, 2008, which will lead to a regulated salt water inlet (Peijs, 2004). The regime will be such that salt water intrusion will not be allowed to cross the imaginary line 'Middelharnis – mouth Spui', which is approximately 11.5 km land inwards from the 'Haringvliet dam'. Therefore, sluices will be opened relative to river discharges, and more during low tide than during high tide. When very low river discharges are expected, there will only be outlet of water from the basins to the North Sea (Jacobs et al., 2003; Bavelaar and Ligtenberg, 2004). In 1997 a five days during experiment ('Chink-experiment') was executed as salt water was let in to measure and model the salt intrusion. The salt intrusion as a result of the experiment was indeed restricted to the western part of the Haringvliet (Jacobs et al., 2003).

2.2. Macrozoobenthos sample characteristics

From the period between 1959 and 2001, 1333 macrozoobenthos samples taken in the Rhine-Meuse estuary were available. The samples were distributed over the whole area (Fig. 1). Table 1 showed that there were differences in sample intensity between the basins and the years. Sample availability was concentrated around the 1960s and 70s. Additional information concerning the spatial distribution of these samples is given in Table 1. In this study, samples from parts of the surrounding rivers (Dordsche Kil, Nieuwe - and Boven Merwede, Amer, Maas, Waal, and Afgedamde – and Bergse Maas) were also reckoned to the Biesbosch area.

The samples were all taken during monitoring studies and surveys executed by researchers of the NIOO-CEME and the precursor of this institute. Far most samples were taken with a 0.1 m^2 'van Veen' grab, a few samples (6) were taken with a 'van Veen' grab of 0.093 m^2, a few samples were taken with corers (15 cm diameter) stuck straight in the sediment (12), or by corers (8 cm diameter) stuck in the bucket of a Reineck Boxcorer (40) in 3-fold. All samples were sieved through a 1 mm mesh and fixed with 4 % buffered formalin. Exceptions are the Boxcorer samples which were sieved through 0.5 mm mesh, and also stained with Bengal Rose. All data were stored in our local database BIS (Benthos Information System, database, version 1.20.0), and recalculated into densities per m^2 at the species level. The 40 samples taken with the Boxcorer were all taken in 2001 in the Haringvliet. It should be noticed that besides the mesh of the sieves, the most important difference between the used techniques is the sample depth, which is approximately (depending on the sediment cohesion) 15 cm for grab samples, and 25 to 30 cm for the corers. Although, the larger part of the animals lives in the topmost few centimeters (Wolff, 1973), densities and species numbers might be slightly underestimated in all years compared to 2001.

2.3. Abiotic data

For all macrozoobenthos samples the date and the NAP (Dutch Ordnance Level) corrected sampling depths were known. Sampling dates were aggregated into years and seasons (Winter = December – February; Spring = March – May; Summer = June – August; Autumn = September – November). Sampling depths were divided in the strata of 0 – 2, 2 – 5, 5 – 10 and >10 meters. The sampling sites were grouped according to the following sub-regions: Haringvliet (West, Central-West, Centrum, Central-East, East), Hollands Diep (West, East), and Biesbosch (Biesbosch East, Nieuwe Merwede, Beneden Merwede, Boven Merwede, Amer, Bergsche Maas, Dordsche Kil, Maas, Afgedamde Maas, Waal, and Biesbosch West) (Fig. 1).

Most abiotic data were extracted from Waterbase, the online database service from the Institute for Coastal and Marine Management from the Dutch Ministry of Public Works and Transportation (RWS-RIKZ) (Waterbase, 2006), in which data for our research area were available from 20 monitoring stations (positions indicated in Fig. 1). We did only extract variables for which sufficient data from the period 1959 – 2001 were available; these are at least 680 measurements, but generally more than 1000 measurements spread over the three basins (Fig. 2). In this way sufficient information was available to get insight in general trends of abiotic factors in the basins. The link between the abiotic data and the biotic data (for the regression analyses) was made at the level of the sub-regions where the monitoring stations were situated. The abiotic data obtained from each sub-region were averaged by year and season before analysis. Due to missing data in the abiotic measurements, a fraction of the biotic dataset was excluded prior to perform the multivariate analyses. The 1333 macrozoobenthos samples were therefore reduced to 458 samples with associated environmental characteristics. We also used the differences in biotic values between the strata for regression analyses, however, these were connected to the same abiotic values for the whole depth range as separate values for strata were not available.

Due to the *ad hoc* collection of information, the abiotic dataset suffered from several shortcomings. For some potentially highly relevant abiotic parameters (e.g. NO_3 and PAHs), not sufficient data were available for the detection of changes over the study area and for their connection with biotic information. The concentrations in metal ions were only available in sufficient amount for analysis from the surface water, whereas sediment concentrations are expected to be more relevant for the macrozoobenthic communities. The analytical precision for the abiotic measurements (too rough) did not always allow the detection of changes in concentrations against time (Waterbase, 2006). The salinity and salinity range data were compiled from chlorinity graphs given by Wolff (1973) and Bavelaar and Ligtenberg (2004), from chloride data given by Smit et al. (1995), unpublished chloride data from occasional measurements at macrozoobenthos sample sites, and conductivity data from Waterbase (2006), all recalculated into salinity values after Millero (1984). The salinity range was calculated per basin taking the maximum value minus the minimum value per year.

2.4. Data analysis

The abiotic parameters were plotted per basin over the research years to allow a visual inspection for possible trend-breaks. The trends that were detected were analyzed with linear regression models and compared for differences between basins using co-variance analysis with general linear models (Systat 11 for Windows).

Average macrozoobenthos densities (D in n m^{-2}), species numbers (S; number of species per sample) and percentage share of specimens per taxonomical class per year, season, stratum and/or sub-region were calculated (Microsoft Office Access 2003). It has to be noticed that the species numbers in the cored samples (with sampled surfaces of 0.030 or 0.035 m^2) might be slightly underestimated relatively to the other samples (sampled surfaces 0.1 or 0.093 m^2), however these are also exactly the samples taken to a larger depth. The Shannon diversity index (H') (Shannon and Weaver, 1949) was calculated as a measure of species diversity taking the number of species and the balance in numbers between species into account (Primer 5.2.8 for Windows). Due to the high number of missing monitoring data over the years, no trend analysis could be performed on the densities, species numbers and diversity. The differences between the years were analyzed instead by using the non-parametric Kruskal-Wallis test. When significant differences were found, the differences between pairs of years were separately tested by means of ANOVA (Systat 11). In order to compensate for the artifact of multiple testing the Bonferroni correction was applied on the significance levels used for the test: the significance level for separate tested pairs was therefore $0.05/(n(n-1)/2)$, with n = number of tested groups/years per tests of the same context (Sokal and Rohlf, 1995).

To detect the relations between the environmental and the biotic variables and relations between each of the biotic variables, linear regressions were executed (after visual inspection using the SPLOM option in Systat 11) on the $\ln(x+1)$-transformed densities and species numbers data called D' and S' (species diversity data (H') are already log-transformed), including only those data where D and S are larger than 1, to exclude the low-density and low-species number samples. Relations between abiotic variables and the low-density and low species number samples are tested for with ANOVAs (Systat 11) comparing the 'low' and 'high' density, species number and diversity samples. A Shannon-diversity below 0.1 is considered 'low'. In principle, Bonferroni corrections on the multiple tests are taken into account, however as significance levels were very low (for the tests concerning the abiotic variables $p < 1.67*10^{-5}$), and Systat did not discriminate between levels smaller than 0.001, we regard these as significantly different. The significance levels of 0.05 and 0.01 are also shown as these might also indicate possible relations between abiotic variables. We also tested for possible effects of the sample date, grouped into seasons (winter, spring, autumn, summer), and sample depth, grouped into four strata (0-2m, 2-5m, 5-10m, >10m) on densities, species numbers and diversity using ANOVAs (Systat 11). Spatial effects on D, S and H' were tested for comparing the sub-regions using ANOVAs in Systat.

To detect changes in species composition and similarities in communities between basins and years, a Non-metric Multi-dimensional Scaling (MDS) was applied to Bray-Curtis similarity matrices calculated from the presence-absence species data per basin and year combination (Bray and Curtis, 1957). The quality of the representation obtained with 2D-plot created with the MDS analysis (Primer 5.2.8 for Windows) is indicated by the value of the so-called stress factor (potentially useful picture for values lower than 0.2) (Clarke and Warwick, 2001). Before MDS plots were made we checked for possible spatial differences within basins by ANOSIM analyses of the deviation in sub-regions applied to Bray-Curtis similarity matrices (Primer 5.2.8 for Windows), taking Bonferroni corrections into account.

3. Results

3.1. Developments in biotic variables

The macrozoobenthos densities in the Haringvliet were significantly higher in 1983 and 2001 than in the 1960s and 70s (Fig. 3). There was a trend towards lower densities between 1972 and 1977, than in the period before 1972, however no significant differences between these years were found. In general, the number of species and Shannon diversity increased during the research period, with the lower diversity indices in the 1960s and the higher values for 1971. Also in the Hollands Diep and Biesbosch high average densities were found in the 1960s with a trend of lower densities from 1973 to 1977, but the variance was large resulting in few significant differences (Fig. 3). The number of species and the Shannon diversity in the Hollands Diep were low in the 1960s and much higher from 1972 onwards. Higher species diversity was in the Biesbosch already found in 1965 and 1966.

Although periods of high densities and low species numbers and diversity (which are per definition positively related as H' is partly determined by S) were observed, e.g. during the 1960s and early 70s, overall densities and species numbers and diversity were positively related (Table 2a). Where generally samples were taken at various depths and dates each year, it has to be noticed that the number of species between the 0-2 meters was significantly higher than the number in the strata below 2 meters (Table 2b). This also accounted for the species diversity. The densities appeared to be highest in the 2-5 m stratum. Significant differences between seasons were found for the densities, which were higher in spring than in the other three seasons. When basins were tested separately (Fig. 4), in general we find the same trends as for the whole Rhine-Meuse estuary. The number of species, however, appeared to be significantly higher in summer than in autumn, and in both seasons higher than in winter, in the Haringvliet. Species diversity in the Haringvliet was higher in summer and autumn than in spring. Additionally in the Hollands Diep also significant differences in densities were found between the eastern and the western part with higher densities in the East, and higher species numbers and diversity were found in three sub-regions of the Biesbosch than in some of the other sub-regions in this basin (results not shown).

3.2. Trends in the abiotic variables

As a consequence of the construction works in the area, the salinity level and – variability sharply decreased during the 1960s in the Rhine-Meuse estuary (Fig. 2). After 1970, when the salt water inlet was completely blocked, the salinity did not exceed the 2.4, and generally also not the 1.5. In the Biesbosch a slight increase in salinity was observed during the 1970s until halfway the 80s. Simultaneously with the closure of the 'Haringvliet dam', the tidal range, which was about 1.85 to 1.95 meters for the whole Haringvliet to Biesbosch area (Peelen, 1970; Wolff, 1973), sharply dropped to 50 centimeters or less (Peelen, 1974; Jacobs et al., 2003). The water temperature gradually increased with 1.6 to 2.4 ºC during the period 1959-2005 over the whole research area (Fig. 2), to which all seasons contributed. The oxygen content of the water decreased initially till the beginning of the 1970s, after which the oxygen level gradually increased again. It has to be noticed that till 1971 only data for the Hollands Diep and the Biesbosch were available (Fig. 2). Also the variability of the oxygen level is less large between approximately 1979 and 2005, than before 1979. The pH increased in the 1970s slightly over the whole range. The clarity (Secchi) of the water generally decreased during the 1970s, and increased again during the 1990s. The suspended matter content was highest before 1971, although it has to be noticed that only data from the Hollands Diep and the Biesbosch were available for that period. The chlorophyll a content appeared to be higher during the 1970s and early 1980s, after which it gradually decreased. DOC and TOC decreased during the 1970s till 1993/1994, after which TOC stabilized and DOC contents increased again in the whole estuary. The nutrient data before 1971 show an initial increase of the NH_4, total P and PO_4 concentrations during the 1960s, while the values for phosphate also increased in the 1970s. Silicate levels remained rather stable at a low level. For all metals (or metal pollutants) and As, a clear decrease (especially in the 1970s and 80s) in surface water concentrations was found between 1972/73 and 2005 (Fig. 2).

Due to their respective locations along the sea-river gradient the separate basins might show differences in the changes of their abiotic conditions against time. The abiotic conditions, however, generally followed similar trends in all the basins. But for instance changes in salinity were stronger closer to the sea in the Haringvliet and the Hollands Diep than in the Biesbosch. Whereas in the Haringvliet and the Hollands Diep the salinity and salinity variability dropped sharply between 1961 and 1969 after which it remained more or less stable, in the Biesbosch the salinity significantly increased till halfway the 1980s (the salinity variability till the end of the 70s) after which it decreased again. Where the clarity of the Haringvliet and Hollands Diep water decreased during the years 1971-1988, when the suspended matter content increased, these parameters were more or less stable in the Biesbosch during these years. After 1988 the clarity of the water improved and suspended matter content decreased in the Biesbosch, while in the Haringvliet and the Hollands Diep stabilization of these parameters was visible. Whereas clarity during the 1970s and 80s was highest in the Haringvliet, it is nowadays highest in the Biesbosch. Chlorophyll a content was especially high in the Hollands Diep and the Biesbosch at the end of the 1970s and beginning of the 80s, after which the contents gradually decreased to similar levels in the whole research area (Fig. 2). There was also a clear spatial gradient with increasing concentrations in metals from the Haringvliet to the Biesbosch. In time the spatial differences were however decreasing. For several metals the surface water concentrations are nowadays more or less similar within the whole estuary.

In summary we found for the abiotic factors in the studied area 1) in the 1960s some strong changes as lowering salinities and salinity variances (due to increasing enclosure of the area), decreasing suspended matter content, yet increasing ammonia concentrations (from polluted fresh water input), 2) in the 1970s a transition stage with slight changes as an increasing salinity in the Biesbosch and phosphate levels indicating poorer water quality, yet at the other hand decreasing ammonia and metal concentrations indicating improving water quality, and 3) more and more stabilizing values of most parameters with significantly improving water quality from the beginning of the 1980s.

3.3. Reaction of biotic variables to environmental changes

The shifts in the biotic characteristics might be related to the changes in abiotic conditions as a consequence of the construction works in the area. For the samples where more than an occasional species or specimen was observed (D and S >1), we see that the densities are significantly related ($p<0.001$) to several of the abiotic variables (Table 2c). Higher densities are especially related to lower As and metal (i.e. Cr, Pb and Cu) contents, and lower TOC and nutrient (i.e. P_{tot} and NH_4) contents, but to higher Fe and Silicate contents. The number of species is also positively related to the Fe content of the water, and negatively to the Ni contents. High diversity was more often observed in waters with higher oxygen contents and lower chlorophyll a contents. In search for which biotic variables determine the absence, or almost absence, of macrozoobenthos in our samples, it is found that none of the abiotic variables alone is responsible for very low densities, but the oxygen content connected to the low number of species and low diversity samples is significantly lower than for the other samples and at low species numbers the salinity and salinity variance is significantly higher (Table 2d).

Comparing the communities on basis of the species presence/absence we first checked for possible differences between sub-regions within basins (ANOSIM). Visually, especially the data for the Biesbosch are non-homogenous spatially distributed (Fig. 1; Table 1). Spatial intra-basin differences of communities appeared to be non-significant and much smaller than the differences between years (results not shown), which allowed us to aggregate. When the communities of the different years were compared using MDS (Fig. 5), some general patterns between the basins were observed. The lower pane shows the general trend for the three basins in time, which is however a smoothening of the patterns shown by measured data (upper pane). The communities of the 1960s of both basins, Haringvliet and Hollands Diep, are situated on the left side of the plot. In the 1970s the communities of these basins are moving to the right and lower middle part of the plot. The communities of 1983 of the two basins are almost identical in species presence, situated in the middle – lower middle part of the plot, after which the communities of the Haringvliet move to the upper right. The Biesbosch however shows a different pattern. It is striking that the communities in the beginning of the 1960s in the Biesbosch and the Hollands Diep were comparable, and that the communities of the second half of the 60s showed more similarities with the situation in the Haringvliet in 2001. The community of 1972 in the Biesbosch showed large similarities with those found in the other two basins in 1983. This means that the communities already present in the 1960s in the Biesbosch appeared with a time-lag in the more western Hollands Diep and Haringvliet. Positioning of the communities seemed to be most related to changes in salinity (in the MDS plot from bottom left to upper right) and suspended matter content (in the MDS plot from lower right to upper left).

Since the sole use of species presence/absence can mask certain patterns, as no difference was made in the densities of species, we also analyzed the shifts in density distributions between taxonomical classes. Figure 6 shows that the Oligochaeta and Polychaeta, dependent whether the environment was more salt or freshwater related, were highly dominant in densities in the communities of the 1960s, till 1971, of the Haringvliet and the Hollands Diep, and in 1963 in the Biesbosch. Only in the Haringvliet in 1960 and 61, the Bivalvia and the Crustacea appeared to be the dominant classes. After 1971, more and more other classes like the Bivalvia, the Insecta, the Gastropoda and the Crustacea became relatively more numerous. It is striking that this trend towards an increase in diversity changes in the second half of the 1970s, especially visible in the Hollands Diep in 1975, and in the Haringvliet in 1977, by a dominance of the Bivalvia. After that a more equal distribution of the densities over several taxonomical classes returned. In the Biesbosch a phase of dominance by Bivalvia and Crustacea is found halfway the 1960s, whereas more diverse communities could already be found in 1972.

4. Discussion

4.1. Effects of waterworks on macrozoobenthos

During the 1960s, a gradual reduction of the salt water inlet, and therewith the reduction of the salinity variance and the increase of the river (fresh-) water influence, led to an increase in the macrozoobenthos densities in the Hollands Diep and the Haringvliet. The communities were highly Oligochaeta and/or Polychaeta dominated at that time. With the completion of the blocking of the saltwater inlet in 1970, the water in the basins became more or less stagnant. Initially at the beginning of the 1970s salt and freshwater species could co-exist in the new stagnant water environment, probably due to horizontal and vertical stratification. This resulted in an increase of the species numbers and diversity. As the conditions became more stable, especially with respect to the salinity variability but also for other parameters, the waters became suitable for a wider range of species, gradually colonizing the Hollands Diep and the Haringvliet from the Biesbosch and from the surrounding polders which drain to the basins (Ferguson and Wolff, 1984). This led to a further increase of the species numbers and diversity, while the macrozoobenthos composition changed from Oligochaeta and/or Polychaeta dominated communities to more diverse communities with more Bivalvia, Gastropoda, Insecta and Crustacea. Simultaneously the densities decreased, as a consequence of lower Oligochaeta and Polychaeta numbers. It is very likely that this transition results in an increase in biomass. As during the 1970s large quantities of pollutants were imported with the river water and depositing in the Hollands Diep and Haringvliet basins, the densities remained low and also the species numbers and diversity decreased again during the second half of the 70s. Bivalvia appeared to dominate the communities. The slow and gradual colonization of the Hollands Diep and Haringvliet was also recorded by Ferguson and Wolff (1984), who characterized the benthic fauna seven years after the disconnection from the sea as generally still poorly developed. But a significant improvement of the water quality (e.g. nutrient and pollutant reduction and higher oxygen levels) during the 1980s led to the establishment of high density high diversity communities, already observable in 1983, but clearly present in 2001 in the Haringvliet.

Studying the northern delta area as a whole, or the three basins independently, the developments started since the 1960s could always be observed, first in the east (the Biesbosch) and than gradually moved to the west. Thus, species from the Biesbosch might gradually colonize the Hollands Diep and the Haringvliet. This corroborates the colonization pattern of non-indigenous species that since 1985 colonize the former estuary coming from the east (Bij de Vaate et al., 2006).

The results show that actually six phases in the macrozoobenthos development can be distinguished. The first phase, an estuary community of marine and brackish water species tolerant to large salinity variability, with relatively low densities and species numbers is actually only present in the Haringvliet during 1960 and 61. The early transitional (second) phase in the mid 1960s, at which the variability of environmental conditions gradually decreases, is characterized by slightly increasing densities and species numbers, where Oligochaeta and/or Polychaeta are dominating the communities, dependent whether it is a salt or freshwater related system. The transitional (third) phase (in the end 1960s) is actually a phase of more stable water conditions, in which the variability in water conditions is gone, and therefore densities of species already present, mainly Oligochaeta, get high. However, at high nutrient conditions in these stable waters, oxygen levels might decrease resulting in mortality. In the next fourth phase (in the beginning of the 1970s) still some ‘marine’ species are present (having opportunities to maintain due to horizontal and vertical stratification) together with freshwater species that gradually colonize the newly created freshwater basins. This results in increasing species numbers and diversity in the initial freshwater phase. As the water quality in terms of nutrients and pollutants was poor, and these substances increasingly accumulated to the bottom of the basins, densities and species numbers and diversity did not increase further, or even decreased during the freshwater poor quality (fifth) phase around the midst 1970s. By the improvement of the water quality, densities and species numbers and diversity gradually increased, and increased further when also the sediment quality improved by covering up and mixing with cleaner sediments. As the other phases, also this (sixth) phase is first seen in the Biesbosch, and finally also in the Haringvliet. In this stable freshwater phase, conditions with high diversity communities and high densities were reached.

4.2. Data availability and uncertainties

As monitoring data on macrozoobenthos from the 1980s to 2005 were scarce, we can not be sure on how densities and species numbers and diversities have been developed during that period. A gradual increase in the three biotic parameters is expected, but highs and lows during the period, or even a recent change of the trend can not be excluded, although radical changes in trends of abiotic parameters are not observed. For instance the so-called Sandoz-accident in 1986 with a huge impact on the macrozoobenthic communities of the river Rhine, might also have caused a decrease in densities and/or species numbers in the former estuary (Bij de Vaate et al., 2006). The densities (300-10000 ind m^{-2} with higher densities at the Haringvliet sites than at the Biesbosch sites) recorded in 1988 (Smit et al., 1995), are in line with our findings in 1983 and 2001. They also recorded similar assemblages as we found, dominated by Oligochaeta (Tubificidae) and Bivalvia (Mollusca) with locally higher numbers of Insecta (Chironomidae).

In our study strong relations between abiotic parameters and biotic data are absent. This is partly the result of the fact that they are not measured at the same sites. As we only have a few monitoring sites of abiotic variables for each basin, we consequently assume that the abiotic parameters are similar throughout the basin. Spatial variability of several of the parameters might, however, be large. That this aspect plays a role is shown by the large variances shown by most of the parameters, which are decreasing when the highly variable estuary is gradually changing into more stable freshwater basins. It is, however, expected that the monitoring stations of abiotic parameters give a representative view for the basins, and as measurements are repeatedly executed over longer periods at the same stations, the long-term trends and changes are not overlooked. The salinity range is calculated with pulled data for whole basins. Therefore the recorded salinity ranges will probably be representative for the western parts of the basins, but overestimates the ranges in especially the eastern Haringvliet and Hollands Diep during the 1960s. Climatic aspects affecting discharges like dry summers or a large number of freezing days are only partly taken into account. Effects of these temporal events on macrozoobenthic communities can be substantial. Their effects might be visible in variables like salinity and temperature, but can become unnoticeable in the year averages and long-term trends. Also sedimentation can have a large influence on the macrozoobenthic communities. In waters without tidal influence and relatively stable currents, the amounts of suspended matter can give an indication of sedimentation. However, the higher amounts of suspended matter before closure of the estuary do not mean that sedimentation rates were higher. Smit et al. (1995) mentions that sedimentation significantly increased after closure of the estuary, although shoals started to erode. Sedimentation rates were highest in the eastern Hollands Diep during the 1980s. Further, the abiotic parameters are measured in the surface water, while for several parameters, and the oxygen contents and metal pollutants in particular, sediment concentrations might be more relevant. It is expected that trends in the water phase and in the sediment are related. It can take much longer before an increase in the oxygen content in the surface waters is also found at larger depths. It is very likely that finally a decrease in metal content in the water phase will lead to a decrease in the sediment, due to mixing processes and the persistent character of metal pollutants. A delay in the reaction of macrozoobenthic communities (or at least certain species) to changes in the water conditions can therefore be expected. This is confirmed by the study of De Lange et al. (2004), showing that the productivity of the macroinvertebrate communities is not hampered, but the species composition and diversity is still affected by the presence of contaminants in the Biesbosch. Important substances with potential effects on macrozoobenthic communities, but not taken into account, are the organic micro-pollutants. It is however expected that these substances show similar trends, that is a decrease since the 1970s, as the metal pollutants. This is supported by measurements in the river Rhine (Bij de Vaate et al., 2006), the main supplier of water entering the Biesbosch, the Hollands Diep and the Haringvliet delivering approximately 3/4th of the total water mass (Peelen, 1974).

The strong changes in community structures in the early 1960s in the Hollands Diep and the Haringvliet (Fig. 6) might partly be the result of the limited number of samples available. It is shown in North Sea studies that benthic communities are highly variable already on small temporal and spatial scales (Armonies, 2000). The variability might be less in a more stable environment of freshwater basins. We should however keep this variability in mind, especially at the time of the area being an open estuary. In our study for instance in the Hollands Diep the Polychaeta and Oligochaeta dominated during the early 1960s. The Oligochaeta are more related to freshwater environments, while the Polychaeta are salt water related. For instance in 1965 all samples were taken in the eastern part of the Hollands Diep. The positioning and the limited number of sample sites is very likely a reason for the deviations of the general observed patterns.

The used methodology of sampling macrozoobenthos (Boxcorer instead of 'van Veen' and sieving over 0.5 mm mesh instead of 1 mm) might have led to a slight overestimation of the densities and species numbers for 2001 compared to the other years. On the other hand much smaller surfaces, influencing the species numbers, were sampled. For instance the presence of Nematoda in 2001 and not in the other years might be a result of the different methodology. But the huge increase of the importance of other groups than Oligochaeta compared to for instance 1983, while Oligochaeta might be an important group of which substantial numbers are missed when sieving over 1 mm mesh (Ysebaert et al., 2005), shows that large changes in the communities have occurred.

4.3. Potential diversity of the area

The number of species per sample significantly increased in time, in all of the three basins. Only for the Haringvliet we have an indication about the current level (numbers in 2001). As the species numbers in the Hollands Diep and Biesbosch were similar to those in the Haringvliet throughout the research period, and the water quality in the three basins has become more similar, we do not expect substantial higher numbers in the Hollands Diep and the Biesbosch nowadays. The year-round salinity variance in the Haringvliet and Hollands Diep was about 16 and 7 respectively (range of 29-0.1 dependent of the location), and 0.4 (range; 0.6-0.1) in the Biesbosch, in the begin 1960s (Wolff, 1973), and decreased during the late 60s to less than 2.4 in the begin 70s in all basins. Considering these salinity variances, the number of species in the Rhine-Meuse estuary are always much lower than the potential number recorded for estuaries, for instance the river Thames estuary (Attrill, 2002). As it is expected that the α-diversity is comparable to the number of species per sample, the potential is about 3, 9.5 and 14 species per m^2 for the Haringvliet, Hollands Diep and Biesbosch respectively in the begin 1960s. These numbers gradually increase to 15 species per m^2 towards the begin 70s. This means that only local measurements in a limited number of years reached the potentials. Generally the recorded species numbers are at the lower end of the range recorded by Attrill (2002). Wolff (1973) indicates that the potential diversity in the 1960s is reached, and in large areas in the Haringvliet even surpassed, when the maximum number of species occurring per sample for series of ten samples is taken. This method might be a good measure at high sampling intensity. In that case the potential diversity in the Haringvliet might also be reached at present. To what extent the measurements in 2001 in Haringvliet give an indication of the diversity status of the Hollands Diep and the Biesbosch is uncertain.

4.4. Future developments in the area

On January 01, 2008, a first step towards restoration of the estuary will be taken by a regulated salt water inlet. This means that the salt to freshwater gradient and the associated tidal salinity variability which is now situated in a relatively small area of the Haringvliet Pre-delta (part of the North Sea), at the west side of the dam 'Haringvliet', will extend its area approximately 11.5 km land inwards in the Haringvliet. For the macrozoobenthic communities in the western part of the Haringvliet this will definitely mean that they will change again. Salt water intrusion and enlarged variability will probably lead to a decrease in macrozoobenthic densities. Initially the species numbers and diversity will increase, as 'marine' species will colonize this part of the Haringvliet, and co-occur with the present freshwater communities. On the longer scale, several freshwater species might disappear from the western part of the Haringvliet. For the basin Haringvliet as a whole the new conditions will lead to an increase of the diversity. Crucial for the stability of the communities, and whether higher densities and diversity can be reached on the longer term in the western part of the Haringvliet, is how often and how severe the basin Haringvliet will be flushed to freshwater conditions. Flushing the Haringvliet to get rid of the salt water, is a measure that will be taken during the 'Chink-regime' that will be installed to cope with periods of low water discharges of the rivers, to prevent salt water intrusion land inwards, further than the imaginary line 'Middelharnis – mouth Spui' (11.5 km land inwards from the 'Haringvliet dam'). Calculations based on historic river discharges show, that this will happen 2 to 3 times a year, and might last in total up to several months a year (Bavelaar and Ligtenberg, 2004; Van Leeuwen et al., 2004). Such an arrangement will probably result in a lower number of species and densities than such an estuary area can potentially harbor, which also has consequences for the food availability for species of higher trophic levels.

The 'long-term large-scale experiment' executed in the 1960s and 70s on changing an estuary into a freshwater lake gets a follow-up. At that time ecology was not involved in the decision-making process (Ferguson and Wolff, 1984) and ecologists could only monitor the effects. This time, the goal of the measures is to rehabilitate the estuarine gradient to achieve ecological progress. However, as far as this concerns the macrozoobenthos, which has an important role in the estuarine food webs and forms an important indicator for the status of the environment, it seems that again we are facing a large-scale experiment of which the outcome is not necessarily positive. We are not sure about the current status of the macrozoobenthic communities, as the sampling in 2001 is the only indication we have from the last 20 years. Nevertheless, again there is an opportunity to monitor effects of an interesting and unique 'large-scale experiment'. It seems that economical boundaries, that is ascertaining freshwater intake for agriculture and drinking water from the largest part of the Haringvliet, again overrule ecological arguments.

The current study shows that macrozoobenthos monitoring data of different origin, covering a larger time span, can be very useful for analyzing historic trends and assessing future developments. However, by covering larger time spans with a limited number of sampling years, the uncertainty of the results increases. When new drastic measures are planned, for which an improvement of the ecological quality is the goal, as is the case in the northern delta, at least the situation of before the measures should be established by an inventory. In estuaries an inventory includes the macrozoobenthic communities, as they are important parts of the food webs, and indicators for the status of the environment.

The results show that the executed works and/or the changes in water quality had large effects on the macrozoobenthic communities, the diversity of these communities, the number of species and the macrozoobenthos densities.

5. Conclusions

The construction of waterworks during the 1960s and 70s changing the estuary of the rivers Rhine and Meuse into a freshwater lake had a huge impact on the macrozoobenthic communities. A gradual diminishing of the salt water intrusion and the variability of salinity levels led to an increase of the macrozoobenthic densities. From 1970, the year that the basins became a stagnant freshwater lake, up to 1974 the number of species and the species diversity was high, due to the coexistence of salt and freshwater species. A poor water quality in terms of nutrients and pollutants during the 1970s combined with an increased sedimentation of these and decreased oxygen levels in the stagnant waters, probably led to a decrease in the macrozoobenthos densities. As water quality and very likely also sediment pollution levels gradually improved, the macrozoobenthos densities, species numbers and diversity increased during the 1980s and probably also later on. Concurrently with the changes in water parameters, the communities changed from Oligochaeta and/or Polychaeta dominated communities during the transition from dynamic to stagnant water, to more diverse communities with higher numbers of Gastropoda and especially Bivalvia during the stagnant water phase with poor water quality. Improvement of the water quality led to the high diversity communities with high densities. Changes in the macrozoobenthic communities were always first visible upstream, in the eastern part of the (former) estuary called the Biesbosch. With a time-lag, the developments also occurred in the central part (Hollands Diep) and the west (Haringvliet). Therefore, the current (2001) communities of the Haringvliet are most related to the communities of the late 1960s of the Biesbosch, complemented with a series of non-indigenous species. The planned salt water inlet for January 01, 2008, and especially the frequently flushing of the new salt-freshwater gradient to freshwater conditions will probably lead to a lower number of species than at present and lower densities than an ‘unregulated’ estuary potentially can harbor. The current study also shows the value of historic macrozoobenthos monitoring data available for longer periods. Although abiotic parameters give strong indications for the current status of the Haringvliet – Hollands Diep – Biesbosch basins macrozoobenthic communities, the lack of data from the 1980s till nowadays in this study however increases the uncertainties of assessment of future developments.

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Figure captions

Fig. 1: Situation, subdivision and historic overview of the Rhine-Meuse estuary in the south-western part of the Netherlands. The macrozoobenthos sample sites are indicated with dots, monitoring sites of abiotic factors are indicated with plusses.

Fig. 2: Developments of the abiotic parameters of the Rhine-Meuse estuary during the period 1959-2005. Shown are the trends in year average values and ranges indicated by standard deviations, with exception of the salinity and salinity variance graphs for which separate trends per basin are shown.

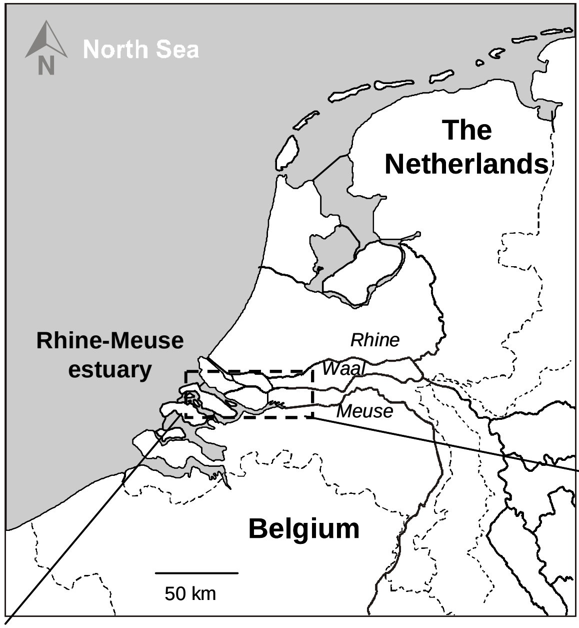
Fig. 3: Macrozoobenthos densities (n m^{-2}), number of species per sample, and diversity (Shannon index) in the Rhine-Meuse estuary for each of the basins Haringvliet, Hollands Diep and Biesbosch during 1960-2001.

Fig. 4: Macrozoobenthos densities (n m^{-2}), number of species per sample and diversity (Shannon index) in various strata (1=(0-2 m); 2=(2-5 m); 3= (5-10 m); 4=(>10 m)) and seasons (1=winter; 2=spring; 3=summer; 4=autumn) for each of the basins Haringvliet, Hollands Diep and Biesbosch of the Rhine-Meuse estuary.

Fig. 5: MDS-plot of macrozoobenthic communities based on presence/absence data of species in the basins Haringvliet, Hollands Diep and Biesbosch per year. Global trends of developments in time are indicated.

Fig. 6: Percentage share of numbers of macrozoobenthos specimens per taxonomical class during the years of monitoring in the basins Haringvliet, Hollands Diep and Biesbosch.

Fig. 1:



Historic overview of waterworks in the Rhine-Meuse estuary:

-June 1958: Start building ‘Haringvliet dam’

-Oktober 1965: Closing of the small mouth ‘Zuiderdiep’

-September 1968: ‘Haringvliet dam’ finished; approx 1 km of sluices stays opened

-November 1968: Closing of the small mouth of ‘Noord-Pampus’

-April 1969: ‘Volkerak dam’ finished

-November 1970: Sluices ‘Haringvliet dam’ only partially opened during low tide, to release freshwater

-March 1997: ‘Chink’-experiment; five days of regulated salt water inlet

-January 2008: Planned start regulated salt water inlet according to adjusted ‘Chink’-regime

Haringvliet:

HV-W = West

HV-CW = Central-West

HV-C = Centrum

HV-CE = Central-East

HV-E = East

Hollands Diep:

HD-W = West

HD-E = East

Biesbosch:

BB-W = Biesbosch West

BB-NieMer = Nieuwe Merwede

BB-BenMer = Beneden Merwede

BB-BovMer = Boven Merwede

BB-BerMaas = Bergsche Maas

BB-Afge = Afgedamde Maas

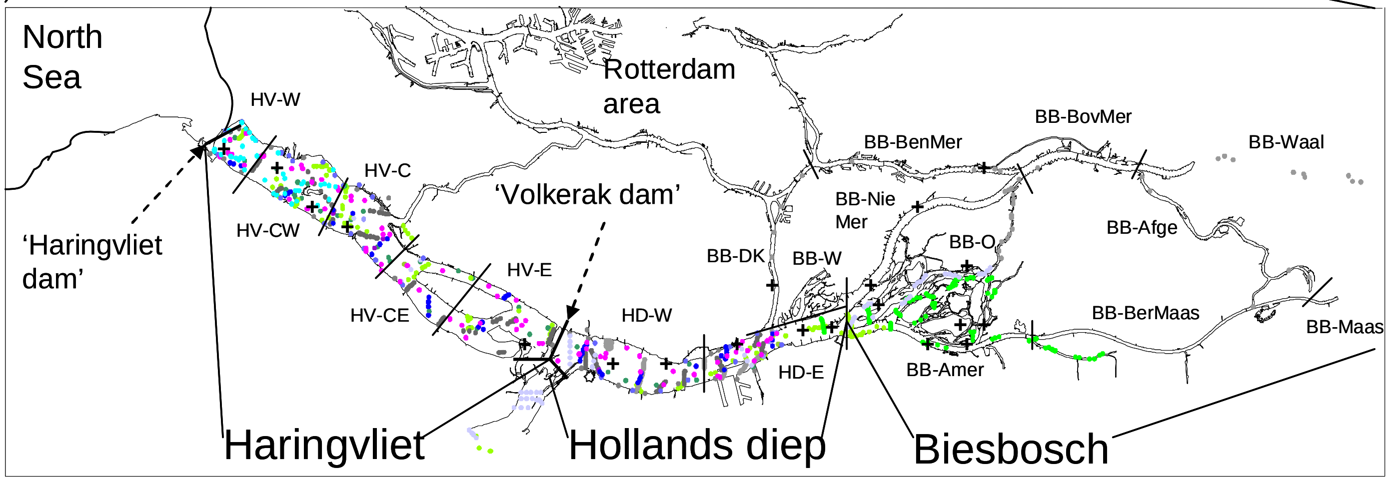
BB-E = Biesbosch East

BB-DK = Dordse Kil

BB- Maas = Maas

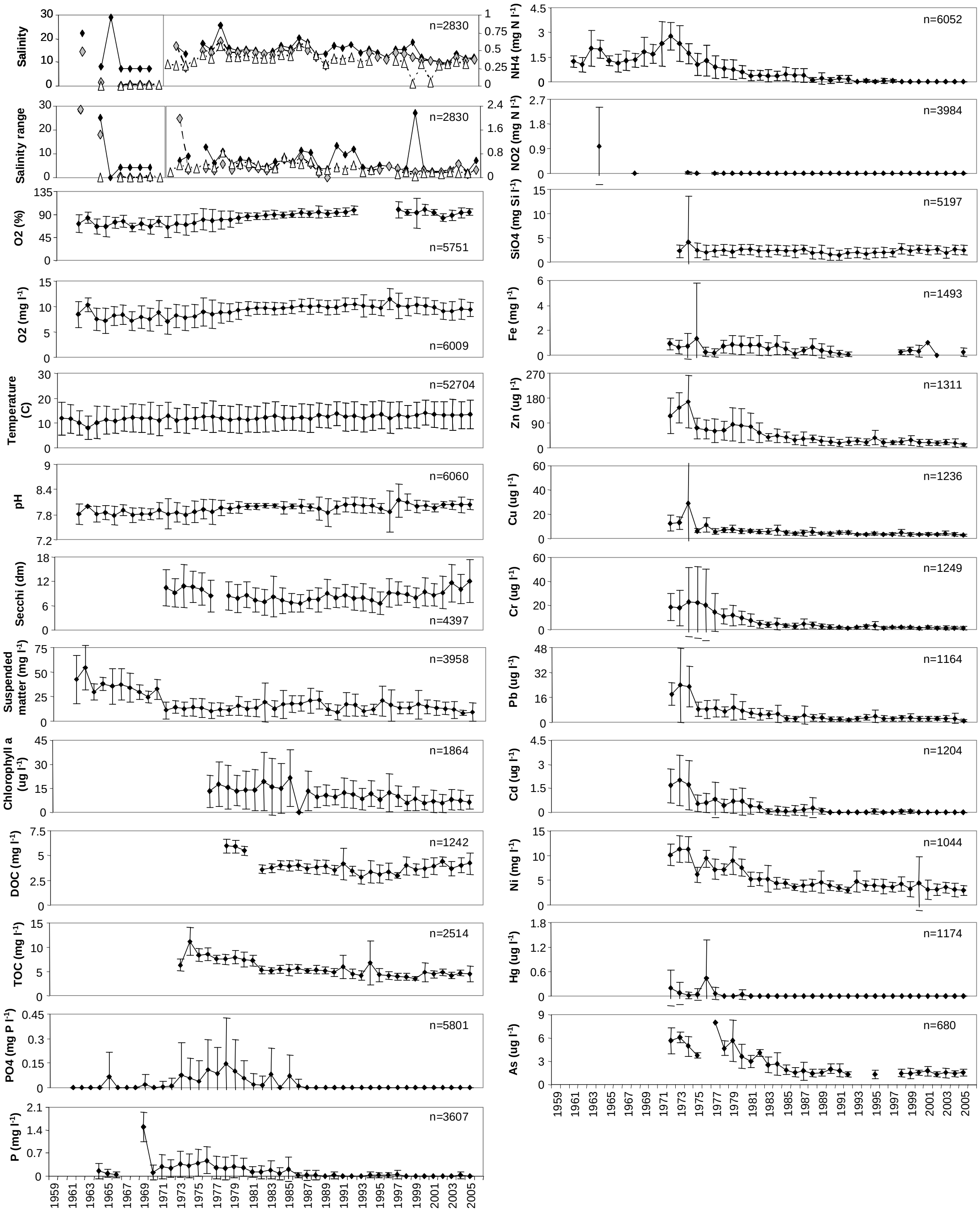
BB-Waal = Waal

BB-Amer = Amer



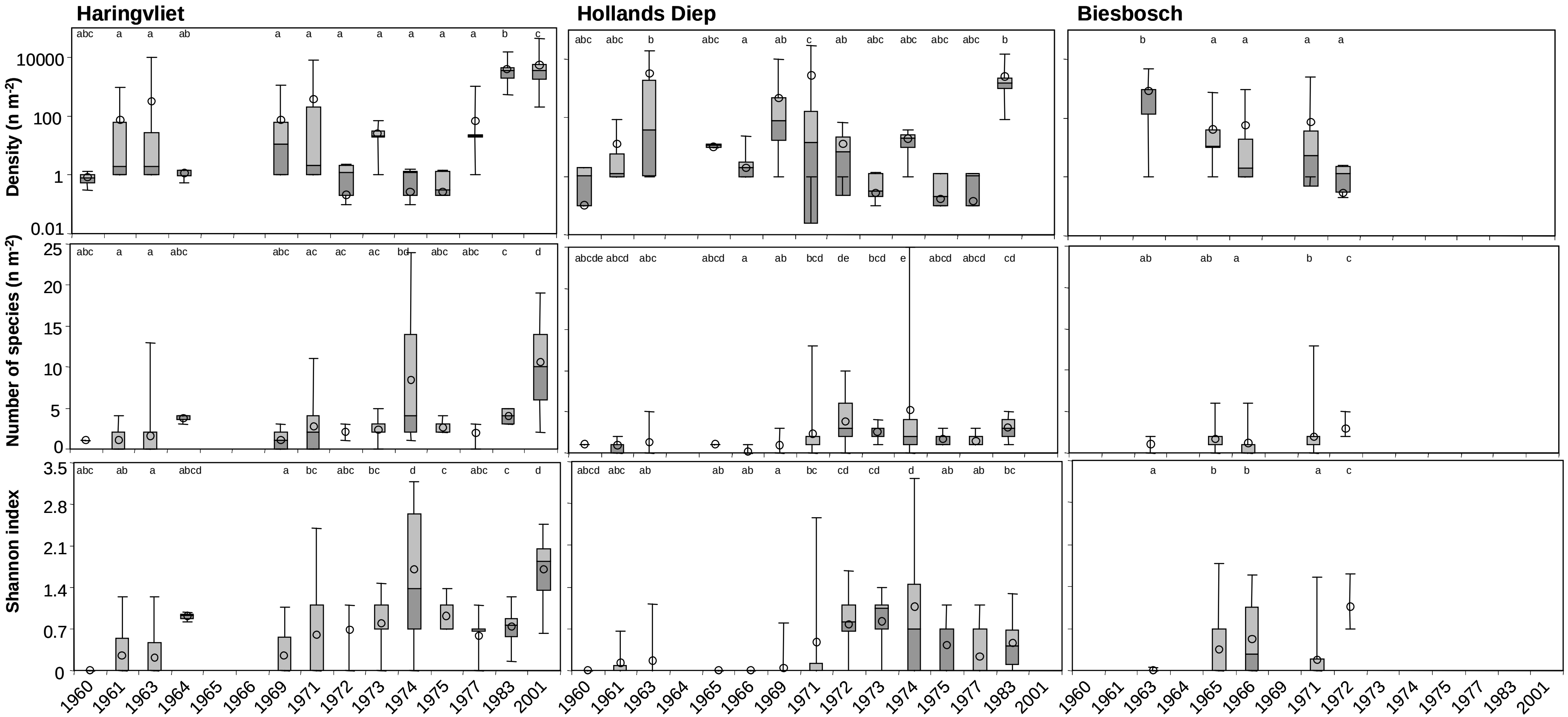
*The two small mouths ‘Zuiderdiep’ and ‘Hole of Noord Pampus’ are situated at the south-western tip of the ‘Haringvliet dam’.

Fig. 2:



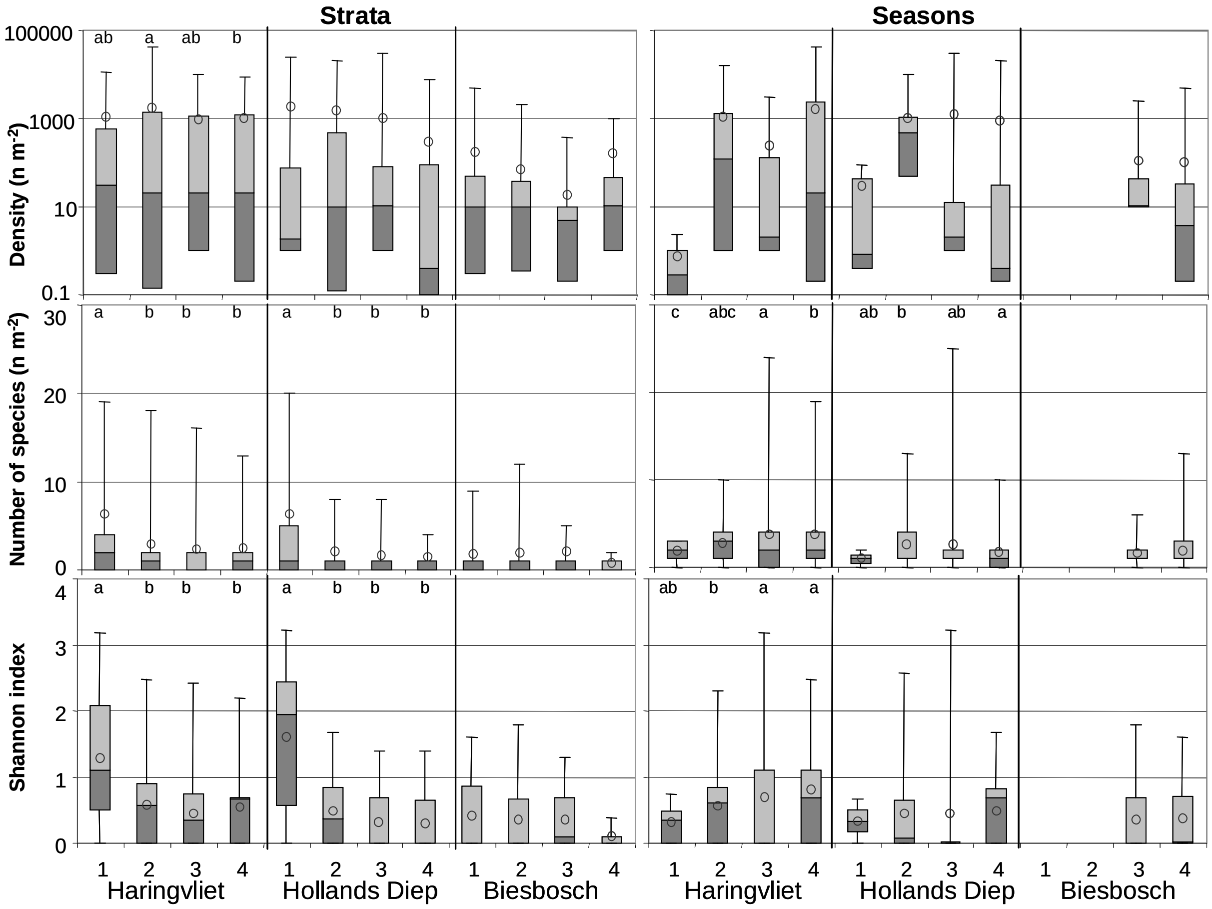
*Graphs made from data extracted from the Waterbase (2006), except for the Salinity (and Salinity range) values, which are combined Conductivity data from Waterbase and Chlorinity data after Wolff (1973), Smit et al. (1995), Bavelaar and Ligtenberg (2004), and unpublished data of the NIOO-CEME. Salinity and Salinity range data are split up in the three basins; Haringvliet (black rhombs), Hollands Diep (grey rhombs) and Biesbosch (white triangles); notice the different scales for the period 1959-1969 and 1970-2005. All other variables show data aggregated from the whole estuary; data covering the whole gradient were not always available for every year (see details in Material and methods).

Fig. 3:



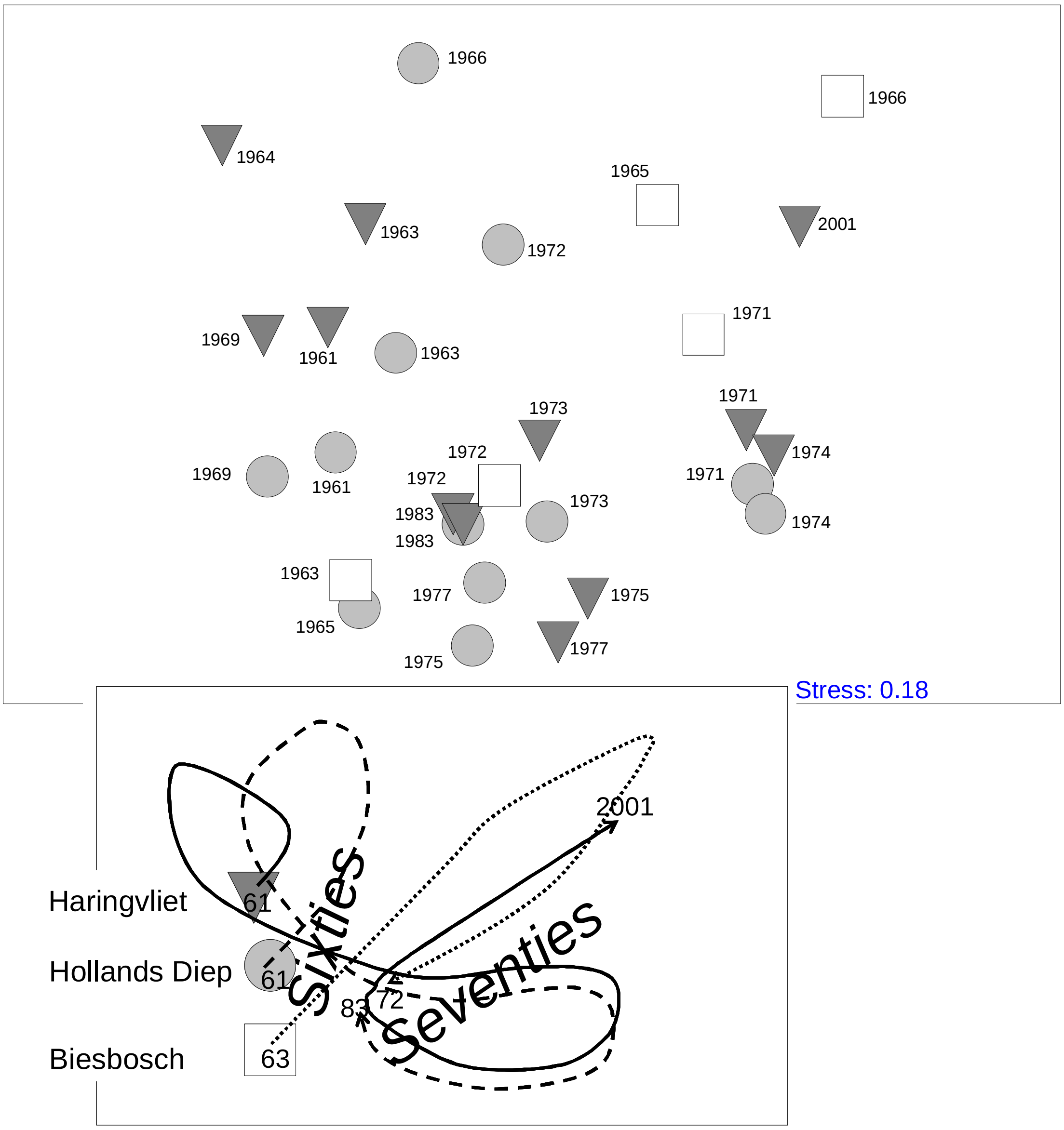
* Different letters indicate significant differences (P < 0.05) between years in a biotic parameter within a basin; identical letters indicate no significant differences.

Fig. 4:



* Different letters indicate significant differences (P < 0.05) in a biotic parameter between strata or seasons within a basin; identical letters or no letters at all indicate no significant differences.

Fig. 5:



1966
1966
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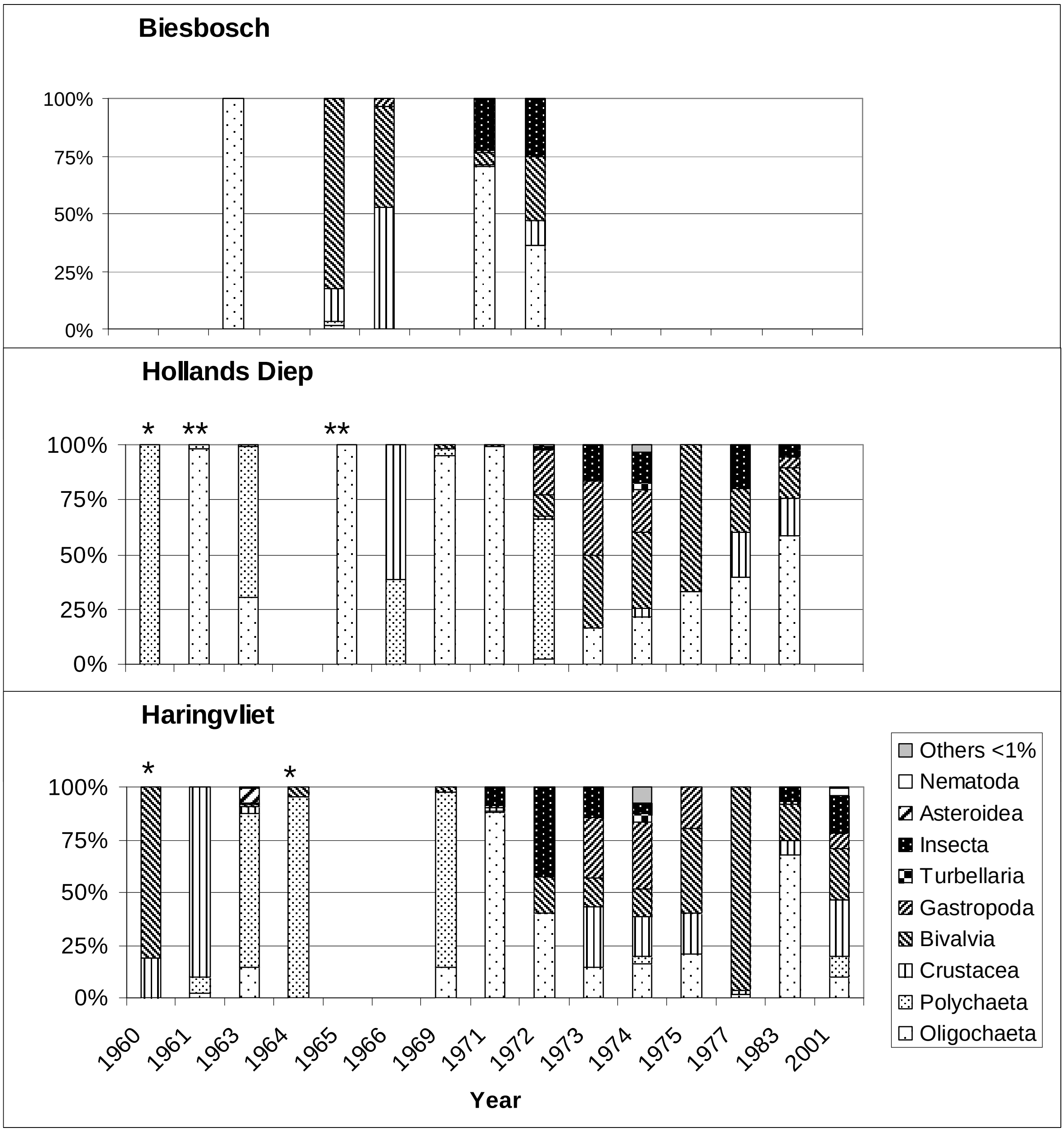
1974
1969
1961
1972
1971
1973
1983
1983
1974
1963
1977
1975
1965
1975
1977
Stress: 0.18
2001
Haringvliet
61
Sixties
Seventies
Hollands Diep
61
83
72
Biesbosch
63

797 Fig. 6:



*A limited number of less than 5 samples is available; **A limited number of between 5 - 10 samples is available (Table 1). Each of the classes Arachnida, Phylactolaemata, Porifera, Hydrozoa and Echinoidea always represent less than 1% of the total number of specimens, and are therefore combined in the class 'Others <1%'.

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Table 1: Number of macrozoobenthos samples available per year and per basin.

Year	Haringvliet	Hollands Diep	Biesbosch	Specifics
1960	2	1		
1961	29	9		
1963	71	32	20	Biesbosch: Only western part sampled
1964	3			
1965		8	91	Hollands Diep: Only eastern part sampled
1966		72	42	Biesbosch: Including river samples
1969	78	52		
1971	118	81	120	Biesbosch: Including river samples
1972	21	46	35	Haringvliet: Eastern to central-western part sampled
1973	21	27		Haringvliet: Eastern to central-western part sampled
1974	45	37		
1975	23	27		
1977	23	27		
1983	80	52		
2001	40			Only western and central-western part sampled
Total	554	471	308	= 1333 samples

Table 2: Relations and trends in biotic and abiotic variables for the gathered data for the whole research period.

a) Results regression analyses between biotic variables for D>1 and S>1; Significance level after Bonferroni correction: p<0.0167				
	n	Regression equation	Sign.	R^2
D'-S'	163	D'=2.27S'+2.27	***	0.192
D'-H'	163	D'=0.366H'+0.278	**	0.057
S'-H'	163	S'=0.787H'+0.827	***	0.726

b) Results comparisons of biotic variables for sample characteristics

	D				S				H’			
Stratum	N=429	Sign.=*	R^2=0.019		N=419	Sign.=***	R^2=0.071		N=399	Sign.=***	R^2=0.078	
	2-5m	<2 and >5m	n-n	Sign.	0-2m	>2m	n-n	Sign.	0-2m	>2m	n-n	Sign.
	1262±2594	707±1547	133-296	*	3.70±4.28	1.97±1.97	97-322	***	0.827±0.872	0.446±0.438	88-311	***
Season	N=429	Sign.=*	R^2=0.019		N=419	Ns			N=399	Ns		
	Spring	Summer-winter	n-n	Sign.								
	1344±1648	805±1977	59-370	*								

c) Results regression analyses between abiotic variables for D>1 and S>1; Significance level after Bonferroni correction: $p<1.67*10^{-5}$

		D’			S’			H’		
	n	Regression equation	Sign.	R^2	Regression equation	Sign.	R^2	Regression equation	Sign.	R^2
Salinity	67	Ns			Ns			Ns		
Sal. range	55	Ns			Ns			Ns		
O_2 (%)	56	Ns			S’=0.008x+0.686	**	0.153	H’=0.011x-0.138	***	0.241
O_2 (mg l^{-1})	56	Ns			S’=0.071x+0.756	**	0.143	H’=0.089x-0.048	***	0.223
Temp.	59	Ns			S’=-0.044x+1.93	*	0.105	H’=-0.048x+1.31	**	0.132
pH	56	Ns			Ns			H’=0.551x-3.69	**	0.123
Secchi	44	D’=-0.222x+8.05	**	0.236	Ns			Ns		
Susp. mat.	56	Ns			Ns			H’=-0.011x+0.793	*	0.074
Chlor. a	25	Ns			S’=-0.050x+1.93	*	0.211	H’=-0.085x+1.46	***	0.517
DOC	16	Ns			Ns			Ns		
TOC	30	D’=-0.431x+8.68	***	0.386	S’=-0.066x+1.86	**	0.334	H’=-0.044x+1.06	*	0.181
PO_4	54	D’=-31.1x+5.34	*	0.101	S’=4.50x+1.38	*	0.087	Ns		
P_{tot}	43	D’=-5.78x+6.27	***	0.328	S’=0.637x+1.42	**	0.156	Ns		
NH_4	56	D’=-1.44x+6.47	***	0.318	Ns			Ns		
NO_2	29	Nv			Nv			Nv		
SiO_4	43	D’=1.62x+1.63	***	0.325	S’=0.149x+1.03	*	0.120	Ns		
Fe	26	D’=5.26x+2.98	***	0.740	S’=0.538x+1.10	***	0.616	Ns		
Zn	23	D’=-0.034x+7.14	**	0.406	S’=-0.005x+1.71	*	0.244	Ns		
Cu	23	D’=-0.415x+8.13	***	0.481	S’=-0.055x+1.84	*	0.275	Ns		
Cr	23	D’=-0.350x+7.63	***	0.552	S’=-0.051x+1.80	**	0.379	Ns		
Pb	23	D’=-0.363x+7.52	***	0.530	S’=-0.052x+1.78	**	0.355	Ns		
Cd	23	D’=-2.46x+6.64	**	0.499	S’=-0.302x+1.63	*	0.247	Ns		
Ni	23	D’=-0.298x+7.83	**	0.325	S’=-0.065x+2.02	***	0.502	H’=-0.047x+1.32	**	0.359
Hg	23	Nv			Nv			Nv		
As	19	D’=-1.33x+10.5	***	0.945	S’=-0.174x+2.16	**	0.510	Ns		

d) Results of comparisons of abiotic variable levels between high and low densities (D), species numbers (S) and species diversities (H'); Significance level after Bonferroni correction: $p<1.67*10^{-5}$

	D		n-n	Sign.	S		n-n	Sign.	H'		n-n	Sign.
	low	high			low	high			low	high		
Salinity				Ns	5.04±8.72	1.41±4.09	107-182	***				Ns
Sal. range				Ns	6.05±10.1	1.89±6.00	89-168	***				Ns
O_2 (%)				Ns	66.2±19.5	77.5±14.8	32-118	**	64.6±21.7	78.5±13.3	32-115	**
O_2 (mg l^{-1})				Ns	6.70±2.17	8.34±1.81	32-118	***	6.46±2.46	8.47±1.60	32-115	***
Temp.	12.9±2.35	13.8±3.01	68-102	*	14.6±3.94	13.2±2.52	40-121	*	15.4±4.19	12.9±2.23	38-120	**
pH				Ns				Ns	7.77±0.37	7.92±0.16	32-115	*
Secchi	13.0±4.21	10.6±5.03	54-71	**				Ns				Ns
Susp. mat.	8.51±4.41	11.7±10.1	62-94	**				Ns				Ns
Chlor. a				Na				Na				Na
DOC				Na				Na				Na
TOC	8.01±1.30	6.22±3.09	31-43	**				Ns				Ns
PO_4				Ns				Ns				Na
P_{tot}				Ns				Ns				Ns
NH_4	1.36±1.01	0.86±0.82	62-94	**				Ns				Ns
NO_2				Nv				Nv				Nv
SiO_4				Ns				Ns				Ns
Fe				Ns				Ns				Na
Zn	122±135	49.7±40.9	22-30	*				Ns				Ns
Cu				Ns				Ns				Ns
Cr	10.7±8.60	6.23±4.78	22-30	*				Ns				Ns
Pb	9.89±3.59	5.99±4.39	22-30	**				Ns				Ns
Cd	1.14±0.84	0.45±0.65	22-30	**				Ns				Ns
Ni				Ns	11.1±0.87	8.67±3.76	4-45	**	11.1±0.87	8.67±3.76	4-45	**
Hg				Nv				Nv				Nv
As	4.63±0.84	3.75±1.58	16-25	*				Ns				Ns

D'=ln(D+1); S'=ln(S+1);
Sign.= significance level; * p<0.05; ** p<0.01; *** p<0.001; Ns = not significant; Nv = no variance; Na = one category not available;
Sal. range = (maximum salinity) – (minimum salinity) when number of samples larger than 1; Temp. = temperature in °C; Secchi = visibility in dm;
Susp. mat. = Suspended matter in mg l^{-1}; Chlor. a = Chlorophyll a in µg l^{-1}; Substances DOC, TOC, PO_4, P_{tot}, NH_4, NO_2, SiO_4 and Fe in mg l^{-1};
Elements Zn, Cu, Cr, Pb, Cd, Ni, Hg and As in µg l^{-1}; n-n = sample sizes at the low and high value of the biotic variable respectively;
Low density: D<1; High density: D≥1; Low number of species: S<1; High number of species: S≥1; Low diversity: H'<0.1; High diversity: H'≥1.
*Beware; significance indications are not necessarily significant when Bonferroni corrections are taken into account!