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Zooplankton oxygen consumption and nutrient release in relation to species composition, animals size and environmental conditions in the Baltic Sea during May and August

by

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ABSTRACT:

Zooplankton metabolism in terms of oxygen consumption and nutrient release (ammonia, phosphate) were measured in the Baltic Sea, a temperate area with high environmental changes both in space and in time.
Plankton of the surface layer were analysed with balance measurements in 4 size classes between 50 and 1000 µm during spring in 1988, 1990 and 1991, in summer 1988 and 1990 as well.
The use of electron transport system (ETS), and the Glutamate Dehydrogenase (GDH) activity as indicators for respiration and ammonia release respectively, enlarged the data density and made a three dimensional resolution available (May 1990, 1991).
Data are in the range of the latitudinal dependend magnitude. They reflect slight interannual, more seasonal and regional aspects. Animals size, temperature, food concentration, and species composition influence the specific rates.

METHODS:

Oxygen consumption, interpreted as respiration, and phosphate and ammonia release (excretion) were measured in the Baltic Sea, mostly from Mecklenburg Bight to the northern Gotland Sea, sometimes from the transitition area (Skagerrak / Kattegat) to the Gulf of Finland (**Fig.1**). Determination were carried out by means of balance or "bottle" methode (Omori and Ikeda, 1984).
Samples were careful collected in the surface layer down to the thermocline using a WP-2-net, equiped with a special cod end to prevent, that animals get dry.
After dark adaptation of about 3 to 5 hours in maximum, under oxygen saturated conditions, using water from the same station, the plankton was fractionated in 4 size classes (55 - 100, 100 - 200, 200 - 500, 500 - 1000 µm), washed carefully with filtered seawater to clean it from nutrient particles, and transfered in 1 l bottles. Bottles were incubate now in a container, cooled by sea surface water, at a rotating wheel (2. 5rpm). At least one control bottle with the same filtered, and oxygen saturated seawater is to add, to be able to calculate the difference of oxygen and nutrients of the bottles with and without zooplankton after the 6 to 15 hours lasting incubation periode. The enrichment of animals in comparison to in situ conditions is about 20 to 1000 times, depending from the temperature.
Finally oxygen, and nutrients will be determined, the zooplankton stored in buffered formaline, to analyse the species composition, the dry mass, using conversion factors as recommended by BMB (1985). Dry mass is needed to calculate the specific metabolic rates.
For the calculation of in situ rates the in situ biomass is to determine in the above mentioned size classes. To be sure, that all the different size classes are quantitatively collected, nets of 55 µm, 100 µm and 200 µm mesh size were used for that purpose . From the 200 µm net the two larges fractions were produced.

With the same nets plankton for the determination of metabolic rates by means of enzymatic activity (ETS, GDH) were catched in 3 depth levels (surface -thermocline, thermocline - halocline , halocline - bottom). Size fractionation and storage in liquid nitrogen were done immediately.
ETS means Electron Transport System activity and is in relation to respiration ,
GDH means Glutamate Dehydrogenase, to be in relation to ammonium release.

The single steps are described in a flow diagramm (**Fig. 2**).

The ETS assay was runned in the laboratory according to Packard (1969, 1971), Owens and King (1975) and modified by Kenner and Ahmed (1975). The samples were homogenized in teflon-glass at 0 - 4 ·C using a phosphate buffer 0.1 M, pH = 8 containing Triton X-100 (Sigma Chem. Co.) for the solubilization of the enzymes (Owens and King, 1975). An aliquot of the crude homogenate was incubated for 20 minutes at 12 ·C in darkness conditions in the presence of NADH, NADPH, succinate and an artificial electron acceptor, the tetrazolium salt INT (Biomedical Lab.). After this time, the reaction is stopped with a solution containing phosphoric acid and formaline and centrifugued between 0 - 4 ·C for 10 minutes at 4000 rpm. The reaction colour was measured at 490 nm with a turbidity base line at 750 nm, and respect to a

blank without substrates and treated as the sample. For the INT coefficient factor, the value of 1.42 was taken from the molar absorption coefficient obtained from Kenner and Ahmed (1975). Final activity values were recalculated for the "in situ" temperature using the Arrhenius equation and an activation energy of 15 Kcal/mol.·C (Packard et al., 1975). Biomass as proteins was determined according to the method of Lowry et al. (1951), using Bovine Sero Albumin (BSA, Sigma Chem. Co.) as standard. The modification of the Lowry method by Peterson (1977, 1983) was used for the samples with low protein content.
The GDH assay was runned in accordance with Bidigare and King (1981) and Bidigare et al. (1982), including certain modifications: One milliliter of crude homogenate (same as ETS assay) is diluted in Tris buffer, pH = 8.6 and centrifugued (0 - 4 ·C) for 10 minutes at 4000 rpm. In a Perkin-Elmer 551-S UV/VIS spectrophotometer equipped with an 1 cm water-jacketed cuvette thermostatized at 12 ·C, an aliquot of 0.5 ml of the supernatant is placed in the presence of NAD and ADP. When absorbance lecture at 340 nm is stable, the assay is runned by adding glutamate, and changes in absorbance were recorded continuously for 2 minutes. The slope is proportional to GDH activity. Total volume in the cuvette was 3.0 ml and NAD, ADP and glutamate concentrations were 1.2, 2 and 40 mM, respectively (Bidigare et al., 1982).

RESULTS:

1. Methodological aspects:

In general there is a body mass - temperature dependency of metabolic rates. A coincidence with calculated rates in magnitude should be a quality mark of the own measurements, because 700 to 1200 observations are the basis of the Ikeda's (1985) equations.
Using May and August 1988 as an example a satisfying correspondence is to observe:

		May	August
in vitro temperature		7.5° C	18.3° C
spec. oxygen consumption per individuum	observation	**24.64** +/- 10.8; N=25	**15.06** +/- 7.76; N=16
μm^3 O_2/ Ind.* h	calculation	**10.82** +/- 2.07;	**17.99** +/- 1.67;
spec. PO_4 release per individuum	observation	**15.17** +/- 7.76; N=27	**6.90** +/- 6.15;
pM / Ind. * h	calculation	**4.85** +/- 1.22;	**6.25** +/- 0.41;
spec. NH_4 release per individuum	observation	**105.32** +/- 92.32; N=24	**57.94** +/- 50.38; N=14
pM / Ind. * h	calculation	**70.71** +/- 16.54;	**112.57** +/- 11.16;

In May, after or during the spring bloom periode, the observed values are higher, than the calculated. In August there is the opposit case, indicating, that temperature is one influence, but the nutritive basis probably of higher priority.

Next table includes results of correlations of 6 data groups, the comparisons

(1) of oxygen consumption and nutrients release on the raw data level,
(2) of ETS - and GDH - activity on the raw data level,
(3) of dry mass specific oxygen consumption and nutrients release rates (raw data devided by biomass),
(4) of protein specific enzymatic activity,
(5) of (1) and (2),
(6) of (3) and (4),

using the May '91 data as the basis:

raw data: O2 vs. PO4	r = 0.8309	p < 0.001	N=21
O2 vs. NH4	r = 0.9475	p < 0.001	N=21
PO4 vs NH4	r = 0.9035	p < 0.001	N=21
raw data: ETS vs. GDH	r = 0.6730	p < 0.001	N = 23
dry mass specific rates: O_2 vs. PO_4	r = 0.6582	p < 0.001	N = 23
O_2 vs. NH_4	r = 0.4904	p < 0.05	N = 21
PO_4 vs. NH_4	r = 0. 5425	p < 0.05	N = 21
protein specific ETS vs. GDH	r = - 0.0577		N = 21
raw data: O_2 vs. ETS NH_4vs. GDH	r = -0.0476 r = 0.3353		N = 21 N = 21
biomass specific O_2 vs. ETS NH_4vs. GDH	r = -0.0225 r = -0.0937		N = 21 N = 21

Correlations were to expect in all the cases. The significant coincidence between the raw data is a satisfying indication for their quality. This is true for both, the data of bottle methode and those of the enzymatic determinations .
The reason for the lesser correlation of the dry mass specific rates are probably the calculated biomass data. This is worse in the case of protein specific ETS- and GDH-activities. Not to understand is the missing correlation between the raw oxygen consumption and the ETS activity on the same level. That biomass specific rates of both groups in the last two rows don't match does finally not wounder. So the link between the two methods, shown in **Figure 2,** is not active now. Both data sets have to be seperately used.

2. Size classes:

The smaller organisms show an about 7 times higher respiration rate compared with the largest group **(Fig.3)**. The mean values are from August 1990. The columns indicate the standard deviation.

3. Vertical structure:

The determination of the oxygen consumption and the ammonia release in terms of enzymatic activity in all the depth levels allows to estimate the average vertical structure of metabolic rates. . This will be done as a *percentage in comparison to the surface layer* in May 1990 and 1991. There were no remarkable differencies between the both years, between ETS and GDH, especially in the group of protein specific rates. Larger vertical gradients are to observe in the in situ - group. The metabolic rate decreases from the surface layer successive downward by aproximately 30% per depth level. The ETS results from 1991 are presented in the next table as an example:

	ETS spec. 1000 - 500 µm	500 - 200 µm	200 - 100 µm	total mean
surface layer (up to thermocline)	100	100	100	100
intermediate layer (thermocline to halocline)	143	85	79	102
bottom layer (halocline to bottom)	101	69	80	83

	in situ ETS 1000 - 500 µm	500 - 200 µm	200 - 100 µm	total mean
surface layer (up to thermocline)	100	100	100	100
intermediate layer (thermocline to halocline)	61	33	65	53
bottom layer (halocline to bottom)	25	17	31	24

4. Regional Patterns:

Figure 4.1 includes environmental parameters, like temperature, salinity, chlorophyll averaged for the surface layer, above the thermocline, and the dry mass of all size fractions, the dry mass of the most important class between 100 and 200 µm size, and the frequency of the main taxonomic groups within this class. The data are regional averaged, one to ten values are the basis.
SK&KG means Skagerrak and Kattegat area,
MB Mecklenburg Bight,
AS Arkona Sea,
BS Bornholm Sea,
S&CG southern and central Gotland Sea, and
NG Northern Gotland Sea (**c.f. Fig.1**).
Figure 4.2 includes biomass specific metabolic rates, oxygen consumtion, ammonia and phosphate release, ETS and GDH activity, and the assimilation number (primary productivity divided by chlorophyll concentration).
Figure 4.3 includes the in situ rates respectively.The averages are the same like in **Figure 4.1.**
In athe most cases a trend is visible, characterizing the late spring situation in the Baltic Sea, with lower temperatures in the northern part, where the metabolic active centre is located during that time, May 1991. The seasonal succession seems to be orientated from the transitition area of the Baltic Sea to the northern Gotland Sea. The in situ oxygen consumption and the phosphate release show a clear decreasing tendency, in accordance with the biomass patterns in **Fig.4.1.**

5. Seasonal and interanual variation

To discribe these variability data from two seasons, May and August, are available, from at least two years.
In **Table 1 (Annex)** the mean situation is included for
in situ dry mass, incubation temperature, in situ frequency of Copepods and of Cladocerans, the dry mass specific and the zooplankton in situ rates of respiration and remineralisation, partly of different size classes.

Comparing the May situation 1990 was significant warmer, than 1988 and 1991. Also the frequency of Copepods / Cladocerans is different. The biomass of the larger organisms is more developed in 1990, than in the other two years. The seasonal cycle started earlier in that year. This has no significant influence on the biomass specific metabolic rates, if they are averaged for the whole Baltic. But it has an input on the in situ rates, in dependency to the biomass.

Remarkable differences are to observe in the biomass specific rates, if May and August will be compared. During August the plankton composition is quit different in comparison to May, the temperature. also,

It is to conclude, that seasonal variability is more significant than interannual. Temperature may have an effect, but nutrition and species composition of zooplankton is also important. Changes between 8 to 12_C (May 88, 91 / May 90) have a slight influence to the specific rates. A comparison with data collected in 1989 in the subtropical Atlantic show more significant differences, but on the basis of a 10 K temperature change! In that case a specific oxygen consumption rate of about 13 mm^3 O2 /mg dry mass * h in the Atlantic is to compare with 5.55 from August 88. This is a relation of 2.3.
(From Ikeda (1985) a factor of 2.8 is to expect, if an area of 21° N (Atlantic) and another of 50° N (Balitc Sea) will be compared.)

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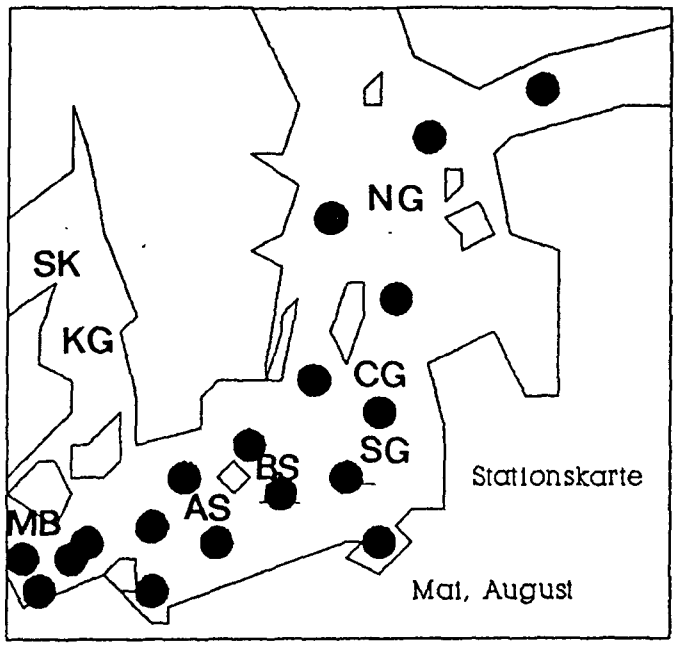


Fig.1: The Study site (explanation of abreviations in chapter 4)

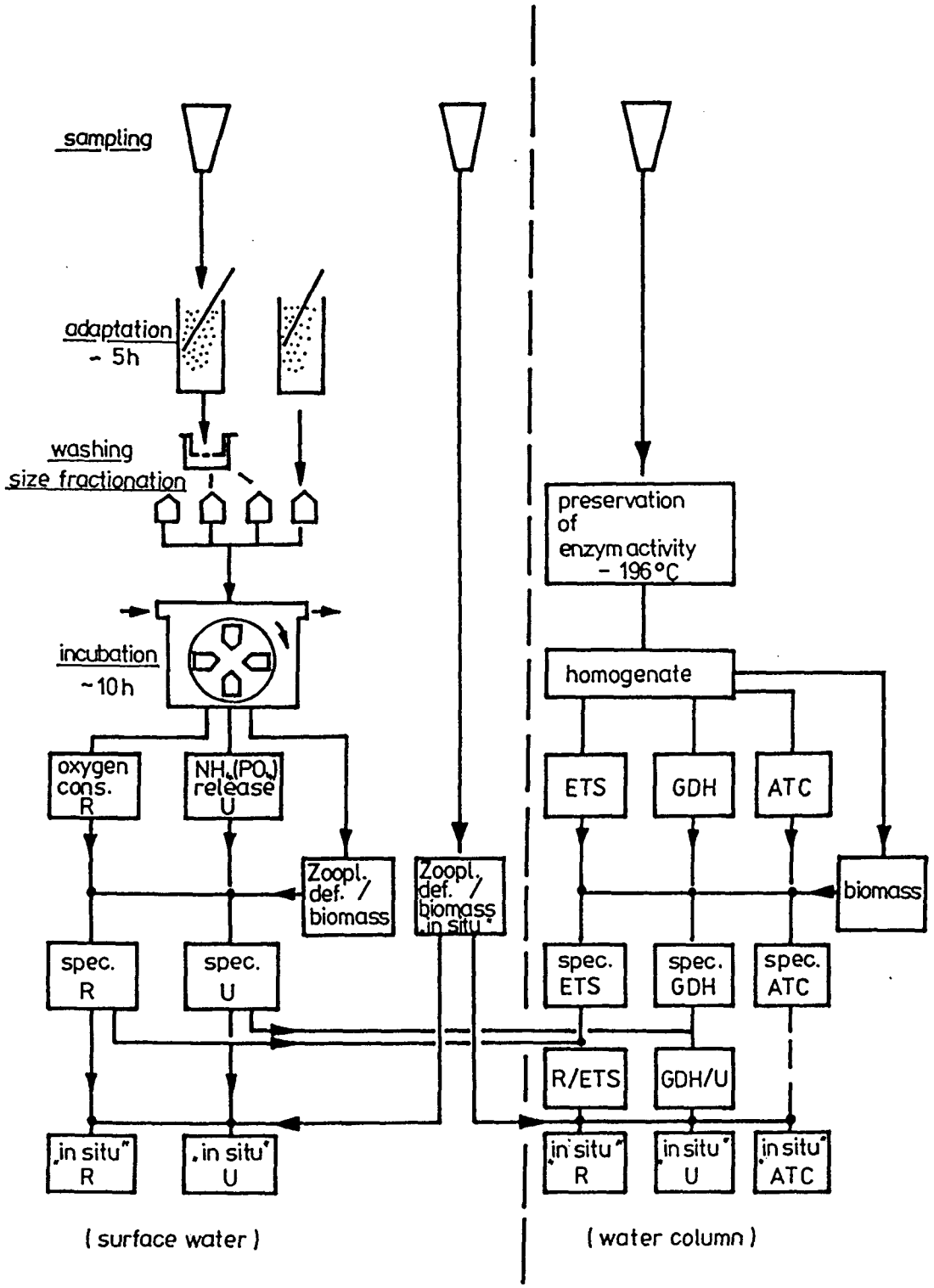


Fig.2:Flow diagramm: the field and laboratory work

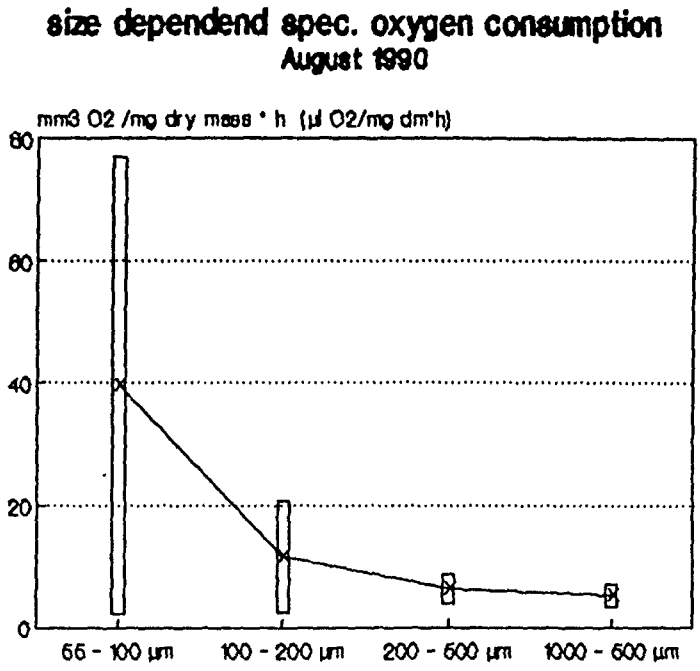


Fig.3: Size dependend specific oxygen consumption,

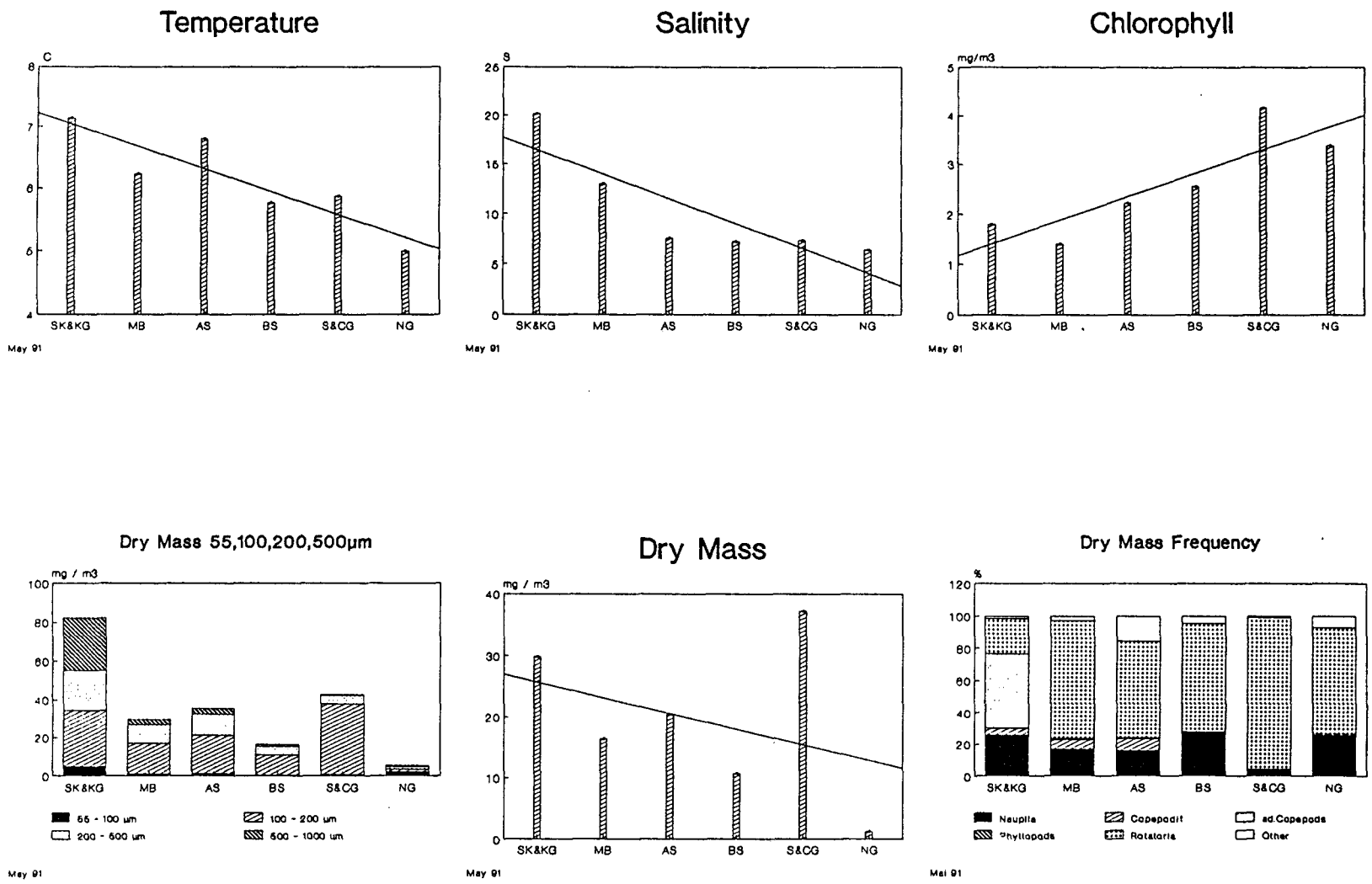
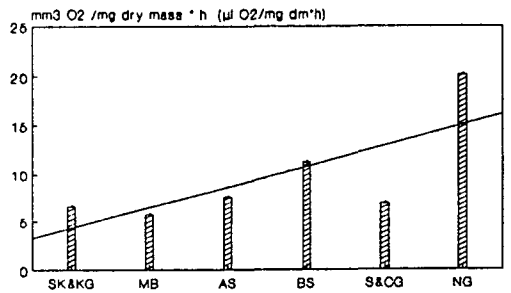


Fig.4.1 ...4.3: Regional aspects of methabolic rates in the Baltic, explanation see text (Paragr. 4).

O2 spec.



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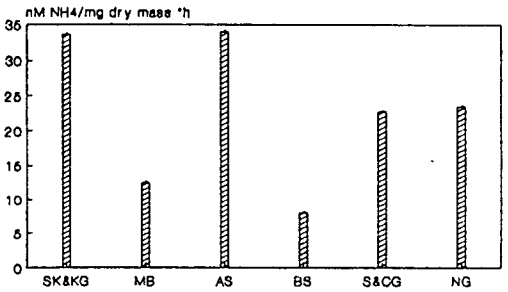
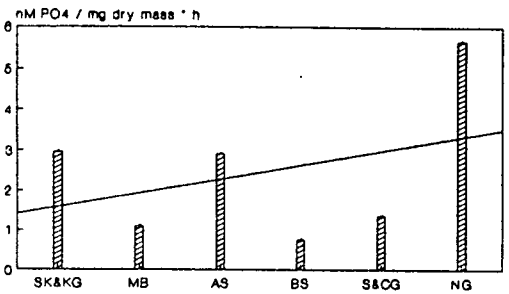
NH4 spec.



PO4 spec.



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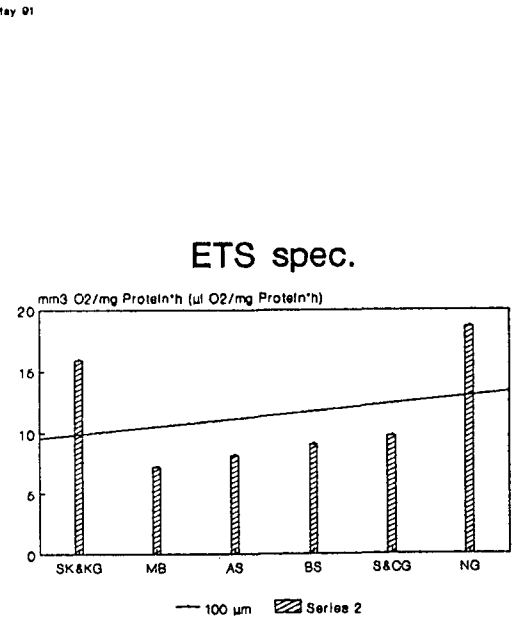
ETS spec.



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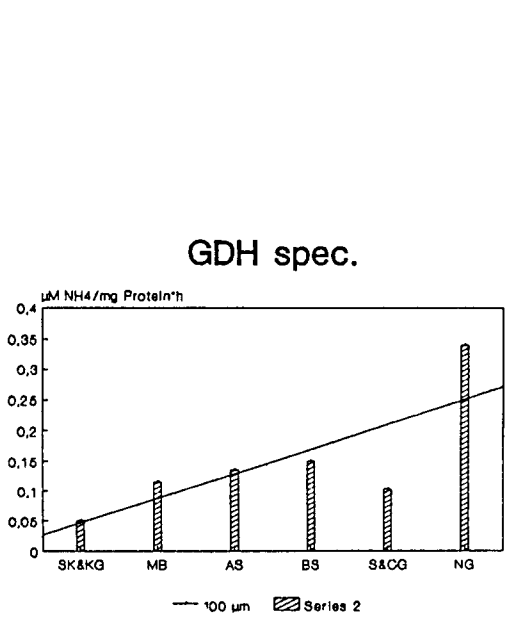
GDH spec.



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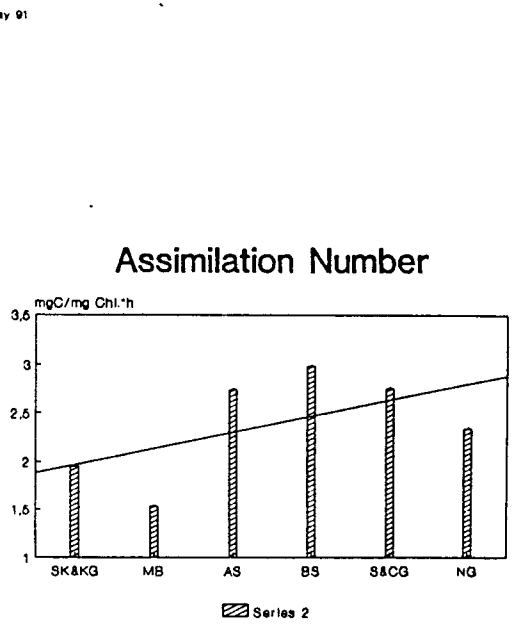
Assimilation Number



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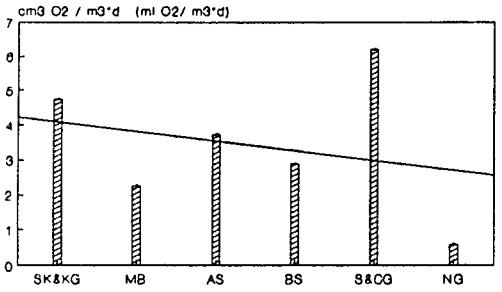
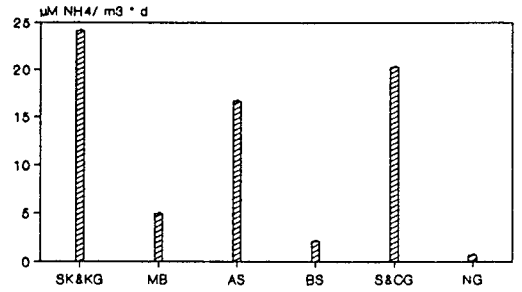
O2 in situ



May 91

NH4 in situ



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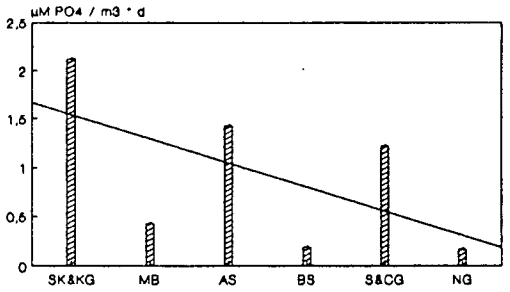
PO4 in situ



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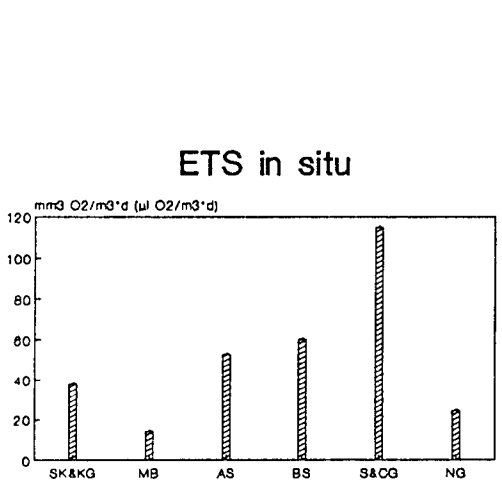
ETS in situ



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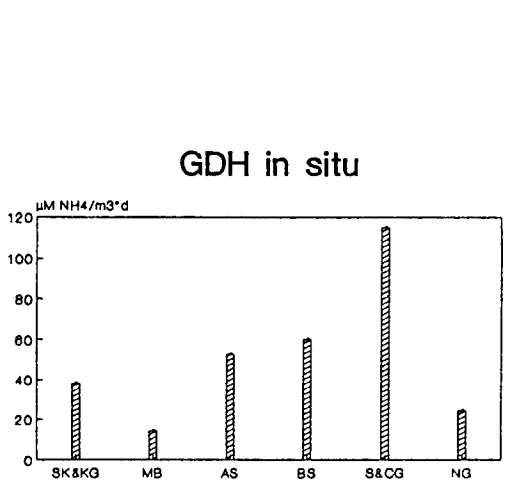
GDH in situ



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	May '88	May '90				May '91				Aug. '88	Aug. '90			
N =	26	16				12				16	5			
mg dry mass·m^{-3}	27	4,19	19,50	4,32	-	4.77	8.59	19,26	1,61	100	8,86	65,37	12,51	1,09
incubation temperature/°C	7,5	12,2				8,6				18,3		18,2		
frequency of Copepods/ %	76	43	40	10	13	80	34	3	0	30	32	29	29	41
frequency of Cladocerans	16	46	41	12	23	11	22	0	0	70	67	70	69	57
Oxygen consumption														
dry mass specific/ mm^3 O_2·mg^{-1}·h^{-1}	9,12	13,93	4,77	8,95	32,33	8,16	5,18	8,20	18,28	5.55	5,26	6,29	11,61	39,68
in situ/ mm^3 O_2·mg^{-1}·h^{-1}		1,4	2,23	0,93		0,93	1,07	3,70	0,71		1,29	8,48	3,98	1,64
	7,24	Σ = 4,56				Σ = 6,5				13,37			Σ = 15,39	
Phosphate release														
dry mass specific/ nM·mg^{-1}·d^{-1}	5,40	4,30	3,12	7,65	21,70	3,78	1,92	2,19	5,01	2,55	0,65	1,49	2,60	4,06
in situ/ μM·m^{-3}·d^{-1}		0,43	1,46	0,87		0,43	0,40	1,01	0,19		0,14	2,34	0,79	0,11
	3,50	Σ = 2,76				Σ = 2,03				5,41			Σ = 3,38	
Ammonia release														
dry mass specific/ nM·mg^{-1}·h^{-1}	34,68	53,48	16,51	29,76	38,65	30,48	17,35	22,67	23,06	21,00	24,66	20,08	15,25	71,70
in situ/ μM·m^{-3}·d^{-1}		5,38	7,73	3,08		3,49	3,58	10,48	0,89		5,25	31,50	4,64	1,99
	42,66	Σ = 16,19				Σ = 18.44				53,61			Σ = 43,38	
Size fraction/μm	>200	1000-500	500-200	200-100	100-55	1000-500	600-200	200-100	100-55	>200	1000-500	500-200	200-100	100-55
		>200				>200					>200			

Table : Comparisons of mean values of environmental conditions, zooplankton biomass, frequency of Copepods and Phyllopods, specific and in situ metabolic rates in May 1988,1990, 1991 and in August 1988,1991.