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Feeding of the hyperbenthic mysid *Neomysis integer* in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries

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Abstract

The diet of the mysid *Neomysis integer* in the maximum turbidity zone (MTZ) of three European estuaries (Elbe, Westerschelde and Gironde) was investigated in spring 1993. The quality and quantity of the diet were assessed through measurement of the stomach fullness and microscopical analysis of the stomach content combined with image analyses. *N. integer* was found to be an omnivore which mainly utilizes mesozooplankton and detritus carbon pools. The quality of the diet did not differ between the sexes or between different developmental stages, although smaller individuals consumed fewer items. In all three estuaries the diet was dominated by Copepoda Calanoida (5–10 *Eurytemora affinis* ind^{-1} for adults; 2–5 ind^{-1} and 2–3 ind^{-1} for subadults and juveniles, respectively) and was supplemented with Rotifera and Cladocera. Phytoplankton and benthic organisms, though present in the stomachs, were negligible. Macrophytal detritus and amorphous material, the latter unidentifiable under the light microscope, were very abundant food items. The amorphous detritus was found to originate from the suspended sediment flocs which are characteristic for the MTZ and mainly consist of clay minerals. The energetic value of the flocs for *N. integer* remains unclear. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Neomysis integer; feeding; turbidity

1. Introduction

Recently, an increased research interest has focused on the description of the food web at the freshwater–seawater interface of estuaries. Since these systems receive large quantities of allochtonous organic matter (natural inputs and discharges from various effluents), their food webs have been described as heterotrophic, i.e., respiratory processes exceed the in situ autotrophic production (e.g., Heip et al., 1995). Moreover, in the oligohaline zone at the freshwater–seawater interface, sediment and organic matter are accumulated by flocculation and sedimentation of suspended matter (e.g., Eisma, 1986; Wolanski, 1995). These phenomena result in a zone of increased turbidity (maximum turbidity zone or MTZ).

The suspended particulate matter (SPM) in the brackish zone of estuaries consists mainly of detritus and inorganic sediment particles next to living matter (bacteria, fungi, phytoplankton, zooplankton, . . .).

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The amount of seston present in the watercolumn of an estuary depends on (1) local primary and secondary production in the estuary proper and in adjacent intertidal areas, (2) the import of marine and fluvial materials and (3) the amount of sediment resuspended in situ (Ketchum, 1983; McLusky, 1989). In the Westerschelde estuary (SW-Netherlands), for example, 1.5 million tons SPM enter the brackish zone per year, 25.10^4 tons of which are detritus and organic matter (Wollast, 1976). Soetaert and Herman (1995) calculated that 20% of the imported organic particles degrade or flocculate and precipitate in the MTZ. SPM concentrations in the watercolumn at the MTZ of the Westerschelde vary between 0.06 and 0.4 g l^{-1} (Soetaert and Van Rijswijk, 1993). In the MTZ of the Elbe (NW-Germany) SPM concentrations vary between 0.1 and 0.2 g l^{-1} (Brockmann, 1992), while in the Gironde (SW-France) concentrations higher than 1 g l^{-1} at the surface and 10 g l^{-1} near the bottom are regularly recorded (Jouanneau and Latouche, 1981).

The biogeochemical cycling in heterotrophic food webs has been shown to behave according to distinctly specific patterns (Smith et al., 1989) and the food webs tend to be based on detritus (Hummel et al., 1988; Hall and Raffaelli, 1991; Hamerlynck et al., 1993). Heterotrophic bacteria are not only responsible for the remineralisation of the nutrients (Goossen et al., 1992), they simultaneously constitute the basis of the food web for higher trophic levels (Azam et al., 1983; Sherr and Sherr, 1988; Billen et al., 1990). Detritus and/or their associated bacteria are consumed, directly or indirectly, by the microzooplankton, the mesozooplankton and the hyperbenthos (Fenchel, 1988; Hamerlynck et al., 1993). Fish and epibenthic macro-invertebrates can then feed at this ‘secondary energy level’.

There is a great need for the description of the feeding ecology of key species in estuarine environments for the development of accurate C-flux models and the description of detritus based food web patterns, including the quantification of transfer coefficients. To date, few studies have taken medium sized hyperbenthic animals into account. Notable exceptions are Hall and Raffaelli (1991) and Soetaert and Herman (1995). Still, the trophic position assigned to the hyperbenthos seems to be rather guessed than derived from field data. The structure of the hyperbenthic community of the freshwater–seawater interface has been described for quite a few Western European estuaries (Mees and Jones, 1997), but studies on the functional impact of the hyperbenthos on suspended particles in the MTZ are lacking.

In the MTZ of West-European estuaries the hyperbenthic community is dominated, both in terms of density and biomass, by the brackish water mysid *Neomysis integer* (Mees et al., 1993, 1995). This species probably has a key function in the energy transfer to higher trophic levels in the ecosystem (Mees et al., 1994). Therefore, *N. integer* was chosen as a model to assess the impact of the feeding of the hyperbenthic community on particles in the MTZ’s of the Elbe, the Westerschelde and the Gironde.

Mysidacea are generally described as omnivores, feeding on detritus, algae and zooplankton (e.g., Mauchline, 1980). They can feed selectively on different zooplankton species and size groups (e.g., Cooper and Goldman, 1980; Murtaugh, 1981a), and thus have the potential of structuring zooplankton communities (Fulton, 1982b; Rudstam et al., 1989). The phytoplankton (Kost and Knight, 1975; Siegfried and Kopache, 1980) and tychoplankton (Webb et al., 1987; Wooldridge, 1989) are possibly also influenced through selective grazing by mysids. Mysid predation has even been reported as a possible control on meiofaunal densities (Siegfried and Kopache, 1980; Grossnickle, 1982; Johnston and Lasenby, 1982). Most mysids utilize organic detritus to a considerable extent and they can be responsible for the remineralisation of a substantial proportion of the non-refractory detritus (Kost and Knight, 1975; Jansen, 1985).

Literature about the diet of *N. integer* is scarce, and only qualitative information is available. According to Lucas (1936) and Tattersall and Tattersall (1951) the species is an efficient filter feeder, grazing on organic detritus and/or planktonic diatoms. According to these authors it only feeds on zooplankton when concentrations of other suspended food items are too low. More recent studies describe *N. integer* as an omnivore consuming detritus, algae, diatoms, rotifers, copepods, amphipods, and other crustaceans, carrion, fragments of leaves and of macroalgae, spores and seeds, terrigenous materials and insect larvae (Kinne, 1955; Mauchline, 1971, 1980; Jansen, 1985). Chitinases and cellulases have been found in the gut of *N. integer* (Zagursky and Feller, 1985), so it can be assumed that they are capable of digesting macrophyte detritus. Still, the growth efficiency of *N. integer* has been shown to be highest (27%) with animal food (dead mysids) and lowest (7.5%) with detritus (Zagursky and Feller, 1985).

This paper describes a methodology for quantitative and qualitative diet analyses of mysids by means of stomach fullness measurements and microscopical stomach analyses. These techniques are applied for a comparison of the diet of *N. integer* in the MTZ of three West-European estuaries. Sexual and ontogenic shifts in the diet are also investigated.

2. Methods

2.1. Samples

The *N. integer* populations of the maximum turbidity zones of the Elbe (NW-Germany), Westerschelde (SW-Netherlands and Belgium) and Gironde (SW-France) estuaries were sampled in spring 1993. All samples were collected in a 1-month period. In each estuary, a station in the MTZ was sampled during daytime with a hyperbenthic sledge in the main estuarine channel (for a description of the sampling gear and the sampling strategy see Hamerlynck and Mees, 1991). In the Elbe a station near Brunsbüttel (53°52′30″N–09°09′55″E) was sampled on April 22, 1993. In the Westerschelde the sampling point was located near Bath (51°23′40″N–04°12′00″E; May 6, 1993). Since upstream of Bath dissolved oxygen concentrations are too low for hyperbenthic life (e.g., Mees et al., 1995), this station—a few kilometres downstream of the MTZ—was chosen because it was characterized by highest mean *N. integer* densities in previous studies (Mees et al., 1993, 1994. In the Gironde a station near Pauillac (45°14′15″N–00°44′50″W) was sampled on May 23, 1993. The salinity at the time of sampling was 4.84, 11.60 and 1.20 psu in Elbe, Westerschelde and Gironde respectively.

Catches were immediately fixed in a 7% neutral formaldehyde solution. In the laboratory, the samples were rinsed over a 1-mm sieve. Adults, subadults and juveniles of *N. integer* were picked out for quantitative and qualitative diet analyses. Sexes and developmental stages were identified according to Mees et al. (1994). No gravid females were used for the diet analysis. Individual *N. integer* were rinsed in distilled water to remove salts, formaldehyde crystals and other impurities. Additionally, the standard length (distance from the basis of the eyestalk to the last abdominal segment) was measured for around 100 individuals per stage and sex.

In order to obtain valuable information on the diet of a species it is advisable to combine several (objective) methods of stomach analysis: at least one method measuring the amounts of the different food items (here named qualitative analysis) and one measuring the bulk of the food material present (quantitative analysis). Ideally, the latter must be linked with the size of the individual (Hyslop, 1980).

2.2. Qualitative diet analyses

Information on the diet composition of *N. integer* was obtained by light microscopic analysis of the stomach contents, in combination with image-analysis techniques. To obtain semi-permanent microscopic slides of the stomach contents, each mysid was first dehydrated (Seinhorst, 1959). A gradual dehydration series from a formaldehyde solution to glycerin causes no risk for abrupt shrinkage of the stomach or intestine: no ingested particles are pushed from the stomach to the intestine nor does digested material return from the intestine into the stomach. The carapax was then removed and the gut was cut just after the round stomach. The stomach (oesophagus included) was dissected out and pulled open in a drop of glycerine on a microscopic slide. Analysis of the slides was performed by light microscope (magnification ×250) connected to an Image Analyzer (Leica Quantimet 500 +). Per estuary, 15 adult males, 15 adult females, 15 subadult males, 15 subadult females and 30 juveniles were processed.

The identification and processing of the different prey categories present in the stomach of *N. integer* was done according to the following procedure.

The chitinous body of adult and copepodite stages of calanoid copepods were usually found to be fragmented (Fig. 1b), depending on the degree of digestion. Mandibles (Fig. 1a) were found to be the most

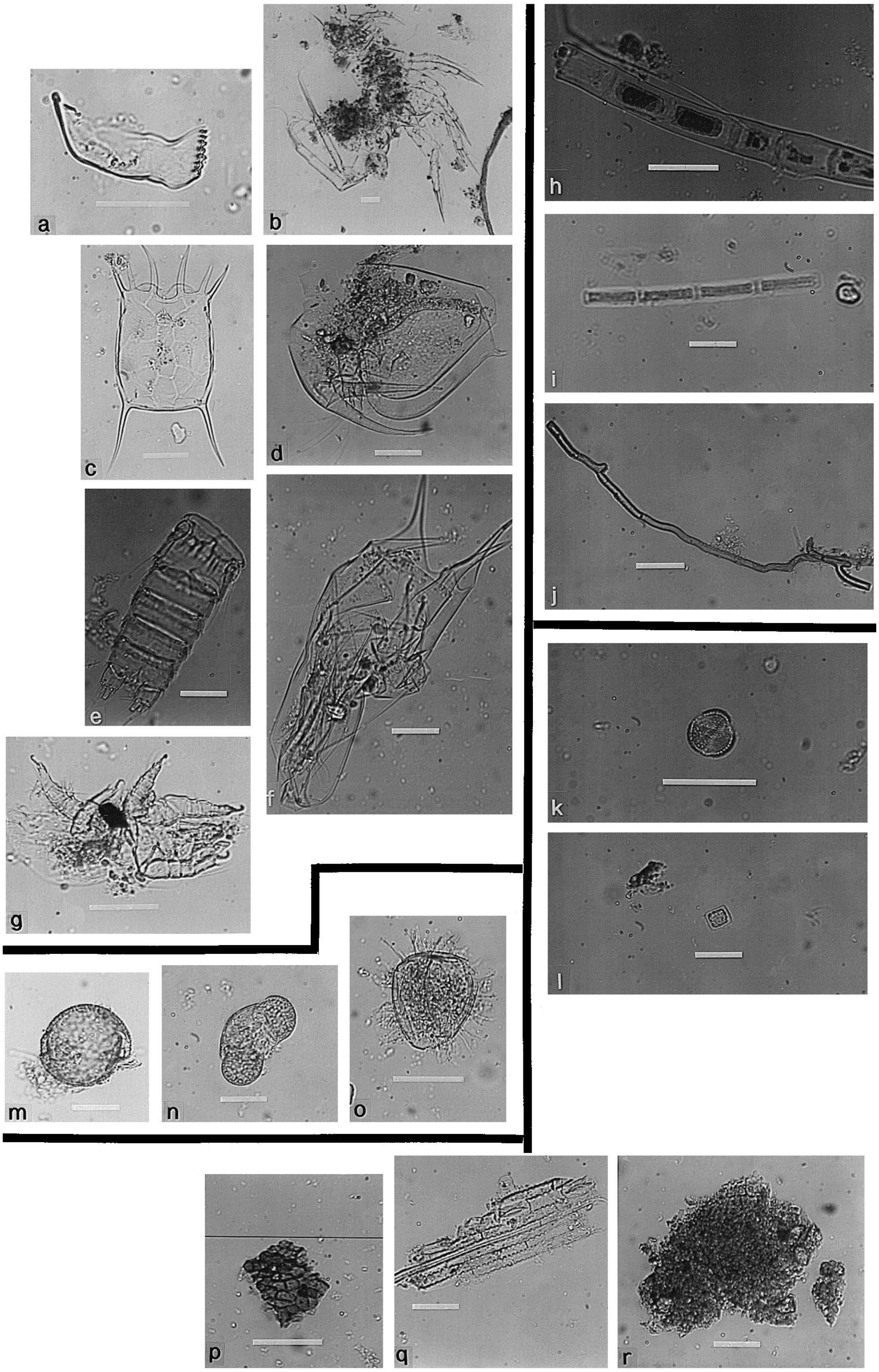
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Table 1
Absolute and relative density (in N/m^2 and %) and biomass (in mg ash free dry weight/m^2 and %) of *N. integer* in the MTZ of Elbe, Westerschelde, and Gironde in spring 1993

	Elbe		Westerschelde		Gironde	
	N/m^2 (%)	mg/m^2 (%)	N/m^2 (%)	mg/m^2 (%)	N/m^2 (%)	mg/m^2 (%)
Adult female	9.4 (26.1)	70.5 (38.2)	2.0 (21.3)	16.9 (43.3)	0.7 (6.5)	2.3 (10.7)
Adult female (gravid)	5.0 (13.9)	41.7 (22.6)	0.9 (9.6)	7.8 (20.0)	0.3 (2.8)	0.9 (4.2)
Adult male	3.6 (10.0)	13.6 (7.4)	1.6 (17.0)	7.7 (19.7)	0.1 (0.9)	0.2 (0.9)
Subadult female	12.7 (35.3)	44.6 (24.2)	1.8 (19.1)	3.0 (7.7)	6.3 (58.3)	13.1 (60.9)
Subadult male	5.1 (14.2)	13.8 (7.5)	1.9 (20.2)	2.9 (7.4)	1.8 (16.7)	3.2 (14.9)
Juvenile	0.2 (0.6)	0.4 (0.2)	1.2 (12.8)	0.7 (1.8)	1.6 (14.8)	1.8 (8.4)
Total	36.0	184.6	9.4	39.0	10.8	215

persisting parts. The number of ingested copepods and copepodites was estimated by counting the mandibles and dividing this figure by 2. Uneven counts were rounded off upwards. The width of the mandible's cutting edge was measured with the image analyzer to investigate possible size selectivity of the different ontogenic stages. The calanoids were identified to genus or species level based on other recognisable parts: the caudal rami (Fig. 1b), the antennae and the fifth pleopods.

Rotifera (Fig. 1c and f), Cladocera (Fig. 1d), Harpacticoida (Fig. 1e) and nauplii of Copepoda (Calanoida and Harpacticoida) were usually found intact. Most specimens present could be identified to genus level and counted. Nauplii were noted as such. Halacaridae (Fig. 1g) and insect larvae were found occasionally, but were not used in further analyses.

Phytoplankton cells were usually found intact and were counted as such. Based on size and shape, a distinction between different types was made: solitary phytoplankton cells (Fig. 1k and l) and colonial cells or filamentous algae (Fig. 1h,i,j) were counted separately. Only a minority of the specimens found could be identified to genus level. Still a distinction could be made between species originating from intertidal areas, freshwater or brackish water in most cases (Muylaert and Sabbe, 1999, this issue).

Pollen were common in the stomachs of *N. integer*. They were counted and divided into round forms (Fig. 1m and o) and pollen of gymnosperms (Fig. 1n). The round forms could not all be identified beyond doubt, and possibly resting stages or cysts of zooplankton are included in the counts.

Large particles with a plant cell structure were denoted as 'macrophytal detritus' (Fig. 1p and q). Particles with no regular cell structure were classified as 'unidentifiable detritus' (Fig. 1r). All the detritus particles present in the stomach were counted by means of an image analyzer and the surface areas and maximal lengths of the particles were measured.

The numerical abundance of each dietary item present in the stomachs was tested for differences between estuaries, ontogenic stages and sexes by means of Kruskal–Wallis tests and subsequent multiple comparisons (Conover, 1980). For macrophytal and unidentifiable detritus, the surface areas and length-frequency distributions of the particles were compared. The frequency of occurrence of the food items present in the stomachs was calculated as the proportion of stomachs containing a certain prey item (Hyslop, 1980). Also, possible size selectivity of the different ontogenic stages of *N. integer* on Calanoida was tested with a Kruskal–Wallis test and subsequent multiple comparisons. No attempt was

Fig. 1. The dominant prey items found in the stomachs of *Neomysis integer*. Zooplankton: (a) mandible of *E. affinis*; (b) part of the calanoid copepod *E. affinis*; in the bottom left corner the caudal rami are recognisable; (c) *Keratella* species; (d) *Bosmina* species; (e) caudal part of a harpacticoid copepod; (f) *Brachionus* species; (g) *Halacaridea* species. Algae: (h) Filamental Chlorophyta; (i) filamental centricate diatom; (j) the intertidal benthic *Vaucheria* species; centricate diatom in frontal (k) and lateral (l) view. Pollen: (m and o) round forms and (n) pollen of gymnosperms. Detritus: (p and q) macrophytal and (r) 'unidentifiable' detritus particles.

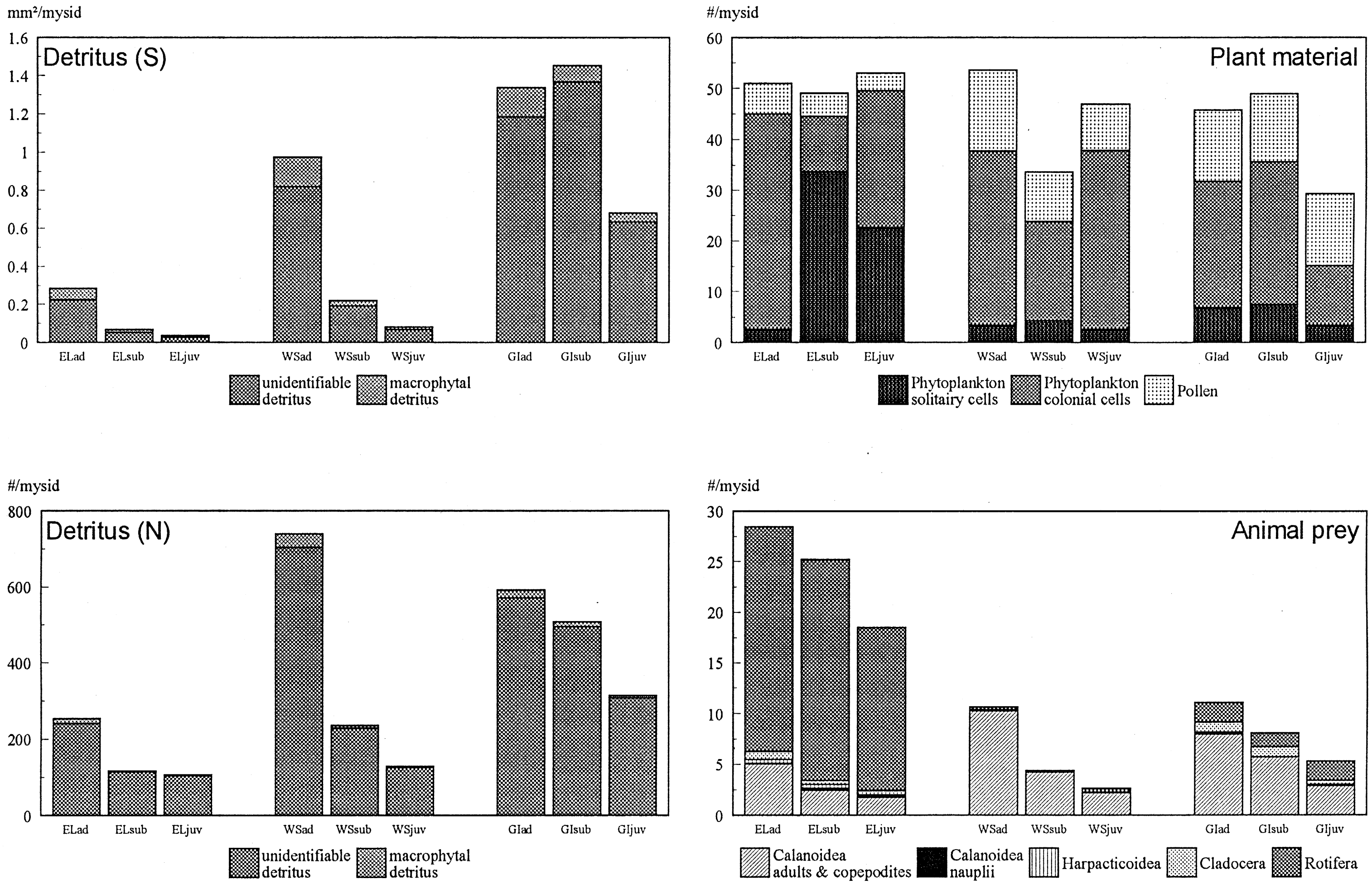


Fig. 2. Absolute composition of the diet of adult, subadult and juvenile *N. integer* in the Elbe, Westerschelde and Gironde. The surface of the detritus particles in mm^2 per mysid (upper left corner); plant material, animal prey and detrital particles in numbers per mysid. See Table 2 for the explanation of the abbreviations.

Table 2
Mean and standard error of different dietary items per adult, subadult and juvenile *N. integer* in the Elbe, Westerschelde and Gironde. Second line: frequency of occurence (%) and, between brackets, the number of individuals analyzed

	Elbe adult (ELad)	Elbe subadult (ELsub)	Elbe juvenile (ELjuv)	Westerschelde adult (WSad)	Westerschelde subadult (WSsub)	Westerschelde juvenile (WSjuv)	Gironde adult (GIad)	Gironde subadult (GIsub)	Gironde juvenile (GIjuv)
Standard	12.53 ± 0.27	9.70 ± 0.13	7.27 ± 0.20	13.44 ± 0.26	7.16 ± 0.12	4.69 ± 0.10	9.42 ± 0.09	7.95 ± 0.11	6.26 ± 0.10
length (mm)	(90)	(89)	(22)	(90)	(89)	(45)	(90)	(75)	(45)
Unidentifiable detritus	0.224 ± 0.038	0.052 ± 0.009	0.030 ± 0.007	0.821 ± 0.125	0.192 ± 0.026	0.079 ± 0.013	1.184 ± 0.126	1.369 ± 0.149	0.634 ± 0.052
Surface (mm^2)	100% (15)	100% (15)	100% (15)	100% (15)	100% (15)	100% (15)	100% (15)	100% (14)	100% (15)
Unidentifiable detritus	241.53 ± 54.83	113.47 ± 13.37	103.87 ± 11.62	704.13 ± 140.98	229.93 ± 23.13	155.20 ± 13.06	572.20 ± 61.50	496.36 ± 34.57	308.13 ± 32.88
Number	100% (15)	100% (15)	100% (15)	100% (15)	100% (15)	100% (15)	100% (15)	100% (14)	100% (15)
Macrophyte detritus	0.061 ± 0.020	0.016 ± 0.006	0.007 ± 0.003	0.152 ± 0.048	0.027 ± 0.006	0.013 ± 0.003	0.152 ± 0.035	0.084 ± 0.014	0.047 ± 0.011
Surface (mm^2)	93% (15)	77% (30)	60% (30)	100% (15)	93% (30)	73% (30)	100% (15)	100% (14)	93% (15)
Macrophyte detritus	12.57 ± 1.89	3.43 ± 1.02	1.60 ± 0.41	36.50 ± 7.04	6.40 ± 1.12	3.23 ± 0.77	20.30 ± 2.05	12.62 ± 1.99	6.57 ± 0.91
Number	97% (30)	77% (30)	60% (30)	100% (30)	93% (30)	73% (30)	100% (27)	100% (29)	93% (30)
Calanoidea	5.07 ± 0.62	2.43 ± 0.32	1.77 ± 0.18	10.27 ± 0.68	4.20 ± 0.32	2.20 ± 0.21	8.00 ± 0.73	5.72 ± 0.31	2.90 ± 0.32
Adult and copepodite	100% (30)	97% (30)	93% (30)	100% (30)	100% (30)	100% (30)	100% (27)	100% (29)	97% (30)
Harpacticoidea	0.40 ± 0.12	0.40 ± 0.11	0.20 ± 0.07	0.07 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.05	0.00 ± 0.00	0.03 ± 0.03
Adult and copepodite	33% (30)	33% (30)	20% (30)	7% (30)	0% (30)	0% (30)	7% (27)	0% (29)	3% (30)
Calanoidea and Harpacticoidea	33.46 ± 0.93	35.82 ± 1.71	36.94 ± 2.04	43.47 ± 0.66	39.38 ± 0.84	38.04 ± 1.34	36.79 ± 0.58	35.08 ± 0.68	32.45 ± 0.90
Mandible width (μm)	(206)	(85)	(53)	(407)	(204)	(84)	(320)	(271)	(137)
Calanoidea and Harpacticoidea	0.03 ± 0.03	0.23 ± 0.09	0.07 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.07	0.00 ± 0.00	0.10 ± 0.06
Nauplii	3% (30)	20% (30)	70% (30)	0% (30)	0% (30)	0% (30)	15% (27)	0% (29)	10% (30)
Cladocera	0.80 ± 0.11	0.37 ± 0.10	0.40 ± 0.09	0.07 ± 0.05	0.00 ± 0.00	0.03 ± 0.03	0.96 ± 0.16	1.03 ± 0.14	0.40 ± 0.12
	70% (30)	33% (30)	40% (30)	7% (30)	0% (30)	3% (30)	70% (27)	76% (29)	33% (30)
Rotifera	22.17 ± 2.50	21.83 ± 2.05	16.10 ± 1.43	0.27 ± 0.10	0.17 ± 0.07	0.40 ± 0.14	1.93 ± 0.38	1.38 ± 0.22	1.87 ± 0.29
	100% (30)	100% (30)	100% (30)	23% (30)	17% (30)	27% (30)	67% (27)	79% (29)	83% (30)
Phytoplankton	2.53 ± 0.42	33.73 ± 6.72	22.63 ± 6.03	3.40 ± 0.50	4.30 ± 0.78	2.67 ± 0.39	6.93 ± 2.35	7.45 ± 3.86	3.40 ± 0.47
solitary	77% (30)	100% (30)	97% (30)	87% (30)	93% (30)	90% (30)	85% (27)	66% (29)	93% (30)
Phytoplankton	42.50 ± 17.63	10.80 ± 4.85	26.90 ± 8.69	34.40 ± 12.75	19.57 ± 9.27	35.20 ± 7.23	24.89 ± 6.52	28.24 ± 5.40	11.83 ± 3.33
colonial	77% (30)	47% (30)	63% (30)	83% (30)	57% (30)	97% (30)	96% (27)	97% (29)	73% (30)
Pollen	5.93 ± 0.53	4.50 ± 0.51	3.47 ± 0.44	15.80 ± 1.41	9.80 ± 1.05	9.07 ± 0.92	14.00 ± 1.02	13.31 ± 0.79	14.20 ± 1.18
	97% (30)	97% (30)	90% (30)	100% (30)	100% (30)	100% (30)	100% (27)	100% (29)	100% (30)

Table 3

(a) Results of the Kruskal–Wallis tests (KW) and subsequent multiple comparisons for the different dietary items for adult, subadult and juvenile *N. integer* of Elbe, Westerschelde and Gironde (latitudinal and ontogenic effects). See Table 2 for the explanation of the abbreviations. (with: *: $p < 0.05$; * *: $p < 0.01$; * * *: $p < 0.001$; NS: $p > 0.05$)

	KW	Latitude									Ontogeny								
		ELad vs. WSad	ELad vs. GIad	WSad vs. GIad	ELsub vs. WSub	ELsub vs. GIsub	WSsub vs. GIsub	ELjuv vs. WSjuv	ELjuv vs. GIjuv	WSjuv vs. GIjuv	ELad vs. Elsub	ELad vs. ELjuv	ELsub vs. ELjuv	WSad vs. WSsub	WSad vs. WSjuv	WSsub vs. WSjuv	GIad vs. GIsub	GIad vs. GIjuv	GIsub vs. GIjuv
Unidentifiable detritus Surface (mm^2)	* * *	* * *	* * *	*	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	*	* * *	* * *	* * *	NS	* * *	* * *
Unidentifiable detritus Number	* * *	* * *	* * *	NS	* * *	* * *	* * *	*	* * *	* * *	* *	* * *	NS	* * *	* * *	*	NS	* * *	* * *
Macrophyte detritus Surface (mm^2)	* * *	*	* *	NS	*	* * *	* * *	NS	* * *	* * *	* * *	* * *	NS	* * *	* * *	*	NS	* *	NS
Macrophyte detritus Number	* * *	* * *	* *	NS	* *	* * *	* * *	NS	* * *	* * *	* * *	* * *	NS	* * *	* * *	* *	* *	* * *	* *
Calanoidea Adult and copepodite	* * *	* * *	* * *	*	* * *	* * *	* *	NS	* *	NS	* * *	* * *	NS	* * *	* * *	* * *	NS	* * *	* * *
Harpacticoidea Adult and copepodite	* * *	* * *	* *	NS	* * *	* * *	NS	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Calanoidea and Harpacticoidea Nauplii	* *	NS	NS	*	* *	* *	NS	NS	NS	NS	* *	NS	*	NS	NS	NS	*	NS	NS
Cladocera	* * *	* * *	NS	* * *	* *	* * *	* * *	* *	NS	* *	* * *	* *	NS	NS	NS	NS	NS	* * *	* * *
Rotifera	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	NS	NS	NS	NS	NS	NS	NS	NS	NS
Phytoplankton solitary	* * *	NS	*	NS	* * *	* * *	NS	* * *	* * *	NS	* * *	* * *	*	NS	NS	NS	NS	NS	NS
Phytoplankton colonial	* * *	NS	NS	NS	NS	* * *	* * *	* * *	NS	* * *	* *	NS	*	*	*	* * *	NS	*	* *
Pollen	* * *	* * *	* * *	NS	* * *	* * *	* * *	* * *	* * *	* * *	NS	* *	NS	* * *	* * *	NS	NS	NS	NS

(b) Results of the Kruskal–Wallis tests (KW) and subsequent multiple comparisons for the different dietary items for adult and subadult male (mal) and female (fem) *N. integer* of Elbe, Westerschelde and Gironde (sexual effect). See Table 2 for the explanation of the abbreviations. (with: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; NS: $p > 0.05$)

	KW	Sex					
		ELad mal vs. ELad fem	ELsub mal vs. ELsub fem	WSad mal vs. WSad fem	WSsub mal vs. WSsub fem	GIad mal vs. GIad fem	GIsub mal vs. GIsub fem
Unidentifiable detritus Surface (mm^2)	no data	no data	no data	no data	no data	no data	no data
Unidentifiable detritus Number	no data	no data	no data	no data	no data	no data	no data
Macrophyte detritus Surface (mm^2)	no data	no data	no data	no data	no data	no data	no data
Macrophyte detritus Number	* * *	* * *	*	* * *	NS	*	NS
Calanoidea Adult and copepodite	* * *	* * *	NS	NS	NS	NS	NS
Harpacticoidea Adult and copepodite	* * *	NS	NS	NS	NS	NS	NS
Calanoidea and Harpacticoidea Nauplii	* *	NS	NS	NS	NS	* *	NS
Cladocera	* * *	NS	NS	NS	NS	NS	NS
Rotifera	* * *	NS	NS	*	NS	* *	NS
Phytoplankton solitary	* * *	NS	NS	NS	NS	NS	NS
Phytoplankton colonial	* * *	NS	NS	NS	NS	NS	NS
Pollen	* * *	NS	NS	NS	NS	NS	NS

made to determine the relative importance of the various food items to the total energy intake of the mysid population.

Further characterisation of the 'unidentifiable detritus' was done by EDAX analysis, using a JEOL JSM-6400 scanning electron microscope with a Voyager II 2100/2110 microanalysis system (Noran Instruments). The stomachs of 10 adult animals per estuary were dissected out. The content was rinsed out in a drop of distilled water, placed on specimen mounts and dried in an oven (40°C) for 30 min. The mounted samples were subsequently coated with carbon. The elemental composition of the detritus flocs was determined, recalculated for the eight most abundant elements (excluding C and O), and compared between estuaries.

2.3. Quantitative diet analyses

The stomach of each mysid was carefully dissected out after removing the carapax. The stomach (and its content) and the mysid were dried separately in small aluminium weighing pans for 4 days at 60°C, after which the dry weight of both was determined with a microbalance to the nearest 1 μg. For the comparison of the three estuaries, 20 adult females and 20 adult males from each estuary were processed. For the ontogenic diet comparison five times three subadult and five times five juvenile individuals were pooled.

Additionally, the empty stomachs of 30 adults per estuary were weighed after carefully emptying the dissected stomach. A linear regression analysis was done on the dry weight of the mysids and the dry weights of the corresponding empty stomachs. The dry weight of the stomach content itself was then calculated as follows:

$$DW_{content} = DW_{stomach} - DW_{empty}$$

$$\text{where: } DW_{empty} = a + b * DW_{mysid}$$

with $DW_{content}$ the dry weight of the stomach content, $DW_{stomach}$ the dry weight of the stomach with its content, DW_{empty} the dry weight of the empty stomach derived by a regression from DW_{mysid} (the dry weight of the mysid without its stomach).

A fullness index (FI) was calculated with these data. This relative measure is frequently used in fisheries research for the comparison of stomach contents of fish taken from different size classes (e.g., Hyslop, 1980). The amount of food present in the stomach of *N. integer* at a given time t is then expressed as the fullness index FI_t:

$$FI_t = \frac{DW_{content}}{DW_{mysid}} \times 100$$

Latitudinal, ontogenic and sexual differences in FI's were assessed with Kruskal–Wallis tests and subsequent multiple comparisons (Conover, 1980).

3. Results

N. integer was found with densities of 36.0, 9.4 and 10.8 individuals per m^2 and biomasses of 184.6, 39.0 and 21.5 mg ash free dry weight per m^2 in the MTZ stations of the Elbe, Westerschelde and Gironde, respectively. The absolute and relative density and biomass of all sexes and stages of *N. integer* present in the MTZ in the three estuaries (spring 1993) are shown in Table 1. The standard length of the mysids differed significantly between estuaries, ontogenic stages and sexes (ANOVA and contrast analysis).

3.1. Qualitative diet analysis

3.1.1. Comparison between estuaries

In the three estuaries, the diet of all ontogenic stages of *N. integer* was composed of zooplankton, phytoplankton and detritus (Fig. 2; Table 2). In the Westerschelde and the Gironde the diet of *N. integer* was numerically dominated by adult and copepodite stages of the calanoid copepod *E. affinis* (Fig. 1a and b) with respectively 10.27 and 8.00 calanoids consumed per adult, 4.20 and 5.72 per subadult and 2.20 and 2.90 per juvenile mysid. In the Elbe rotifers were the most abundant animal prey items for the three ontogenic stages. Here, only 5.07 calanoid copepods were consumed per adult, 2.43 per subadult, and 1.77 per juvenile mysid. Adult and copepodite stages of harpacticoids (Fig. 1e) were rare in the stomachs. They were only encountered in 7% of the adult individuals of the Westerschelde and 3–7% of the juveniles and adults in the Gironde. In the Elbe harpacticoids occurred in 20–33% of the stomachs, though always in low numbers. Nauplii (of calanoids and harpacticoids) were present in low numbers in the stomachs from the Elbe (0.03–0.23 ind^{-1}) and the Gironde (0.10–0.15 ind^{-1}); they were not consumed in the Westerschelde. Their frequency of occurrence was low (3–20% in adults and subadults of the Elbe and 10–15% in the Gironde), except for the juveniles in the Elbe (70%). Cladocera of the genus *Bosmina* (Fig. 1d) were encountered in 33–76% of the stomachs of Elbe and Gironde, but rarely in those of the Westerschelde (max. 7%). Rotifers of the genera *Keratella* (Fig. 1c) and *Brachionus* (Fig. 1f) were the most abundant prey items for *N. integer* in the MTZ of the Elbe (16.10–22.17 ind^{-1}). In the Gironde and the Westerschelde they were consumed in lesser numbers (1.38–1.93 ind^{-1} and 0.17–0.40 ind^{-1} respectively). The frequency of occurrence of the rotifers was 100% in the Elbe, 67–83% in the Gironde and 17–27% in the Westerschelde. Other zooplanktonic prey were very rarely encountered and were excluded for further analyses. Halacaridae (Fig. 1g) were infrequently encountered in the Westerschelde and the Gironde (a total of six observations) and one larval Homoptera (Insecta) was found in the Gironde.

For each prey item the overall longitudinal effects were significant. The average numbers per stomach were tested for significant differences between the three estuaries for the three ontogenic stages (Table 3a) by multiple comparisons. Except for copepod nauplii and harpacticoids in all ontogenic stages and calanoids in juveniles, most of the differences were highly significant.

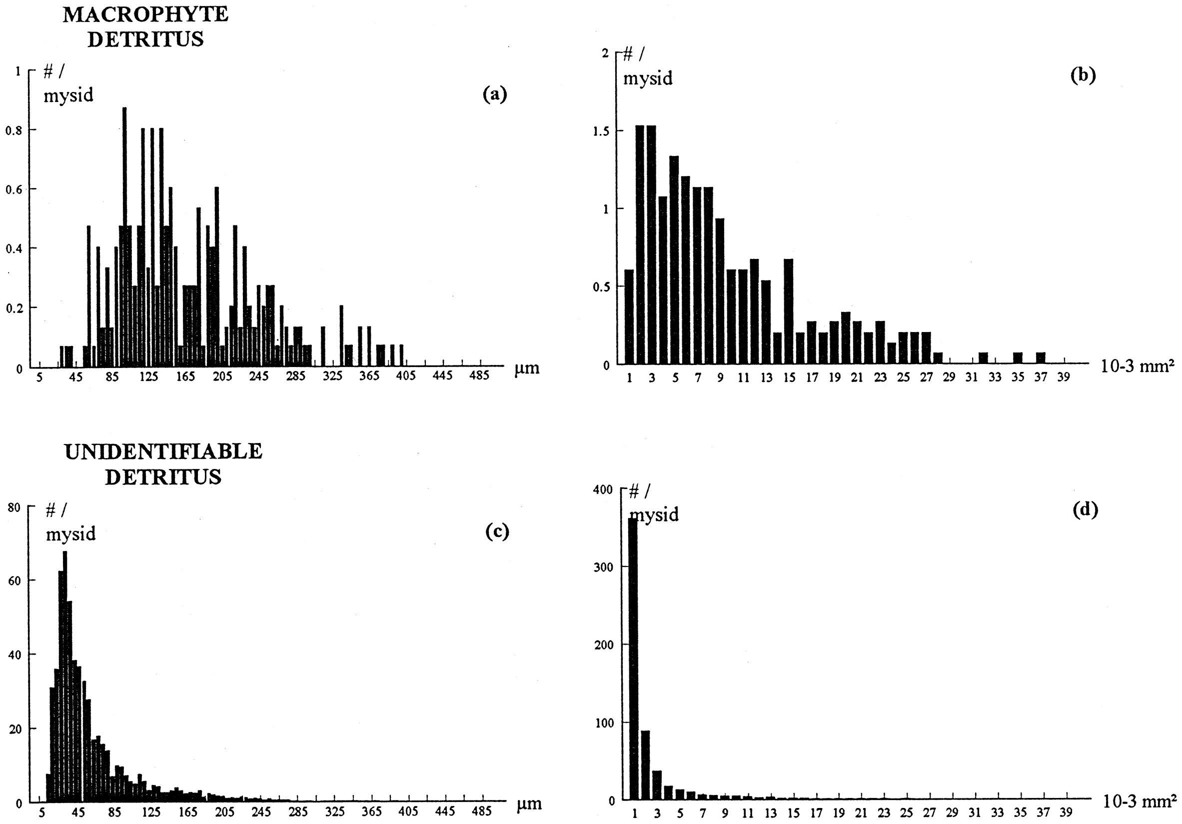
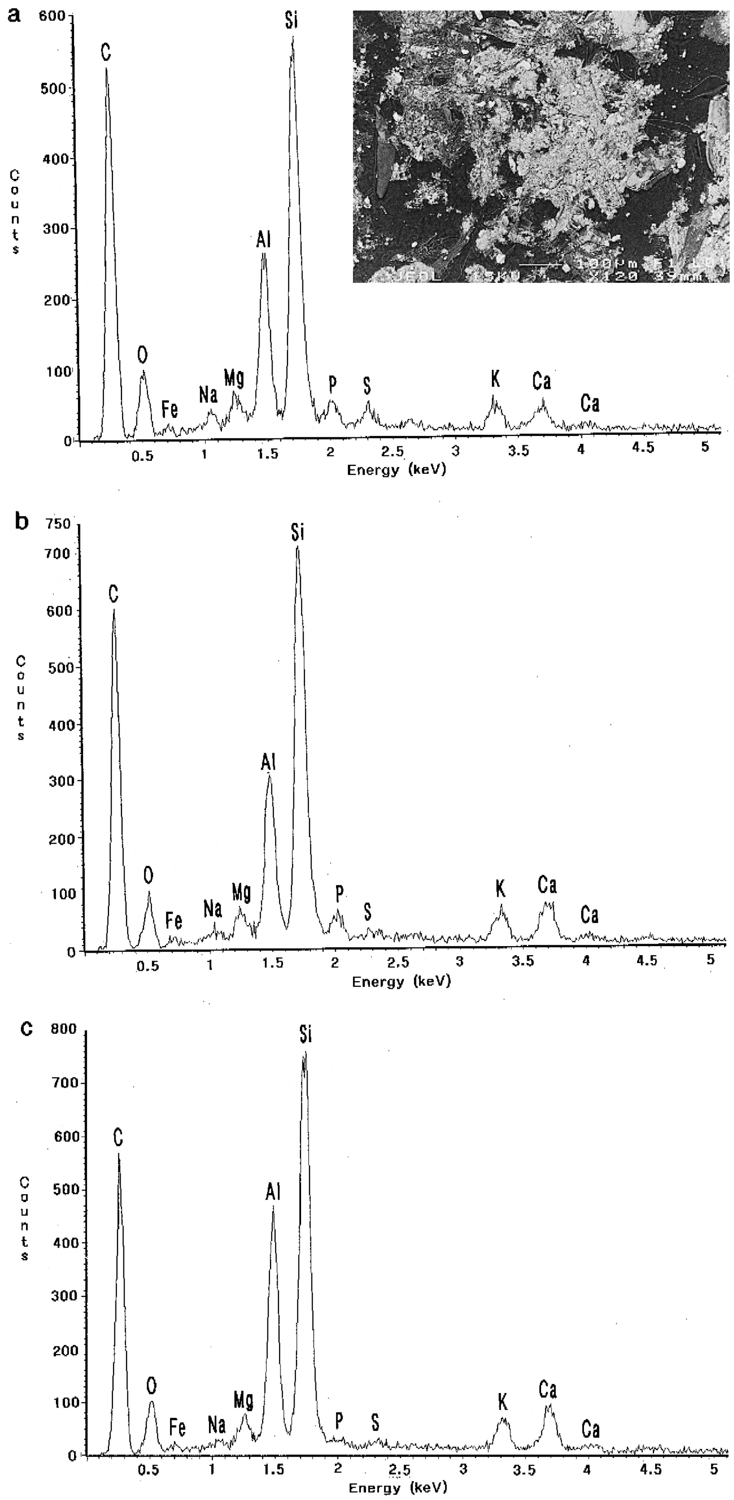


Fig. 3. Length (left) and surface area (right) frequency distribution of macrophyte detritus (top) and ‘unidentifiable’ detritus (bottom) present in the stomach of adult *N. integer* in the MTZ of the Gironde.


a
b
c
Counts
Energy (keV)
0
100
200
300
400
500
600
700
750
800
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
C
O
Fe
Na
Mg
Al
Si
P
S
K
Ca
Ca
100µm
JEOL 15kV ×120 39mm

Also some phytoplankton was consumed by *N. integer* in the three estuaries. Solitary (Fig. 1k and l) and colonial phytoplankton (Fig. 1h,i,j) species could be recognized in the stomachs with a mean frequency of occurrence of 82%. Colonial and filamental algal strands were the most abundant (Table 2). For adults, e.g., 42.5, 34.4 and 24.9 cells (i.e., 4.6, 5.1 and 4.8 strands) were found per mysid in Elbe, Westerschelde and Gironde respectively, while solitary cells only amounted to 2.5, 3.4 and 6.9 counts per adult. Still, most of the differences were not significant (Table 3a). Similar trends were found for subadults and juveniles in the Westerschelde and the Gironde. Only in the Elbe, these ontogenic groups had consumed significantly higher amounts of solitary phytoplankton cells (Table 3a) as compared to the other estuaries.

Pollen was found in 90 to 100% of the stomachs. Average numbers per stomach were 4.6, 11.6 and 13.8 in Elbe, Westerschelde and Gironde, respectively. Two general types were distinguished. Pollen grains with air sacs on either side (Fig. 1n) were recognized as originating from gymnosperms. These were especially abundant in the Gironde (62.53%) and the Westerschelde (37.3%), while in the Elbe only 6.3% of the pollen originated from gymnosperms. The round forms (Fig. 1m and o) could not be identified and possibly resting stages of mesozooplankton or cysts of microzooplanktonic species are included in the counts.

The frequency with which macrophytal detritus was consumed in the three estuaries was always higher than 60%, although macrophyte detritus accounted for less than 5% of the total number of detritus particles consumed. The size distributions of the macrophytal detritus in the stomachs (e.g., for adults in the Gironde, Fig. 3a and b) were comparable over the estuaries: the majority ($> 90\%$) was smaller than 0.020 mm^2 for adults, 0.013 mm^2 for subadults and 0.015 mm^2 for juveniles. Maximal particle sizes of 0.039 mm^2, 0.061 mm^2 and 0.064 mm^2 were recorded in Elbe, Westerschelde and Gironde, respectively. Highest numbers of macrophyte detritus particles were found in adults in the Westerschelde (36.50 ind^{-1}), while subadults and juveniles in the Gironde contained significantly higher numbers (12.62 and 6.57 ind^{-1} respectively) as compared to the same ontogenic stages in the other estuaries.

Table 4
Relative elemental composition (weight percentage ± standard error) of the flocs found in the stomachs of adult *N. integer* from Elbe, Westerschelde and Gironde. Note that the relative abundance was calculated with the eight most abundant elements, excluding C and O

Element	ELad	WSad	GIad
Mg	2.65 ± 0.51	1.30 ± 0.48	2.09 ± 0.35
Al	15.56 ± 0.82	13.52 ± 0.85	20.56 ± 0.70
Si	41.87 ± 1.16	43.61 ± 1.28	44.71 ± 0.99
P	4.87 ± 1.21	5.57 ± 1.38	1.45 ± 0.89
S	5.05 ± 0.82	1.78 ± 0.90	1.71 ± 0.59
K	5.59 ± 0.73	5.85 ± 0.93	6.76 ± 0.67
Ca	6.10 ± 0.86	13.24 ± 1.20	11.13 ± 0.85
Fe	18.20 ± 1.91	15.13 ± 2.36	11.58 ± 1.60

The size frequency distributions of the unidentifiable fraction of the detritus (Fig. 3c and d for adults in the Gironde) showed the same patterns in all estuaries. The bulk (90%) of the particles found in adult stomachs were smaller than 85 μm (Elbe), 90 μm (Westerschelde) and 125 μm (Gironde). In subadults and juveniles the bulk of the particles had smaller sizes. Particles with a maximal length up to 300 μm (Elbe), 500 μm (Westerschelde) and 600 μm (Gironde) were regularly found in the stomachs. In the three estuaries, the modes of the size frequency distributions were located around 30–35 μm. Adult *N. integer* of the Westerschelde consumed the highest number of unidentifiable detrital particles (704 particles ind^{-1}), while for subadults and juveniles highest numbers were found in the Gironde (496 and 308 ind^{-1} respectively). Mean total numbers of detritus particles consumed by the three ontogenic stages were 153, 363 and 458 ind^{-1} in Elbe, Westerschelde and Gironde.

A more detailed analysis of the ‘unidentifiable fraction’ of the detritus was done using a petro-

Fig. 4. Output of an EDAX analysis on ‘unidentifiable’ detritus particles from the stomachs of adult *N. integer* in Elbe (a), Westerschelde (b) and Gironde (c). The *X*-axis was cut off short of the primary Fe-peaks (located around 6.4 and 7.1 keV). Upper right corner: back-scattered electron (BSE) image of an analyzed floc.

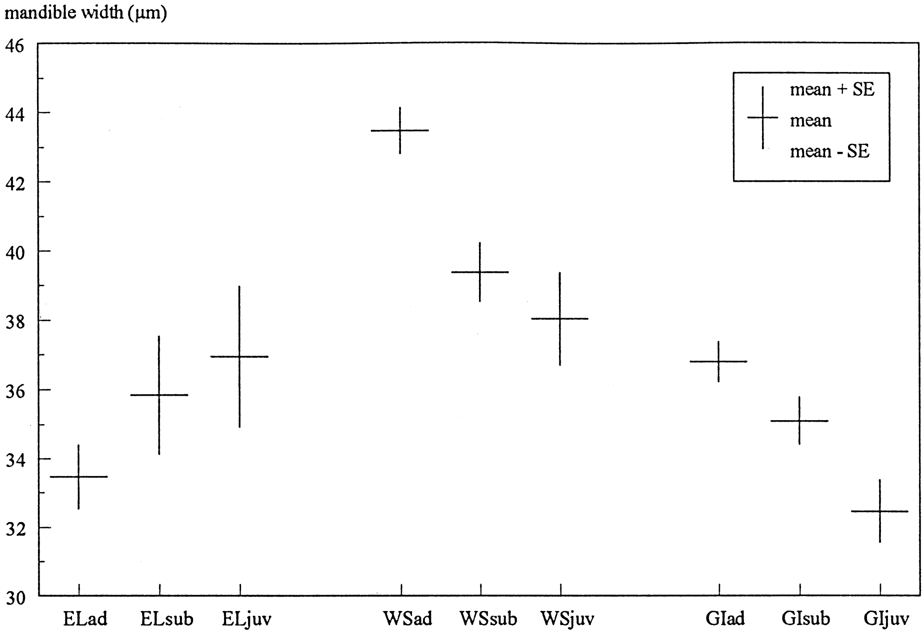


Fig. 5. Mean mandible width (and standard error) of the calanoid copepods *E. affinis* consumed by adult, subadult and juvenile *N. integer* in Elbe, Westerschelde and Gironde. See Table 2 for the explanation of the abbreviations.

graphic optical microscope. The particles showed a high mineral content. The elemental composition of the detritus was assessed by EDAX analysis. In back-scattered electron (BSE) images of the samples, the flocs were easily identified as aggregates with much lighter grey values, indicating a major difference in composition between the flocs and other components (photograph on Fig. 4). For EDAX analyses, only flocs with lengths of 150–300 µm were used. The composition of the flocs was very similar in the three estuaries (Fig. 4 and Table 4). The elemental composition, dominated by silicon and aluminium (around 60% by weight) and with subordinate amounts of magnesium, potassium and iron, demonstrate that the flocs mainly consist of clay minerals. Part of the iron occurs in the form of pyrite (FeS_2), whose presence as individual crystals or grains, was often directly observed. Because a car-

Table 5

Results of the Kruskal–Wallis tests (KW) and subsequent multiple comparisons for the mandible width of the copepods consumed by adult, subadult and juvenile *N. integer* of Elbe, Westerschelde and Gironde (ontogenic and sexual effects). See Table 2 for the explanation of the abbreviations. (with: ∗: $p < 0.05$; ∗∗: $p < 0.01$; ∗∗∗: $p < 0.001$; NS: $p > 0.05$)

Ontogeny

KW	ELad vs. ELsub	ELad vs. ELjuv	ELsub vs. ELjuv	WSad vs. WSsub	WSad vs. WSjuv	WSsub vs. WSjuv	GIad vs. GIsub	GIad vs. GIjuv	GIsub vs. GIjuv
∗∗∗	NS	NS	NS	∗∗∗	∗∗∗	NS	NS	∗∗∗	∗

Sex

KW	ELad mal vs. ELad fem	ELsub mal vs. ELsub fem	WSad mal vs. WSad fem	WSsub mal vs. WSsub fem	GIad mal vs. GIad fem	GIsub mal vs. GIsub fem
∗∗∗	NS	NS	NS	NS	NS	NS

bon coating was used, the carbon content of the flocs could not be quantified, but the EDAX spectra and BSE images show that their carbon content is not high. No diatoms or other unicellular organisms could be found attached to the flocs.

3.1.2. Comparison of developmental stages

The diet of subadult and juvenile *N. integer* consisted of the same prey categories as that of adults, but generally a lower number of particles was consumed by the smaller mysids (Fig. 2; Tables 2 and 3). Stomachs of juveniles in the Westerschelde and Gironde contained significantly less detritus, calanoid copepods and colonial phytoplankton cells, as compared to these of adults and subadults. In the Elbe the diet of juveniles resembled that of the subadults, whereas in the Gironde the diet of adults rather resembled that of the subadults. Ontogenic differ-

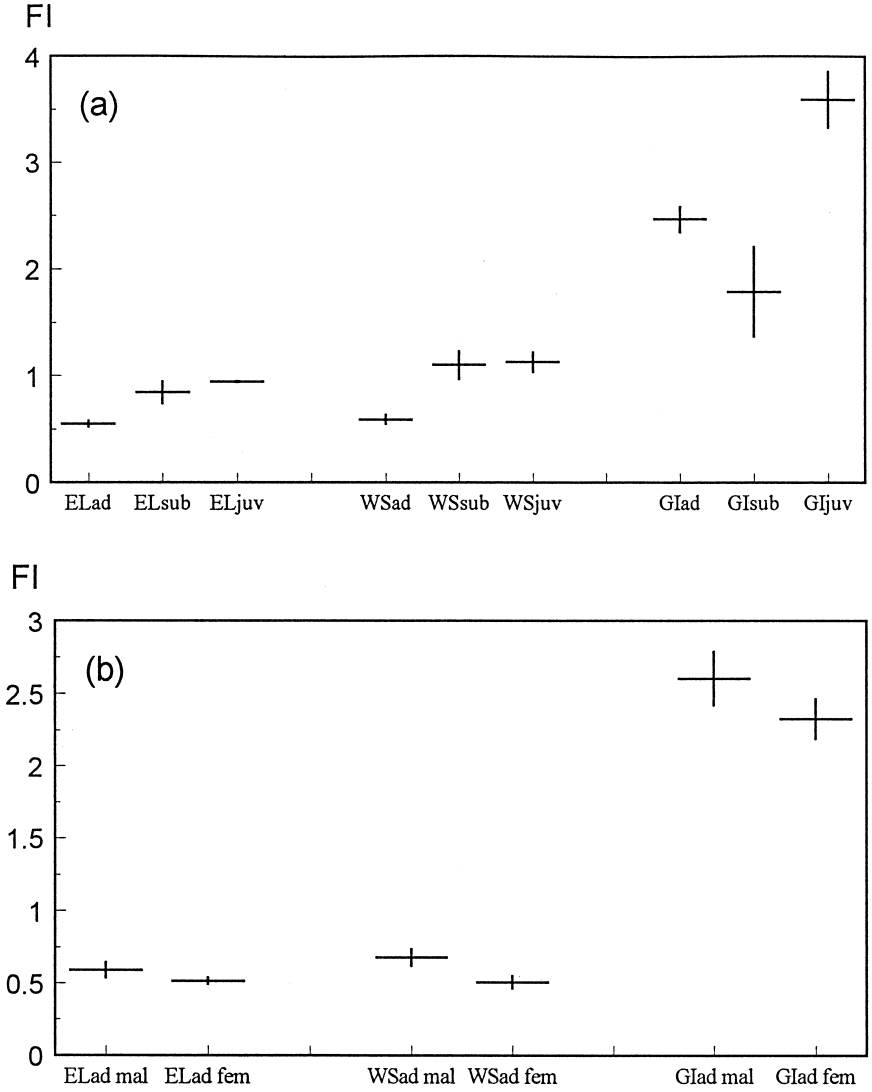


Fig. 6. The mean fullness index FI (and standard error) for (a) adult, subadult and juvenile and (b) adult male and female *N. integer* in Elbe, Westerschelde and Gironde. See Table 2 for the explanation of the abbreviations.

ences in the number of solitary phytoplankton consumed where only found in the Elbe. The number of pollen consumed only differed in the Elbe and the Westerschelde, and Cladocera only in the Elbe and the Gironde.

Adults and copepodites of *E. affinis* were the most important zooplankters consumed by all the mysid stages, except in the Elbe where rotifers were the most abundant taxon in the diet. In the Westerschelde and the Gironde the smaller mysids selected significantly smaller copepods, whereas in the Elbe no significant difference in copepod size selection was found between the ontogenic stages (Fig. 5;Tables 2 and 5). This is possibly correlated with the fact that in the Elbe the number of mandibles measured was significantly lower than in the other estuaries.

The total number of detritus particles consumed was comparable for adults and subadults in the Gironde (593 and 509 particles ind^{-1}), while the stomachs of juveniles contained significantly less particles (315 ind^{-1}). In the Westerschelde the three ontogenic stages consumed different amounts of detritus: 741, 236 and 158 particles ind^{-1} for adults, subadults and juveniles respectively. In the Elbe adults consumed significantly higher numbers of detritus particles (254.10 ind^{-1}) than subadults and juveniles (116.90 and 105.47 ind^{-1}). Macrophytal particles only accounted for a minor part of the total detrital fraction in the diet (3–5% for adults, 2–3% for subadults and juveniles). The mean size of the macrophyte detritus particles was independent of the size of the mysid: for all ontogenic stages the mean surface area per macrophyte particle was around 0.004 mm^2 in Elbe and Westerschelde and 0.007 mm^2 in the Gironde. The size range of the unidentifiable detritus was comparable for the different mysid stages in the Gironde: all stages mainly contained particles with a surface area smaller than 0.005 mm^2 (modal length 35 μm). In the Elbe and Westerschelde the size of the unidentifiable detritus particles found in the stomach decreased with the size of the mysid: modal length of 30 μm in adults, 15 μm in subadults and 10 μm in juveniles.

3.1.3. Comparison of the sexes

For most dietary items no sexual difference was found (Table 3b). An exception is that in all estuaries the numbers of macrophytal detritus particles were higher in the stomachs of adult females as compared to adult males (a factor of 2.3, 3.1 and 1.5 in the Elbe, Westerschelde and Gironde, respectively). Another exception is that adult males in the Elbe consumed only 3.0 calanoid copepods per individual versus 7.1 for the adult females.

Table 6

Results of Kruskal–Wallis tests and subsequent multiple comparisons on the fullness index (FI) for the latitudinal-ontogenic and latitudinal-sexual effects. See Table 2 for the explanations of the abbreviations. (with: ∗: $p < 0.05$; ∗∗: $p < 0.01$; ∗∗∗: $p < 0.001$; NS: $p > 0.05$)

Latitude ∗∗∗		*Ontogeny ∗∗*		*Sex (adults): NS*	
EL vs. WS	NS	ad vs. sub	NS		
EL vs. GI	∗∗∗	ad vs. juv	∗∗		
WS vs. GI	∗∗∗	sub vs. juv	NS		
Latitude and ontogeny ∗∗∗				*Latitude and sex (adults): ∗∗∗*	
ELad vs. WSad	NS	ELad vs. ELsub	∗∗	EL mal vs. EL fem	NS
ELad vs. GIad	∗∗∗	ELad vs. ELjuv	∗∗	WS mal vs. WS fem	∗∗
WSad vs. GIad	∗∗∗	ELsub vs. ELjuv	NS	GI mal vs. GI fem	NS
ELsub vs. WSsub	NS	WSad vs. WSsub	∗∗∗	EL mal vs. WS mal	NS
ELsub vs. GIsub	NS	WSad vs. WSjuv	∗∗∗	EL mal vs. GI mal	∗∗∗
WSsub vs. GIsub	NS	WSsub vs. WSjuv	NS	WS mal vs. GI mal	∗∗∗
ELjuv vs. WSjuv	NS	GIad vs. GIsub	∗	EL fem vs. WS fem	NS
ELjuv vs. GIjuv	∗∗∗	GIad vs. GIjuv	NS	EL fem vs. GI fem	∗∗∗
WSjuv vs. GIjuv	∗∗	GIsub vs. GIjuv	∗∗	WS fem vs. GI fem	∗∗∗

3.2. Quantitative diet analysis

The dry weight of the empty stomachs could be derived from the following regression equation, after which the dry weight of the stomach contents could be calculated by subtraction.

$$ln\,\mathrm{DW}_{\mathrm{empty}} = 5.04722 + 0.513386\,ln\,\mathrm{DW}_{\mathrm{mysid}}$$

$$(N = 89; r = 0.672; p = 0.000)$$

The fullness indices (FI) were compared between the estuaries, ontogenic stages and sexes with Kruskal–Wallis tests and subsequent multiple comparisons (see Fig. 6 and Table 6). General latitudinal effects in the stomach fullness could not be demonstrated between Elbe and Westerschelde, while in the Gironde significantly higher fullness indices were recorded for adults and juveniles. In the three estuaries adult *N. integer* had a significantly lower FI than juveniles. In Elbe and Westerschelde the FI's of subadult *N. integer* were comparable to those of juveniles. In the Gironde the subadults had a lower FI than adults and juveniles. No sexual effect could be detected in the fullness index.

4. Discussion

4.1. Methodology

The size range of adult, subadult and juvenile *N. integer* used for stomach analysis comparison was different in the three estuaries (Table 2). This is not surprising, since the three populations were sampled in different stages of the annual population dynamical cycle. The different length-frequency distributions and population compositions (Table 1) found in the three estuaries can be explained by a seasonal temperature effect. The average water temperature in Elbe, Westerschelde and Gironde at the time of sampling was 9°C, 15°C and 17°C, respectively. Studies on the population dynamics of *N. integer* in the Westerschelde (Mees et al., 1994) and the Gironde (Mees and Sorbe, in preparation) have shown that the adults of the overwintering cohort are larger than those belonging to the summer generations and that the species does not reproduce when water temperature is lower than 10°C. In the Elbe samples, few juveniles were found and many of the females were gravid, indicating that the population was still in a 'winter phase'. The relatively higher abundance of juveniles in the Westerschelde (13% of the total density) and the Gironde (15%) samples was due to the fact that reproduction by the overwintering cohort took place before the time of sampling. Although the sampling campaigns in the three estuaries were executed within a 1-month period, the latitudinal temperature effect was amplified by the North to South sequence of sampling. Also, the length at maturity has been found to increase with increasing latitude (Mees et al., in preparation).

For the preparation of the microscopic slides the mysids were dehydrated from a formaldehyde solution to glycerin, in which the stomach content was subsequently embedded. This procedure yields semi-permanent slides in which artifacts are avoided. The microscopic slides contained parts of the stomach and oesophagus tissue, but their cell structure and armature made them easily distinguishable from parts of zooplanktonic prey categories.

Generally, the gut passage time of mysids has been reported to be in the order of 30–90 min. (Zagursky and Feller, 1985), so the particles present in the stomach will give a good idea about the recently ingested food. The frequencies of occurrence (Table 2) of the ingested prey items were usually higher than 60%, except for calanoid nauplii, harpacticoids and cladocerans. Therefore, the within variation of a sample is low and the analysis of 30 animals per sample sufficed to describe the diet of *N. integer* (Hyslop, 1980).

4.2. General latitudinal, ontogenic and sexual effects

The fullness indices of *N. integer* from the Elbe and the Westerschelde were comparable (0.55 and 0.59 respectively), while in the Gironde a significantly higher FI was measured (2.46). The adults from the former two estuaries showed quite a wide size range distribution (Table 2), but a rather constant FI. Adult *N. integer* from the Gironde had a significantly smaller standard length, a smaller size variation, and a large variation in their fullness index. This phenomenon is demonstrated graphically in Fig. 7.

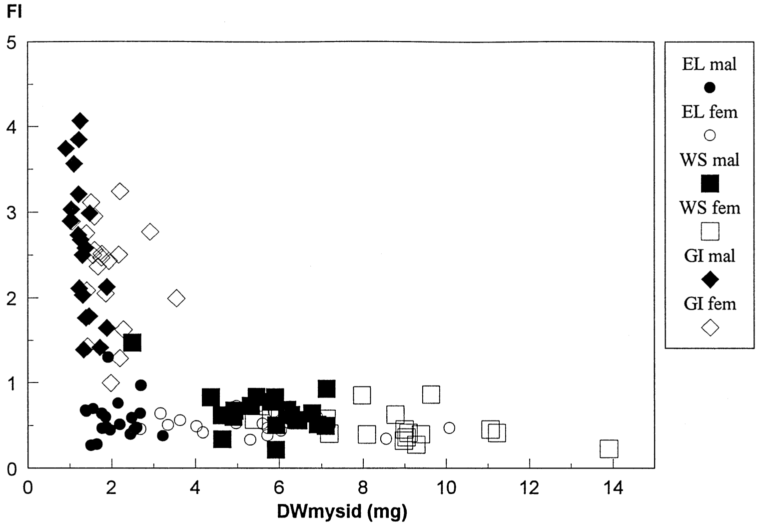


Fig. 7. Relationship between the fullness index (FI) and the dry weight of the mysids (DW_{mysid}) for adult male and female *N. integer* in Elbe, Westerschelde and Gironde.

Irrespective of the ontogenic stage, consumption of detritus particles and pollen by *N. integer* increased from the Elbe, over the Westerschelde, to the Gironde. The numbers of calanoids, harpacticoids and phytoplankton consumed were comparable in the Westerschelde and the Gironde. In both estuaries, calanoids were consumed in higher numbers as compared to the Elbe, while harpacticoids and phytoplankton were found in lower quantities. No Cladocera, Rotifera or copepod nauplii were found in the stomachs of *N. integer* of the Westerschelde. In the two other estuaries, nauplii were always rare in the diet, while Cladocera were most important in the Gironde and Rotifera in the Elbe.

Irrespective of the estuary, large adults had a lower fullness index than smaller individuals. Adults consumed significantly higher amounts of detritus, calanoids, cladocers, colonial phytoplankton cells and pollen. No ontogenic effects could be demonstrated for harpacticoids, copepod nauplii, solitary phytoplankton cells and rotifers.

An ontogenic shift in diet composition has been reported for *N. mercedis* (Kost and Knight, 1975; Siegfried and Kopache, 1980). The main changes were found for the smallest juveniles (2–3 mm), a size class, which was not efficiently sampled in this study (Mees et al., 1994). The mandible widths of the calanoids consumed decreased with the size of the mysid in the Gironde and the Westerschelde. Although juvenile *N. integer* consumed the smallest calanoid copepodite stages, spermatophores were frequently found in the stomachs. Since these are attached to the gonopores of adult females only, this suggests that juvenile mysids at least hunt adult calanoid copepods. In the Elbe *N. integer* did not show a size selectivity for *E. affinis*, but here the number of mandibles measured was significantly lower than in the other estuaries.

No sexual differences could be found in the fullness index, or in the amount of dietary items consumed. The only exceptions were calanoids and macrophytal detritus particles, which were sometimes consumed in significantly higher amounts by adult females.

4.3. Food items

N. integer mainly fed upon mesozooplankton. Late copepodite stages and adults of *E. affinis* were the most important prey item. On average 5.07, 10.27 and 8.00 calanoids per adult mysid were consumed in the MTZ's of Elbe, Westerschelde and Gironde, respectively. Subadults and juveniles consumed less copepods (Table 2). Siegfried and Kopache (1980) calculated that the relative importance of carnivory amounted to 90% of the total nutritional uptake of *N. mercedis*. 90–100% of the diet of *Mysis mixta* consisted of copepods and Cladocera (Rudstam et al., 1989). In both studies however, the detritus was not included in the calculations, thus overestimating the importance of carnivory.

Although the gut passage time of mysids is in the order of 1 h, it has been shown that rigid zooplankton parts (e.g., mandibles) can stay in the stomachs for more than 12 h (Rudstam et al., 1989). The counting of the number of mandibles present in the stomach can therefore result in an overestimation of the actual number of copepods consumed.

During the sampling campaigns, the densities of adult and copepodite stages of *E. affinis* in the MTZ of the Elbe and Westerschelde were in the order of 10 000 and 40 000 individuals per m^3, respectively (Castel et al., pers. comm.). No data were available on copepod densities in the MTZ of the Gironde for spring 1993, although a density between 5000 and 15 000 individuals per m^3 can be expected in March–April (Castel and Veiga, 1990). The results of the qualitative stomach analysis indicate a positive correlation between these densities and the predation by *N. integer* on *E. affinis*. Similar results were found for other mysid species: the predation rate was found to increase with copepod densities (e.g., Siegfried and Kopache, 1980; Bowers and Vanderploeg, 1982). No other calanoid copepod species were found in the stomachs of *N. integer*, although some were recorded in the watercolumn in low densities (*Diaptomus* species in the Elbe: 25 ind m^{-3}; *Acartia* species and *Temora* species in the Westerschelde: 400 ind m^{-3}). In the three estuaries cyclopoid copepods were abundant in the MTZ (300–1000 individuals per m^3). Nevertheless they were never encountered in the stomachs of *N. integer*, probably due to a higher escape response of the cyclopoids as compared to *E. affinis* (Tackx, pers. comm.).

Although Harpacticoida were very abundant in the meiobenthos communities of the subtidal sediments in the MTZ (2000–9000 ind m^{-2}) (Vincx et al., pers. comm.), they were rarely consumed by *N. integer*. Other meiobenthic animals and microphytobenthic diatoms were rarely encountered in the stomachs. This indicates that the mysids feed in the hyperbenthic layer of the watercolumn and do not scrape the bottom while foraging.

In spring 1993 high densities of calanoid nauplii were recorded in the watercolumn (23–79.10^3 individuals per m^3). *N. integer* seems to show a negative selection for nauplii. Also *N. mercedis* (Siegfried and Kopache, 1980; Murtaugh, 1981b) and *Mysis relicta* (Siegfried and Kopache, 1980; Bowers and Vanderploeg, 1982) do not consume nauplii in large amounts. Nauplii can be underrepresented in the diet of the mysids because adult and copepodite stages of calanoids, which are energetically more valuable, are positively selected. Or the nauplii might be more successful in avoiding the mysid feeding current than are the later life stages. Another explanation can be the high digestion rate of the nauplii (Rudstam et al., 1989), which can result in an underestimation of the predation on this prey by means of stomach analysis.

N. integer consumed filamental algae rather than solitary phytoplankton cells. Siegfried and Kopache (1980) reported a higher selectivity of *N. mercedis* for larger algae and filamental cells, while small phytoplankters were not consumed in high numbers although they were very abundant in the environment. In all estuaries some 10 algal cells per *N. integer* (Fig. 2, Table 2) were consumed. Still, the quantitative importance in the diet of the mysids is negligible, although phytoplankton might qualitatively be important for the provision of oligo-elements. Moreover one has to keep in mind that in the turbid zone of estuaries, where peak densities of *N. integer* are encountered, phytoplankton concentrations are generally low (Heip et al., 1995; Muylaert and Sabbe, 1999, this issue). In most cases, it was impossible to identify the phytoplankton up to genus or species level. Still, a distinction could be made between specimens from fresh, brackish or marine origin. In the three estuaries mainly algae from the brackish and freshwater parts of the system were consumed: *Thalassiosira proschkinae*, *Nannochloris coccoides*, *Paralia sulcata*, *Pediastrum species* and colonial chlorophyta (Fig. 1h) were the most common. Phytoplankton from the more marine reaches of the estuary (e.g., *Skeletonema species*) were rarely encountered in the stomachs. Filamental phytobenthic strands from the brackish zone (*Vaucheria species*: Fig. 1j) could be recognised in the stomachs from the three estuaries, indicating a possible horizontal migration of *N. integer* to intertidal areas for feeding.

Pollen of gymnosperms were mainly found in the stomachs of Westerschelde and Gironde individuals. The rivers Schelde and Garonne run through extensive pine forests. It is not known if the pollen are selectively ingested, nor if they can be digested by *N. integer*. Pine pollen were also found in the stomachs of the euryhaline mysid *M. mixta* from the Baltic Sea (Rudstam et al., 1989) and were supposed to be digested. The round pollen could not be identified and possibly resting stages of mesozooplankton or cysts of microzooplanktonic species are included in the counts.

About 3–5% of the total number of detrital particles consumed by *N. integer* in the three estuaries was clearly from macrophytal origin. It is possible that the mysid fragments larger macrophyte detritus particles to a size between 1000 and 20,000 μm^2 before ingestion. *N. integer* possesses cellulase enzymes (Zagursky and Feller, 1985), so the species can theoretically digest the macrophytal detritus. It is not known if they are capable of deriving substantial nutrition directly from macrophyte detritus either via digestion with its own cellulases or by an associated gut microflora. *Mysis stenolepis* has an assimilation efficiency of 30–50% on sterile cellulose (Foulds and Mann, 1978; Wainwright and Mann, 1982). Artificially made macrophytal detritus of *Spartina alterniflora* (Zagursky and Feller, 1985) contains 42.7% C and 2.4% N of the total dry weight. This detritus can serve as a nutritionally significant food item for *N. americana*, especially in periods of low availability of other nutritionally more valuable food items. Hence mysids can be an important link between (marsh-) macrophyte production and higher trophic levels.

The bulk of the 'unidentifiable detritus' originated from sediment flocs suspended in the watercolumn. However some of the particles counted as unidentifiable detritus probably were partly digested zooplankton and phytoplankton or originated from the stomach contents of ingested prey species. According to Eisma (1987) two size groups of flocs can be found suspended in the watercolumn in the MTZ of estuaries. Microflocs are firmly held together and have lengths between 1 and 125 μm. Together with single mineral particles these microflocs are the basic units of the more loosely bound, fragile macroflocs. The latter can reach sizes of 3 – 4 mm in turbid water. The 'unidentifiable detritus' particles in the stomachs of *N. integer* were within the range of 10 to 500 μm length and the fraction smaller than 125 μm was dominant.

EDAX did not allow for quantifications of the relative concentration of carbon in the flocs (because of the carbon coating), although analysis of the particles with diffracted electronic beams suggested that carbon concentration in the flocs was low. The particulate organic carbon (POC) of the river suspended matter is on average between 1 and 5% (Eisma et al., 1985). If the carbon content of the unidentifiable detritus is assumed to be of the same order, the importance in the energy balance of *N. integer* is negligible. The reason why so many flocs are present in the stomach can not be explained. The uptake might occur accidentally when feeding on other prey items. No associated bacteria, fungi, nanoflagelates, Protozoa or diatoms were found on the detritus flocs in the stomachs, but this is probably due to the conservation method used.

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