Temporal variability of a benthic food web: patterns and processes in a low-diversity system

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ABSTRACT: Trophic interactions are not static links, but are dynamic both in space and time. In temperate environments, seasonal patterns in primary production may be pronounced and, hence, the zoobenthic community and the trophic couplings within it change during the main production season. We assessed temporal variability in a benthic food web using stable isotope ratios of C and N. Organisms belonging to 3 trophic levels, producers (e.g. macroalgae and vascular plants), primary consumers (macrozoobenthos) and secondary consumers (epibenthic fishes and *Crangon crangon*), were sampled monthly in a shallow soft-substrate habitat in the northern Baltic Sea. The study focused on intra-seasonal (main production period from June to September 2005) and inter-annual (comparing data collected in June of 2 consecutive years) variability of trophic interactions involving phyto- and zoobenthos. Intra-seasonal changes in stable isotope ratios of food web components occurred both on a horizontal (within a trophic level) and a vertical scale (between trophic levels). Generally, unimodal patterns were evident, with enrichment of ^{13}C and depletion of ^{15}N occurring during the production period. The fluctuation in stable isotope values over the season decreased with increasing trophic level. Predator stable isotope values are probably affected by changes at the base of the food web and changes in consumer diet. The intra-seasonal pattern, together with limited interannual variability, indicates a temporally stable food web structure.

KEY WORDS: Epibenthic predators · Functional diversity · Benthic macrofauna · Trophic relationships · Soft substrate · Stable isotopes · $\delta^{13}C$ · $\delta^{15}N$

INTRODUCTION

The food web remains a focal point in ecology as a central part of the description of ecosystems and interactions between species within a community. Although often portrayed as such, the links in a food web are far from static, but vary in time and space (McCann et al. 2005). Temporal changes in food web interactions can be prominent in aquatic systems due to variation in the biotic and abiotic factors structuring the web (Polis et al. 1996, Riera & Richard 1997, Straile 2005). One of the major challenges in food web studies is to incorporate and interpret variation in community and food web dynamics (McCann et al. 2005, Akin & Winemiller 2006, Winemiller et al. 2007). In temperate environments, a seasonal change in primary production is pronounced and the zoobenthic community consequently

shows succession patterns in structure and trophic interactions along the production season (Möller et al. 1985, Blomqvist & Bonsdorff 1986). Generalist consumers can switch between food sources as the prey species change in availability and profitability within the habitat, and may thereby stabilize trophic dynamics (McCann et al. 2005).

Stable isotope analysis has become an essential tool for ecologists (Peterson & Fry 1987, Fry 2006), functioning as 'short, medium and long-term recorders for ecological processes' (West et al. 2006). Whereas stomach/gut content analysis provides a snapshot of ingested food objects, stable isotope analysis depicts what has been digested and assimilated by the organism and whether seasonal changes in diet are manifested on the tissue level of an organism. The use of $\delta^{13}C$ and $\delta^{15}N$ in trophic interaction studies rely on the

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assumption of specific fractionation of the isotopes. The $\delta^{13}C$ value of an organism provides an estimate of the energy source through a miniscule enrichment over trophic levels, 0 to 1‰, whereas $\delta^{15}N$ indicates the trophic position of an organism as its isotope value is enriched by 3 to 4‰ relative to that of the diet (Peterson & Fry 1987). Thus, dual isotope analysis describes processes in the food web, as well as functions as a tracer. The actual fractionation can vary between species and habitats (Vander Zanden & Rasmussen 2001), and nitrogen fractionation may, in fact, be closer to 2‰ (McCutchan et al. 2003).

The isotopic composition of primary producers may change several times during a year, e.g. plankton in the Baltic Sea exhibit bimodal ($\delta^{13}C$) and trimodal ($\delta^{15}N$) distributions (Rolff 2000). Apart from species turnover, changes in temperature and inorganic nutrient concentrations affect plankton stable isotope values within and between seasons (Goering et al. 1990, Sato et al. 2006). This kind of variation is not restricted to autotrophs, but can, after an eventual time lag, be mirrored in higher trophic levels (Goering et al. 1990, Vizzini & Mazzola 2003). Stable isotope values of animal tissues are influenced by tissue turnover time, temperature, individual size, and health condition or starvation (Yokoyama et al. 2005, Guelinckx et al. 2007). Changes in isotope values can also occur over an individual's life span according to seasonal or ontogenetic shifts in diet. Temporal fluctuations in stable isotopes are easily detectable in small species with high tissue turnover. Large individuals and long-lived species show isotope values representative of a longer time (up to months or a year, Hesslein et al. 1993) and, thus, respond more slowly to fluctuations in the isotopic signature of food items. Temporal variability in stable isotope values has been reported for zooplankton (Rolff 2000), benthic invertebrates (Riera & Richard 1997, Vizzini & Mazzola 2003) and fish (Sarà et al. 2002, Vizzini & Mazzola 2003).

Whereas several studies have revealed differences in $\delta^{13}C$ and $\delta^{15}N$ between seasons, only rarely has the variation of stable isotope values within a season or over the main production period been assessed (but see Goering et al. 1990, Riera & Richard 1997, Rolff 2000). The literature regarding benthic primary and secondary consumers is particularly scarce for this subject. Although spatial variation in stable isotopes is recognized (Deegan & Garritt 1997, Hansson et al. 1997, Norkko et al. 2007), we suggest that it is equally necessary to acknowledge temporal variability (Akin & Winemiller 2006) and its possible implications for the interpolation and extrapolation power of results from stable isotope studies that sample on a limited time scale (e.g. few sampling events, or even a single event).

The prime objective of our study was to evaluate the variability in benthic food web structure over the main production season. This was undertaken by assessing temporal changes in the ratios of stable isotopes, $^{13}C{:}^{12}C$ and $^{15}N{:}^{14}N$, of organisms belonging to 3 trophic levels: producers, primary consumers and secondary consumers of a shallow, coastal, soft-bottom habitat in the northern Baltic Sea. We were particularly interested in (1) intra-seasonal variability (over the main production period, June to September 2005) and (2) inter-annual variability (comparing June 2005 with June 2006) of phyto- and zoobenthos (focusing on benthic invertebrates and fishes). The present study aimed to elucidate the temporal variation of trophic interactions on both a horizontal scale (within a trophic level) and a vertical scale (between trophic levels) in the food web.

MATERIALS AND METHODS

Study site and organisms. Our study site (60° 10′ N, 19° 32′ E, Åland Islands, northern Baltic Sea) is a shallow (mean depth, 2.5 m), exposed, sandy bay. Substrate organic content is low (0.1%, as determined by loss on ignition, 3 h at 500°C). Water temperature was 12.0 to 12.5°C in June (both years) and September 2005. In July and August 2005, the temperature was 17.0 and 19.0°C, respectively. Salinity in this part of the Baltic Sea is 5 to 7.

The low species number in this area allows us to study most of the numerically or functionally important species of this particular habitat; in all, 21 species are included in this study. This biotope, shallow sandy substrate, provides a suitable model system for studying temporal changes in marine coastal food webs. The eastern part of the bay is characterized as bare sand while the western part is vegetated from 1 to 5 m depth (Boström & Bonsdorff 2000). The soft-bottom vegetation consists of meadows of *Zostera marina* L. and *Ruppia maritima* L. as well as *Potamogeton* spp. and *Zannichellia* spp. (Boström & Bonsdorff 2000). Apart from the sandy substrate dominating the bay, rocks are also present, supplying substrate for the annual filamentous algae, *Cladophora glomerata* (L.) Kützing, *Pylaiella littoralis* (L.) Kjellman/*Ectocarpus siliculosus* (Dillwyn) Lyngbye and *Ceramium tenuicorne* (Kützing) Waern, and the perennial macroalga *Fucus vesiculosus* L. Drift algae are common in the bight during the production season. Drift algal assemblages vary in species composition during summer but are usually conglomerates of filamentous brown, red and green algal species (*P. littoralis/E. siliculosus*, *C. tenuicorne*, *C. glomerata*) (Holmström 1998).

Shallow sandy bottoms are important nursery and feeding grounds for juvenile flatfishes, such as *Pla-*

tichthys flesus L. and *Scophthalmus maximus* L., and other epibenthic predators, e.g. *Crangon crangon* L. and *Pomatoschistus minutus* Pallas (Bonsdorff & Blomqvist 1993, Aarnio et al. 1996) (see Table 1). *Macoma balthica* L., *Nereis diversicolor* (syn. *Hediste diversicolor*) Müller and *Bathyporeia pilosa* Lindström belong to the dominant infauna in this habitat, and constitute important food sources for epibenthic predators. See Boström & Bonsdorff (2000) for information on other benthic invertebrate fauna characteristic of the site.

Organism sampling. Sampling was conducted 4 times during 2005 (June 14, July 28, August 22 and September 26) and once in 2006 (June 29) to collect organisms for analysis of the stable isotope ratios, $^{13}C:^{12}C$ and $^{15}N:^{14}N$. Infaunal invertebrates were sampled with a shovel and a bucket sieve (1 mm mesh) at about 1 m depth. Macrophytes and sediment were sampled through snorkeling and SCUBA diving, while drift algae were collected with a net. Samples of sediment organic matter (ca. 2 cm surface sediment) were collected from 1 and 4 m depths using a core. Epibenthic predators such as fish and *Crangon crangon* were collected using a beach seine (4 mm mesh net with 2 mm mesh bag) and a push net (2 mm mesh). Planktonic matter was collected from the water column with 90 µm mesh and 10 µm mesh nets. Additionally, unsieved seawater was collected.

Stable isotope analyses. Macrophytes were rinsed in filtered (20 µm) seawater and cleared of any remaining associated biota by hand under a stereomicroscope. Epiphytes were collected from *Fucus vesiculosus*. Infaunal macroinvertebrates were identified to species or group level and put in filtered seawater for a minimum of 12 h for clearance of gut contents. The sizes of all individuals were measured, and in the case of juvenile *Platichthys flesus*, 2 size groups were analyzed separately (size limit, 46 mm at which *P. flesus* switches from meiofaunal to macrofaunal diet). All samples were stored frozen (–20°C) until they were oven-dried (60°C, 48 h) and ground to a fine powder using a mortar and pestle. Aliquots were packed into 6×8 mm tin capsules for subsequent analysis. All faunal samples consisted of several whole individuals, except fish and crustacean samples that consisted of muscle tissue alone. Mollusc samples were obtained by removing the soft tissue from the shells. Planktonic matter was filtered through pre-combusted (500°C, 4 h) Whatman GF/C or GF/F glass fiber filters. Filters were dried, quickly acidified with 1 M HCl and dried again. We aimed at minimizing HCl treatment of the other samples to avoid a negative influence on stable isotope values. The effects of acidification of samples are contentious; several studies have documented that acid treatment affects $\delta^{13}C$ but not $\delta^{15}N$, or affects both (Jacob et al. 2005, Søreide et al. 2006). C and N stable isotope ratios were determined at the Stable Isotope Facility (University of California, Davis, California), using a Europa Scientific Hydra 20/20 continuous flow isotope ratio mass spectrometer. Stable isotope signatures are reported in delta (δ) notation in units of per mille (‰) according to:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard} - 1)] \times 10^3 \quad (1)$$

where R is $^{13}C:^{12}C$ or $^{15}N:^{14}N$. The standards for C and N are Vienna-PDB and atmospheric N_2, respectively (Fry 2006).

Trophic positioning. Trophic positioning relative to a common baseline allows for evaluation of trophic structure using $\delta^{15}N$ signatures (Cabana & Rasmussen 1996, Vander Zanden & Rasmussen 1999, Post 2002). Unionid mussels are useful in providing baseline $\delta^{15}N$ values for limnic food web studies (Post 2002). We used the measured $\delta^{15}N$ of the tellinid bivalve *Macoma balthica* as our reference value. The bivalve is a facultative active suspension feeder and surface deposit feeder (Ólafsson 1986), giving us a $\delta^{15}N$ baseline integrating pelagic as well as benthic production. The $\delta^{15}N$ values of *M. balthica* were compared with those of the obligate filter feeder *Mytilus edulis* L. (monthly data from 3 simultaneous sampling events, M. Nordström, K. Aarnio, E. Bonsdorff unpubl. data) to test for potential effects of facultative feeding or filter feeding on nitrogen isotopic ratio. No statistically significant difference was found between the species (2-way ANOVA, main factor 'Species': df = 1, MS = 0.071, F = 0.410, p = 0.530, error df = 18). The interaction between Species and Month was also non-significant (n = 4), and as *M. balthica* is a key species in our study area and constitutes an important food object within the benthic food web, we estimated the species to be a suitable reference for predator trophic positions in this particular study. Trophic position for consumers was calculated as following:

$$\text{Trophic position} = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta_n \quad (2)$$

where λ is the trophic level of the organism used to establish $\delta^{15}N_{base}$ (in the present study the primary consumer, i.e. trophic level = 2), $\delta^{15}N_{consumer}$ is measured directly, and Δ_n is the enrichment in $\delta^{15}N$ per trophic level (in this study, 3.6‰ according to the estimate based on our data).

Statistical analyses. Multivariate analyses were performed to reveal natural groupings in the data according to time. Non-metric multidimensional scaling (nMDS) and cluster analyses were performed using Bray-Curtis similarity coefficients based on means of stable isotope values ($\delta^{13}C$ or $\delta^{15}N$). One-way ANOVAs (factor 'month' or 'species') were performed to deter-

mine species-specific changes in stable isotope values during the main production season and inter-specific differences for particular sampling occasions. Two-way mixed model ANOVAs (factors 'Species' and 'Year', fixed and random, respectively) were performed groupwise (primary producers, primary consumers, secondary consumers) on stable isotope data to determine inter-annual changes in isotopes and inter-specific patterns. Linear regression with $\delta^{15}N$ as a function of predator length was performed to estimate the effect of consumer body size on trophic position. Prior to analyses, the data were tested for normality and homogeneity of variances and log transformed if not meeting the assumptions. Transformation was not successful in all cases, and data subsequently were analyzed using the non-parametric Kruskal-Wallis test. All values are reported as mean ± SE unless defined otherwise.

Modeling. Using the IsoSource mass balance model (Phillips & Gregg 2003), we estimated intra-seasonal changes in relative contribution of 3 abundant benthic invertebrate species, *Macoma balthica*, *Nereis diversicolor* and *Bathyporeia pilosa*, to the diet of 2 epibenthic predators, *Platichthys flesus* and *Crangon crangon*. Based on species $\delta^{13}C$ and $\delta^{15}N$ values (mean for each month and mean for total season), IsoSource delivered feasible proportions (0 to 100%) of prey species accounting for the target mixture (predator value). The source increment was set to 1% and the lowest possible mass balance tolerance (0.01 to 1.10) supplying solutions was used. No additional constraints were used. IsoSource estimates based on $\delta^{13}C$ values only required somewhat lower tolerance levels and the results from this analysis are compared with diet estimates based on dual isotope analysis.

RESULTS

Temporal patterns were revealed on both a horizontal and a vertical food web scale. Distinct isotopic values occurred for species and for trophic levels. The stable isotope values ranged from −25.59 to −7.00 for $\delta^{13}C$ and from −4.15 to 11.07 for $\delta^{15}N$ (Table 1). Planktonic matter was the most depleted in ^{13}C (water sample, filtered using 90 μm mesh; average, −25.28) and vascular plants were the most enriched (*Zan-*

nichellia palustris L.; average, −7.26) (Fig. 1). Cluster analysis of $\delta^{13}C$ divided the species into 2 major groups: 1 group with infaunal macroinvertebrates and their predators (relying on benthic production), and 1 group consisting of e.g. planktivorous fish and macroalgae (utilizing C from the water column). Cluster analysis of $\delta^{15}N$ grouped species into 3 categories representative of trophic levels, i.e. primary producers, primary consumers and secondary consumers (Fig. 1). Between benthic primary and secondary consumers, the average $\delta^{13}C$ trophic shift was 0.8 ± 0.1‰ and the corresponding value for $\delta^{15}N$ was 3.6 ± 0.2‰, which is consistent with values found in literature.

Intra-seasonal variability

Values for $\delta^{13}C$ and $\delta^{15}N$ varied over the production period. Inter-annual differences in stable isotope signals also occurred, but were generally minor compared with the changes within the production season.
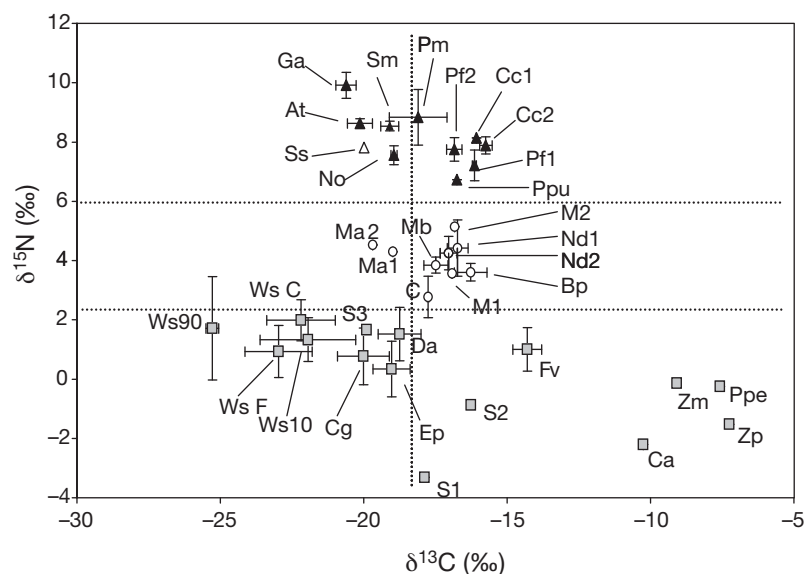

Fig. 1. Isotope values for organisms sampled from June to September 2005 ($\delta^{13}C$ and $\delta^{15}N$, in ‰; means ± SE). ■: primary producers; O: primary consumers; ▲: predators; △: parasite). Ws90: water sample, 90 μm mesh; Ws10: water sample, 10 μm mesh; WsC: water sample, Whatman GF/C filter; WsF: water sample, Whatman GF/F filter; S1: sediment at 1 m; S2: sediment at 1 m taken directly under deposited drift algae; S3: sediment at 4 m: Cg: *Cladophora glomerata*; Ep: Epiphytes; Da: Drift algae; Fv: *Fucus vesiculosus*; Ca: *Chara aspera*; Zm: *Zostera marina*; Ppe: *Potamogeton pectinatus*; Zp: *Zannichellia palustris*; Ma: *Mya arenaria*; Mb: *Macoma balthica*; M: *Marenzelleria* sp.; Nd: *Nereis diversicolor*; C: Chironomidae; Bp: *Bathyporeia pilosa*; Ga: *Gasterosteus aculeatus*; At: *Ammodytes tobianus*; Sm: *Scophthalmus maximus*; No: *Nerophis ophidion*; Pm: *Pomatoschistus minutus*; Ppu: *Pungitius pungitius*; Cc: *Crangon crangon*; Pf: *Platichthys flesus*; Ss: *Schistocephalus solidus.* Number after a species name indicates size (1 = small, 2 = large, see text for details). Horizontal lines separate trophic levels and vertical line separates planktivorous (lefthand side) and benthivorous (righthand side) predators (based on cluster analyses)

Table 1. Species functional groups and minimum and maximum (min, max) stable isotope values (‰) for each sampling (filamentous algae = Fil, leathery alga = L, vascular plant = V, Charophyta = Char; feeding type: filter-feeder = F, carnivore = C, herbivore = H, detritivore = D, predator = Pr, planktivore = plankt, benthivore = benth, piscivore = pisc; degree of mobility: mobile = M, semi-mobile = D, sessile = S; feeding habit: jawed = J, tentaculate = T, other feeding mechanism = X; size: small = 1, large = 2, see text for details). Grouping is according to Fauchald & Jumars (1979), Bonsdorff & Blomqvist (1993), Steneck & Dethier (1994). ns = not sampled

Species	Functional group	June 2005	July 2005	August 2005	September 2005	June 2006
$\delta^{13}C$						
Cladophora glomerata	Fil	−22.99, −22.35	−19.82, −18.81	−19.11, −18.05	−20.35, −18.98	−21.51, −20.75
Fucus vesiculosus	L	−16.39, −14.47	−16.13, −14.98	−14.21, −12.61	−15.14, −12.28	−16.54, −12.95
Epiphytes	Fil	−21.24, −19.21	−18.56, −18.33	−17.60, −17.46	−20.51, −17.47	−18.71, −18.50
Drift algae	Fil/L	−21.01, −17.18	−20.36, −19.82	−19.73, −18.52	−17.66, −16.03	−21.94, −20.51
Zostera marina	V	ns	ns	−9.20, −9.03	ns	−10.89, −10.50
Zannichellia palustris	V	ns	ns	−7.95, −7.36	ns	ns
Chara aspera	Char	ns	ns	−10.55, −9.83	ns	−12.34, −11.91
Potamogeton pectinatus	V	ns	ns	−7.58, −7.00	ns	−10.17, −9.41
Water samples	–	−25.59, −24.70	−22.08, −19.87	−25.48, −23.88	−22.00, −18.45	−23.71, −22.39
Macoma balthica	F/SDX	−18.90, −18.09	−17.30, −16.61	−17.24, −16.29	−18.05, −17.34	−20.43, −18.85
Nereis diversicolor (1)	C/H/F/SMJ	−17.43	ns	−17.44, −16.01	−16.52, −15.89	−18.49, −16.83
Nereis diversicolor (2)	C/H/F/SMJ	−17.85, −16.64	−16.67, −15.86	−19.24, −16.89	−17.18, −16.34	−17.62, −15.88
Chironomidae	SDX	−17.83	ns	−18.28, −17.30	−17.72	−18.14
Bathyporeia pilosa	C/MSX	−16.40, −15.33	−17.85, −16.43	−17.70, −16.70	−15.53, −14.39	−16.41, −16.32
Marenzelleria sp. (1)	F/SDT	ns	ns	ns	−17.03, −16.86	−17.34, −17.03
Marenzelleria sp. (2)	F/SDT	ns	ns	ns	−16.86, −16.79	−17.00, −16.58
Crangon crangon	CMJ	−16.91, −15.93	−15.65, −15.32	−16.09, −15.20	−15.66, −15.30	−16.72, −16.02
Platichthys flesus (1)	Pr (benth)	−17.64, −13.76	ns	−16.81, −15.75	−16.64, −15.49	−17.87, −17.56
Platichthys flesus (2)	Pr (benth)	−18.26, −13.76	−17.03, −16.62	−16.85, −16.50	−16.57, −16.05	−18.31, −17.44
Scophthalmus maximus	Pr (pisc/benth)	−18.93, −18.88	−20.02	−19.47, −17.17	−18.99, −18.17	−19.51
Pomatoschistus minutus	Pr (benth)	−19.11	ns	−17.95, −16.65	ns	−19.22, −19.12
Ammodytes tobianus	Pr (plankt/benth)	−21.70, −20.85	−20.90, −19.47	−20.32, −19.02	−19.94, −18.64	−21.20, −19.13
Gasterosteus aculeatus	Pr (plankt/benth)	−21.61, −20.87	−20.91, −20.23	−20.26, −19.79	ns	−21.30, −20.50
Nerophis ophidion	Pr (plankt)	ns	−19.05, −18.63	−19.49, −18.28	ns	ns
$\delta^{15}N$						
Cladophora glomerata	Fil	2.55, 3.29	0.94, 1.07	−1.78, −1.51	0.26, 1.11	2.22, 2.62
Fucus vesiculosus	L	1.38, 2.29	1.96, 2.86	−1.52, −0.20	−0.16, 0.74	0.66, 1.58
Epiphytes	Fil	2.61, 2.76	−0.56, 0.39	−2.20, −1.71	−1.28, 1.39	2.55, 3.14
Drift algae	Fil/L	3.31, 5.49	0.96, 1.69	−1.02, −0.12	1.38, 1.55	1.94, 2.50
Zostera marina	V	ns	ns	−1.38, 0.62	ns	1.51, 1.96
Zannichellia palustris	V	ns	ns	−0.53, 0.07	ns	ns
Chara aspera	Char	ns	ns	−2.53, −1.67	ns	−4.15, −2.68
Potamogeton pectinatus	V	ns	ns	−1.90, −1.04	ns	1.45, 2.31
Water samples	–	2.76, 3.75	−0.20, 3.05	−1.34, 0.62	−0.15, 2.42	0.00, 4.55
Macoma balthica	F/SDX	4.12, 4.55	3.23, 3.56	3.26, 3.65	4.16, 4.39	4.13, 4.93
Nereis diversicolor (1)	C/H/F/SMJ	5.69	ns	2.09, 3.49	4.32, 4.57	5.02, 5.62
Nereis diversicolor (2)	C/H/F/SMJ	5.85, 6.42	3.23, 3.70	2.87, 3.46	4.30, 4.78	4.98, 5.42
Chironomidae	SDX	4.05	ns	1.28, 1.97	2.60	3.03
Bathyporeia pilosa	C/MSX	4.28, 4.56	3.09, 4.00	2.71, 3.27	3.07, 3.96	3.83, 4.35
Marenzelleria sp. (1)	F/SDT	ns	ns	ns	3.43, 3.66	4.84, 5.15
Marenzelleria sp. (2)	F/SDT	ns	ns	ns	4.96, 5.32	5.44, 6.21
Crangon crangon	CMJ	7.71, 9.26	6.88, 7.38	7.56, 8.36	7.67, 8.33	7.09, 8.35
Platichthys flesus (1)	Pr (benth)	7.31, 8.85	ns	5.43, 7.44	6.93, 7.65	7.67, 8.62
Platichthys flesus (2)	Pr (benth)	8.42, 9.33	7.58, 8.28	6.63, 7.27	6.85, 7.84	7.06, 8.44
Scophthalmus maximus	Pr (pisc/benth)	8.37, 8.60	8.16	7.92, 8.96	8.76, 9.18	7.69
Pomatoschistus minutus	Pr (benth)	9.77	ns	7.71, 8.07	ns	8.05, 8.91
Ammodytes tobianus	Pr (plankt/benth)	7.97, 9.03	8.75, 9.21	7.67, 10.24	8.25, 9.20	7.51, 7.92
Gasterosteus aculeatus	Pr (plankt/benth)	9.52, 10.92	10.05, 11.07	8.05, 10.07	ns	8.48, 10.05
Nerophis ophidion	Pr (plankt)	ns	7.18, 7.29	7.67, 8.09	ns	ns

The $\delta^{13}C$ values recorded in early summer differed from the other sampling dates (nMDS and cluster analysis, in which June 2005 and June 2006 showed the highest level of separation from other months; sim-ilarity > 96%, Fig. 2a). Values for $\delta^{15}N$ revealed a differing temporal pattern as August was singled out from the other months. June 2005 and June 2006 still showed highest resemblance for $\delta^{15}N$ (>95% similar-
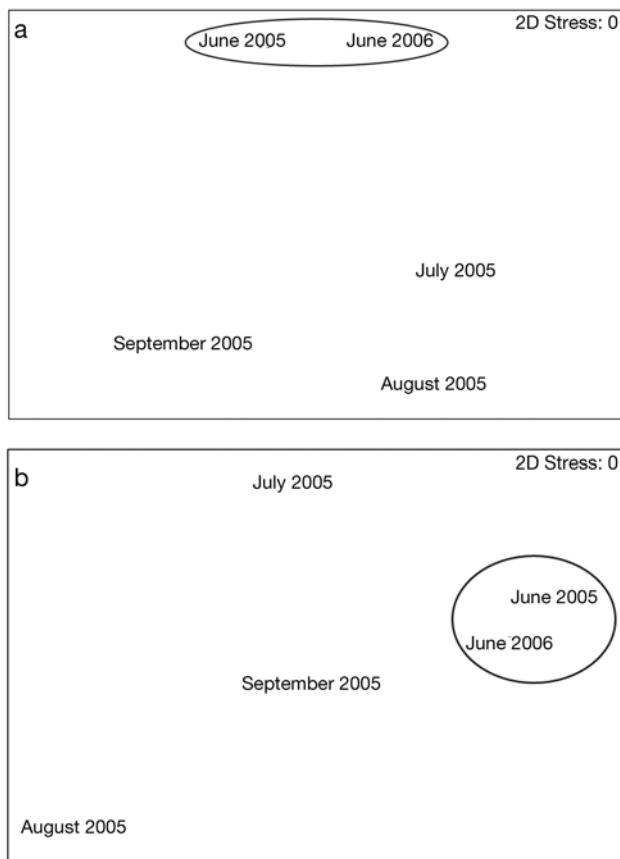
Fig. 2. Non-metric multidimensional scaling (nMDS) ordination over monthly values for (a) $\delta^{13}C$ and (b) $\delta^{15}N$ for assemblage consisting of primary producers, primary consumers and secondary consumers. The circle denotes proximity (>95% similarity based on cluster analyses) of June 2005 and June 2006

Fig. 3. Fluctuation in $\delta^{13}C$ (black bars) and $\delta^{15}N$ (grey bars) observed during production season (mean ± SE). Similar letters indicate non-significant differences, $p \geq 0.05$ (Roman letters for $\delta^{13}C$ and Greek letters for $\delta^{15}N$ comparisons)

ity, Fig. 2b). This indicates changing $\delta^{13}C$ and $\delta^{15}N$ values over the main production period, but also that the values returned by springtime the following year.

Generally, unimodal distributions for stable isotope values over the main production season dominated our data, with opposite patterns occurring for $\delta^{13}C$ (enrichment) and $\delta^{15}N$ (depletion). However, species-specific distributions were evident. The intra-seasonal variability of both isotopes decreased with increasing trophic level (1-way ANOVA: $\delta^{13}C$ MS = 0.830, df = 3, F = 14.755, p < 0.001; $\delta^{15}N$ MS = 0.692, df = 3, F = 14.394, p < 0.001). Water samples and macroalgae showed the highest intra-seasonal fluctuations in $\delta^{13}C$ and $\delta^{15}N$, whereas predators exhibited more stable values (Fig. 3). Water samples showed 2 peaks during the season, with elevated $\delta^{13}C$ values in July and September. Values for $\delta^{15}N$ showed a unimodal pattern with lower values occurring in the middle of the production period (Fig. 4).
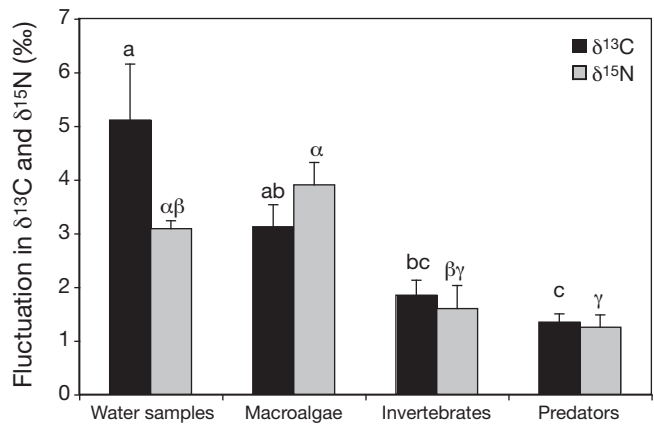
Most of the macroalgae and fauna became significantly enriched in ^{13}C as the production season progressed (Table 2, Fig. 4a–f). The macroalga *Cladophora glomerata* and epiphytic algae growing on *Fucus vesiculosus* showed peaks in $\delta^{13}C$ during July and August. The drift algae $\delta^{13}C$ did not increase before the end of the season (September). *F. vesiculosus* showed the least variation of these species (Fig. 4e). The invertebrates revealed species-specific responses with *Macoma balthica* increasing in $\delta^{13}C$ during July and August, and *Bathyporeia pilosa* showing the opposite pattern with a significant decrease in $\delta^{13}C$. *Nereis diversicolor* showed only minor fluctuations in $\delta^{13}C$ (none according to the post hoc analysis, Bonferroni p > 0.05, Fig. 4c). The secondary consumers increased in ^{13}C during the production period, except for *Scophthalmus maximus*, which showed relatively constant $\delta^{13}C$ values throughout the season (Fig. 4a).

All macroalgae and invertebrates were significantly depleted in ^{15}N in the middle of the summer, particularly in August (Table 2, Fig. 4d,f). The benthivorous predators showed a different pattern compared with the pelagically feeding predators. The benthivores decreased in $\delta^{15}N$, following the trend of the benthic invertebrates, while the planktivorous fishes showed no significant changes in $\delta^{15}N$ values (Table 2, Fig. 4b).

Inter-annual variability

Macroalgae showed species-specific patterns influenced by sampling year for stable isotopes of both C and N (2-way ANOVA, significant interactions; Spe-
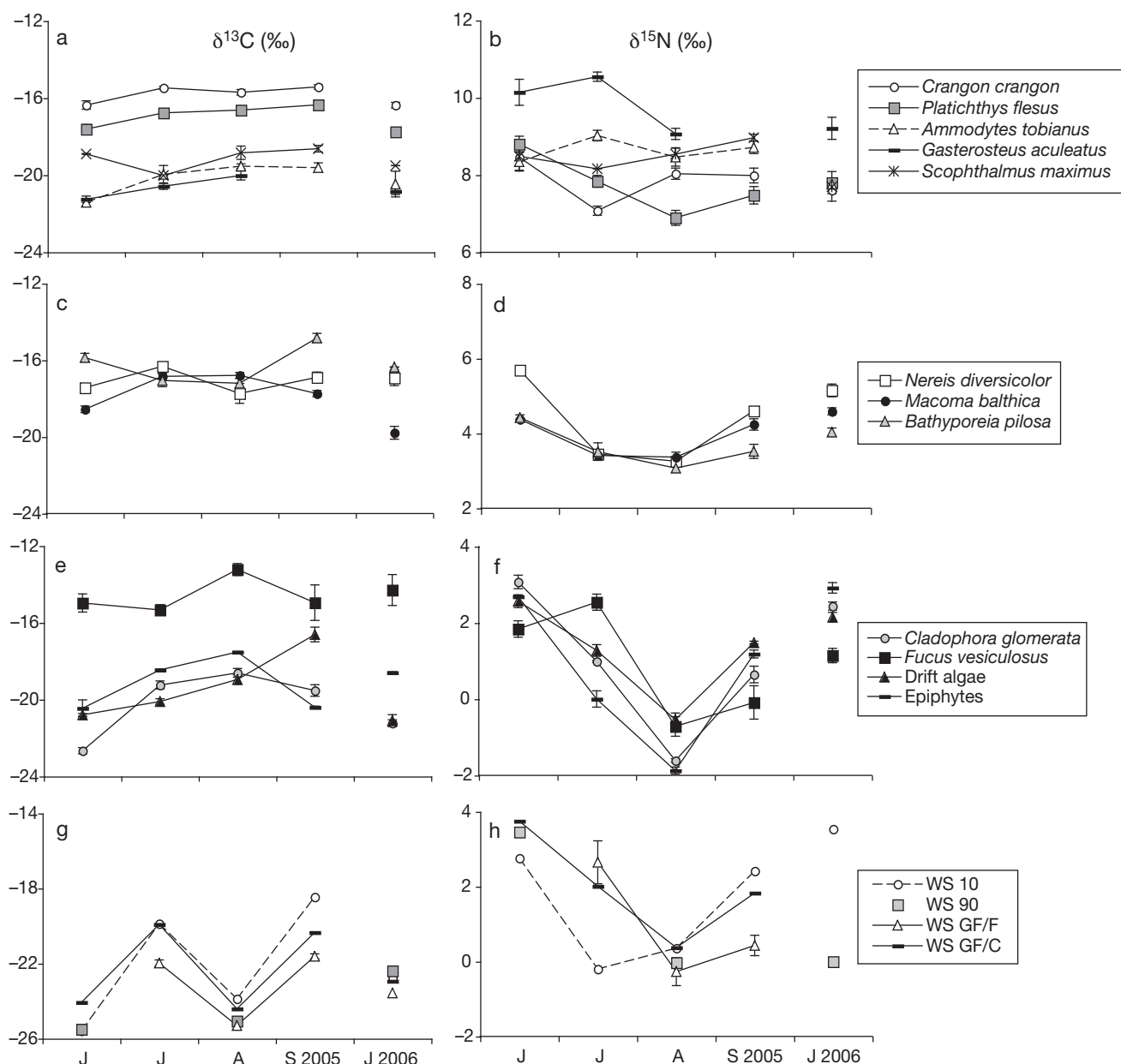
Fig. 4. Within-seasonal and inter-annual values (‰, mean ± SE) of $\delta^{13}C$ and $\delta^{15}N$ for (a,b) secondary consumers, (c,d) primary consumers, (e,f) primary producers and (g,h) planktonic matter. Note that the scale on the *y*-axes differs between graphs

cies × Year, p ≤ 0.01) (Table 3, Fig. 4e–f). Invertebrates also revealed significant interactions for C and N stable isotopes (Species × Year, p ≤ 0.01 and p ≤ 0.001 for C and N, respectively, Fig. 4c,d). Predator $\delta^{13}C$ values were significantly different between species (p = 0.002), but not between years. Predator $\delta^{15}N$ values depended on species identity and year (both p = 0.002), but no interaction between the factors was found. On average, the $\delta^{15}N$ values measured in 2006 were lower than in 2005, but within the intra-seasonal range (Fig. 4b).

Inter-specific resource partitioning and trophic positions

The primary consumer stable isotope ratios for both C and N indicate inter-specific resource partitioning during June (1-way ANOVA: $\delta^{13}C$ df = 2, MS = 7.363, F = 40.945, p < 0.001; $\delta^{15}N$ df = 2, MS = 4.248, F = 104.911, p < 0.001) and September 2005 ($\delta^{13}C$ df = 2, MS = 8.799, F = 47.089, p < 0.001; $\delta^{15}N$ df = 2, MS = 1.089, F = 14.540, p = 0.002), while no partitioning is evident during July and August in that same year (p >

Table 2. Results of 1-way ANOVA for species-specific intra-seasonal variability in $\delta^{13}C$ and $\delta^{15}N$. As heterogeneity increases the probability of Type I error, the non-significant result for *Gasterosteus aculeatus* is reliable. The p-values for *Cladophora glomerata*, epiphytes ($\delta^{15}N$) and drift algae are highly significant (p < 0.00001) and even a conservative approach should find these valid. However, the p-values for epiphytes ($\delta^{13}C$) and *Bathyporeia pilosa* (p ≤ 0.001) may be treated more cautiously. All differences were also significant when tested nonparametrically (Kruskal-Wallis, p ≤ 0.013). Significant values at p ≤ 0.05 are shown in **bold**; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ns = not significant

	df, total	MS	*F*	p
$\delta^{13}C$				
Primary producers				
Cladophora glomerata	3, 16	13.710	60.701	**<0.001****
Fucus vesiculosus	3, 16	4.455	5.896	**0.009****
Drift algae	3, 16	9.008	9.902	**0.001****
Epiphytes	3, 16	7.431	11.007	**0.001****[a]
Primary consumers				
Macoma balthica	3, 16	2.962	26.686	**<0.001****
Nereis diversicolor	3, 15	1.564	3.852	**0.038***
Bathyporeia pilosa	3, 15	5.252	20.018	**<0.001****
Secondary consumers				
Crangon crangon	3, 15	0.717	7.500	**0.004****
Platichthys flesus	3, 14	1.169	12.548	**0.001****
Scophthalmus maximus	2, 11	0.073	0.167	0.849 ns
Ammodytes tobianus	3, 16	3.497	11.111	**0.001****
Gasterosteus aculeatus	2, 9	1.096	9.951	**0.009****
$\delta^{15}N$				
Primary producers				
Cladophora glomerata	3, 16	16.518	216.032	**<0.001****[a]
Fucus vesiculosus	3, 16	9.521	43.669	**<0.001****
Drift algae	3, 16	14.390	42.416	**<0.001****[a]
Epiphytes	3, 16	15.667	38.055	**<0.001****[a]
Primary consumers				
Macoma balthica	3, 16	1.212	49.152	**<0.001****
Nereis diversicolor	3, 15	7.557	125.651	**<0.001****
Bathyporeia pilosa	3, 15	1.423	12.871	**<0.001****[a]
Secondary consumers				
Crangon crangon	3, 15	1.359	7.524	**0.004****
Platichthys flesus	3, 14	2.299	15.607	**<0.001****
Scophthalmus maximus	2, 11	0.258	2.515	0.136 ns
Ammodytes tobianus	3, 16	0.330	1.147	0.367 ns
Gasterosteus aculeatus	2, 9	1.470	2.807	0.127 ns[a]

[a]*C. glomerata* ($\delta^{15}N$), epiphytes ($\delta^{13}C$ and $\delta^{15}N$), drift algae ($\delta^{15}N$), *B. pilosa* ($\delta^{15}N$) and *G. aculeatus* ($\delta^{15}N$) showed unequal variances that were unaffected by transformation

son as well as between years. Interestingly, the N isotope values of predators show a pattern opposite to the one for macroinvertebrates, as the predators show highly similar $\delta^{15}N$ values in June 2005 and 2006, but the values then spread out during the rest of the production season. No significant relationship between predator size (mm) and the $\delta^{13}C$ or $\delta^{15}N$ values of *C. crangon* or *P. flesus* occurred (regression analyses: $R^2 \approx 0$, p > 0.05).

The results from dual isotope modeling indicate that the dietary importance of *Bathyporeia pilosa*, *Macoma balthica*, and *Nereis diversicolor* for *Crangon crangon* and *Platichthys flesus* vary over the season (Table 4a). The model suggests that, of the 3 species compared, *B. pilosa* contributes the most to the diet of *C. crangon* for the entire season, and also in June and September. Monthly estimates give *M. balthica* as an important food item for *P. flesus*, although limited contribution by the bivalve emerges from calculations based on the seasonal average. For both predators, *M. balthica* seemingly constitutes the preferred food source in August. Based on C stable isotope data alone, the model outcome demanded somewhat lower tolerance levels. In general, estimates between dual and single isotope models were similar, but the latter showed high overlap between food sources for *P. flesus* (Table 4b).

Although many species revealed significant decreases in $\delta^{15}N$ during the production season, several predator trophic positions relative to *Macoma balthica* actually increased (e.g. *Crangon crangon* 1-way ANOVA: df = 3, MS = 0.079, *F* = 5.067, p = 0.017; post hoc July < August, p = 0.026) (Fig. 5). During 2005, the lowest observed position, 2.9, was recorded for *Platichthys flesus* in September while *Gasterosteus aculeatus* reached a value of 4.0 in July. *G. aculeatus*, belonging to the pelagically feeding group, showed consistently higher $\delta^{15}N$ values, indicating that the planktonic chain may be longer than the one with benthic macrofauna, although any intermediate steps were not evident in our material due to low resolution of plankton size fractions in our water samples.

0.05). The sampling occasion in June 2006 again demonstrated separate stable isotope values between macroinvertebrate species ($\delta^{13}C$ df = 2, MS = 13.504, *F* = 36.073, p < 0.001; $\delta^{15}N$ df = 2, MS = 1.243, *F* = 18.733, p = 0.001). No mid-season overlap in predator $\delta^{13}C$ values occured. Instead $\delta^{13}C$ values indicate resource partitioning also among the benthic predators *Crangon crangon* and *Platichthys flesus* (2-way ANOVA, main factor 'Species': df = 1, MS = 9.109, *F* = 96.949, p < 0.001, no significant interaction between species and month). This persists throughout the sea-

Table 3. Two-factorial ANOVA for inter-annual changes (June 2005 and June 2006) in $\delta^{13}C$ and $\delta^{15}N$ of primary producers, primary consumers and secondary consumers. Significant values at $p \leq 0.05$ are shown in **bold**; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

Treatment	df	MS	F	p
Primary producers				
$\delta^{13}C$				
Species	3	78.509	14.806	**0.026***
Year	1	2.398	0.452	0.549
Species × Year	3	5.303	5.483	**0.005****
Error	24	0.967		
$\delta^{15}N$				
Species	3	3.826	3.029	0.194
Year	1	3.962	3.136	0.175
Species × Year	3	1.263	5.571	**0.005****
Error	24	0.227		
Primary consumers				
$\delta^{13}C$				
Species	2	19.375	11.998	0.077
Year	1	1.017	0.631	0.510
Species × Year	2	1.615	5.938	**0.010****
Error	19	0.272		
$\delta^{15}N$				
Species	2	4.591	6.923	0.126
Year	1	0.808	1.220	0.384
Species × Year	2	0.663	12.573	**≤0.001*****
Error	19	0.053		
Secondary consumers				
$\delta^{13}C$				
Species	3	42.870	98.248	**0.002****
Year	1	0.727	1.669	0.287
Species × Year	3	0.436	1.74	0.187
Error	23	0.251		
$\delta^{15}N$				
Species	3	4.810	81.666	**0.002****
Year	1	5.427	90.925	**0.002****
Species × Year	3	0.059	0.187	0.904
Error	23	0.315		

DISCUSSION

Our study revealed that trophic interactions varied within season and that temporal patterns in isotopic composition were applicable to food web components within, as well as across, trophic levels, even up to secondary consumers. The indications of changes in interspecific resource partitioning found in our study are of interest regarding species with similar function or trophic flexibility.

Temporal variability of coastal food web components

The temporal patterns observed in our study are consistent with Rolff (2000), who found bimodal ($\delta^{13}C$) and trimodal ($\delta^{15}N$) distributions in size-fractionated plankton sampled during a year, and the peaks and lows reported by Rolff (2000) occurred at similar time intervals as those observed in our study. However, seasonal comparisons in some other studies have revealed contradictory patterns. Higher $\delta^{13}C$ values in summer compared with other seasons has been found for macrophytes and macroalgae, benthic invertebrates and fish (Hentschel 1998, Kang et al. 1999, Sarà et al. 2002, Vizzini & Mazzola 2003), but depleted values have also been observed (Goering et al. 1990, Carlier et al. 2007). We found that $\delta^{15}N$ values generally decrease over the season, as Carlier et al. (2007) also discovered, but Vizzini & Mazzola (2003) could not identify any evident trend for nitrogen. Quarterly sampling occasions seldom provide unambiguous information on temporal changes in food web structure, whereas a more intense frequency of sampling supports a more rigid description (e.g. Goering et al. 1990, Rolff 2000, the present study). The range observed by Rolff (2000) for phytoplankton isotope values from June to September 1994 was about 7‰ for $\delta^{13}C$ and 5‰ for $\delta^{15}N$, whereas the corresponding fluctuations in our study were approximately 5 and 3‰. Diurnal changes in phytoplankton $\delta^{13}C$, e.g. coupled with light conditions, are smaller (≤1.5‰, Burkhardt et al. 1999) than fluctuations occurring within a season. Differences between winter and summer values for invertebrate $\delta^{13}C$ of roughly 3‰ have been reported (Vizzini & Mazzola 2003). In our study, maximum fluctuations in macroinvertebrate $\delta^{13}C$ were about 2‰. Thus, inherent variability observable between or within seasons may be greater than the fractionation levels that supposedly define trophic structure (0 to 1‰ for $\delta^{13}C$ and 2 to 4‰ for $\delta^{15}N$), especially for C.

Our study revealed within-season changes on both a horizontal and a vertical level in a coastal benthic food web. Possible mechanisms behind temporal variability in predator stable isotope values include (1) changes occurring at the base of the food web that are mirrored in higher trophic levels, (2) changes occurring in consumer diet or (3) a combination of both (Wainright et al. 1993).

Changes at the base of the food web

Short-lived autotrophs are affected by seasonal changes in environmental factors to a greater extent than are higher-level organisms (Cabana & Rasmussen 1996). In our study, the variations observed over the season were largest for primary producers. Thus, abiotic factors are probably driving the patterns of enrichment of ^{13}C (unimodal for macroalgae and bimodal for planktonic matter) and a depletion of ^{15}N (unimodal). Dissolved inorganic carbon (DIC) dynamics are important for the isotopic composition of producers, and the

Table 4. Possible percentage (%) contributions by 3 infaunal invertebrates to diets (range) of 2 epibenthic predators. Mass balance model based on (a) $\delta^{13}C$ and $\delta^{15}N$ values and (b) $\delta^{13}C$ values only. Monthly estimates and total season (June to September 2005) estimate provided. No additional constraints applied. Mass balance tolerance is a measure of the range of included solutions (e.g. entering a value of 0.1 specifies that all source combinations that result in predicted mixture signatures within 0.1 of the observed signature are considered as feasible solutions)

	June	July	August	September	Total season
(a) $\delta^{13}C$ and $\delta^{15}N$					
Crangon crangon					
Macoma balthica	23	0–10	98–100	0–5	0–10
Bathyporeia pilosa	57	0–7	0–2	52–56	78–100
Nereis diversicolor	20	90–100	0–1	40–48	0–22
Increment	1	1	1	1	1
Tolerance (‰)	0.01	0.90	1.10	0.40	0.70
Platichthys flesus					
Macoma balthica	50	0–2	93–100	52	0–10
Bathyporeia pilosa	7	96–100	0–7	48	13–31
Nereis diversicolor	43	0–4	0–3	0	66–87
Increment	1	1	1	1	1
Tolerance (‰)	0.01	0.73	0.20	0.02	0.10
(b) $\delta^{13}C$ only					
Crangon crangon					
Macoma balthica	0–19	0–11	98–100	0–12	0–6
Bathyporeia pilosa	67–81	0–8	0–2	71–88	91–100
Nereis diversicolor	0–33	89–100	0–1	0–29	0–9
Increment	1	1	1	1	1
Tolerance (‰)	0.01	0.90	1.10	0.01	0.60
Platichthys flesus					
Macoma balthica	16–65	0–67	93–100	0–30	0–47
Bathyporeia pilosa	0–35	0–48	0–7	26–69	0–75
Nereis diversicolor	0–84	33–54	0–3	1–74	25–53
Increment	1	1	1	1	1
Tolerance (‰)	0.01	0.01	0.20	0.01	0.01

CO_2 concentration in water affects C stable isotope fractionation of marine phytoplankton, macroalgae and seagrass (Hemminga & Mateo 1996, Burkhardt et al. 1999, Riebesell et al. 2000). Autotrophs can discriminate against the heavier C isotope (^{13}C) during photosynthesis, rendering the remaining DIC pool increasingly heavier in its isotopic composition. As growth rates increase, the fractionation against ^{13}C decreases. This is mirrored in the progressively enriched autotrophs and heterotrophs during the season (Rolff 2000, Gu et al. 2006, present study). Irradiance, temperature, limiting nutrient concentrations and other factors related to growth rate also affect $\delta^{13}C$ values of autotrophs (Hemminga & Mateo 1996, Burkhardt et al. 1999, Riebesell et al. 2000).

Species-specific divergence in isotope signatures within a trophic level may be related to differing life history traits, where the response to environmental changes may be detected in annual macroalgae (e.g. *Cladophora glomerata*) before it is evident in perennial species (e.g. *Fucus vesiculosus*). For example, time lags could be induced by N-storage capacity in algal tissue (Lehvo et al. 2001). The large variation in the stable isotope values of planktonic matter are probably a result of the fast turnover of small organisms, but could also be attributed to changes in species composition of the plankton community. In August, the plankton collected with a 10 µm mesh net consisted of cyanobacterial species such as *Aphanizomenon flos-aquae* (L.) Ralfs, *Nodularia spumigena* Mertens and *Anabaena* sp., and had a $\delta^{15}N$ value of 0.36‰. These are heterocyst-possessing species capable of N_2 fixation, and are often dominant in blooms of cyanobacteria in the Baltic Sea (Finni et al. 2001). Cyanobacterial fixation of N_2 is predominately non-discriminative for isotopes, and thus $\delta^{15}N$ of cyanobacteria should be close to that of air, approximately 0‰ (Goericke et al. 1994, Gu et al. 2006). Studies in the Baltic region have reported low $\delta^{15}N$ values in autotrophs during the main production period (Rolff 2000, Deutsch & Voss 2006).

Changes in consumer diet

Due to the strong seasonal changes characterizing the Baltic Sea, the feeding habits of many organisms change during the main production period. For primary consumers, the intra-seasonal pattern of the $\delta^{13}C$ values indicated collective utilization of one food source when this is abundant during the middle of the production period. In the beginning and towards the end of the season, *Macoma balthica*, *Nereis diversicolor* and *Bathyporeia pilosa* seem to rely on different food resources. The omnivorous polychaete *N. diversicolor* showed high $\delta^{15}N$ values during the beginning and end of the production period, indicating that predation might be an important feeding mode during these times. Discontinuities between producer and consumer isotopic ratios may be due to temporal separation of components, e.g. a deposit feeder relying on older rather than freshly deposited planktonic matter (Goering et al. 1990), or active switching between food sources by the (e.g. facultative) consumer (Ólafsson 1986). Unfortunately, data on the temporal variability of stable isotope values of sedimentary organic matter (SOM) during 2005 are lacking due to problems during sampling. Therefore, it is not possible to evaluate
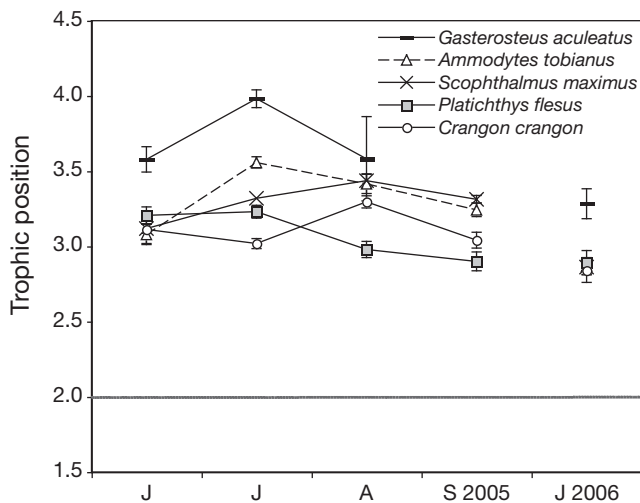
Fig. 5. Monthly trophic positions of secondary consumers (mean ± SE). Grey line shows baseline organism *Macoma balthica*

eventual temporal variations in the contribution of the sedimentary C to the benthic food web. Values from 2006, however, demonstrate that the SOM fall well in line with the δ^{13}C values of benthic consumers (Fig. 1).

All fishes sampled in this study are planktivorous, benthivorous and/or piscivorous. Predators showed stable food preferences (C sources), but there is a separation between the benthivores and the pelagically feeding species, also according to the δ^{15}N values. The δ^{15}N values showed changes at the invertebrate infaunal level mirrored in species like *Crangon crangon* and *Platichthys flesus*. *Ammodytes tobianus* and *Gasterosteus aculeatus* trophic levels were more constant, and somewhat higher, than the position of the benthic predators. *G. aculeatus* feeds on roe of other fishes (Thorman & Wiederholm 1983), a behaviour that possibly increases the δ^{15}N of the species.

Crangon crangon and *Platichthys flesus* (and other commonly occurring epibenthic predators on shallow soft bottoms) may occupy an important position as regulators of the zoobenthic community (Pihl 1985). Gut content analyses have revealed a significant overlap in the diet of the brown shrimp and the flounder. Both feed on meiofauna during their early life stages, after which they switch to macrosized invertebrate prey (Pihl & Rosenberg 1984, Aarnio et al. 1996). Common food items are polychaetes (e.g. *Nereis diversicolor*, *Pygospio elegans* Claparede), crustaceans (e.g. *Bathyporeia pilosa*, *Corophium volutator* Pallas) and bivalves (e.g. *Macoma balthica*, *Cerastoderma edule* L.) (Pihl & Rosenberg 1984, Pihl 1985, Aarnio et al. 1996, Link et al. 2005, Feller 2006). These predators feed primarily on abundant prey species, i.e. the diet follows seasonal changes in prey availability. Interestingly, the δ^{13}C data in this study revealed a consistent separation

between *C. crangon* and *P. flesus*, indicating resource partitioning. Based on the 3 prey species evaluations (*N. diversicolor*, *M. balthica*, *B. pilosa*), the model suggested that the 2 latter species (key species of the habitat) are important in the diet of epibenthic predators. At times, prey species contributions were overlapping, confirming generalist diets. However, the high degree of overlap complicates interpretation of specific contributions. A high mass balance tolerance (0.7 to 1.1) was required for obtaining feasible solutions for *C. crangon* diet on data from the entire season, as well as in July and August when prey δ^{13}C values are highly similar. This indicates indeterminable solutions and the contributions provided are to be regarded only as indicative (Phillips & Gregg 2003). An experimental study found *P. flesus* to prefer (numerically) the bivalve *M. balthica*, but to receive more energy from *B. pilosa* (Mattila & Bonsdorff 1998). *C. crangon* feed on abundant species and *B. pilosa* is a preferred food item at exposed sandy bottoms where it dominates the community, such as at our study site (Pihl & Rosenberg 1984, Boström 2002). Invertebrate recruitment patterns are partially in agreement with the indications provided by the modeling, as *M. balthica* settles around early August and then occurs in abundance in the benthos (Blomqvist & Bonsdorff 1986, Bonsdorff et al. 1995). The abundance of *B. pilosa* peaks once before the middle of the summer and once at the end of the production season (Blomqvist & Bonsdorff 1986), which is in accordance with the high prevalence in diet estimates.

Temporal resolution of stable isotope studies

We observed no time lag in the response between producers and consumers on a month-to-month basis. To reveal such time lags, even more intensive sampling than we undertook would be necessary. Changes in stable isotope values of organisms are ultimately dependent on tissue turnover time. For example, the turnover rate for *Crassostreas gigas* Thunberg and *Pomatoschistus minutus* muscle is approximately 1 mo (half-life) for δ^{13}C and δ^{15}N (Riera & Richard 1997, Guelinckx et al. 2007) and our data confirm that significant changes in isotope composition of invertebrates and juvenile predatory vertebrates can occur on a monthly scale. Changes in the stable isotope composition of muscle tissue of juvenile flatfish are probably a result of somatic growth during the season, and to a lesser degree due to metabolic turnover (Bosley et al. 2002). The isotopic composition of a generalist predator is rarely at any point in definite equilibrium with that of its diet.

Our sampling was frequent enough to reveal temporal changes in stable isotope values of species belong-

ing to 3 trophic levels. One short sampling effort would provide material depicting food web structure. Relying on samples collected at different trophic levels at different times during the main production period could, however, result in misinterpretation of relevant food sources. Sample collection should be targeted according to the biology and ecology of the species. Also, a single sampling event may leave temporal changes in diet unaccounted for, and the inclusion or exclusion of temporal variability must be considered according to the hypotheses in question. Stable isotope analysis is a complement to, but not a replacement for, other food web study methods such as dietary data analysis and experimental studies on trophic relationships (Winemiller et al. 2007).

CONCLUSIONS

A conceptual model of a simplified food web on shallow sandy substrate shows consumers connected by 3.2 trophic links, on average, per species (Fig. 6), similar to the general linkage density reported for aquatic systems (Link 2002). Benthic–pelagic coupling is evident at several trophic levels, with sedimentation of phytoplankton contributing to the availability of organic matter for sedimentary consumers. Higher-level predators, such as *Scophthalmus maximus*, utilize prey from both the planktonic and the benthic food chain.

The present study provides evidence for temporal changes in stable isotopes of food web components on a vertical as well as a horizontal scale. The patterns
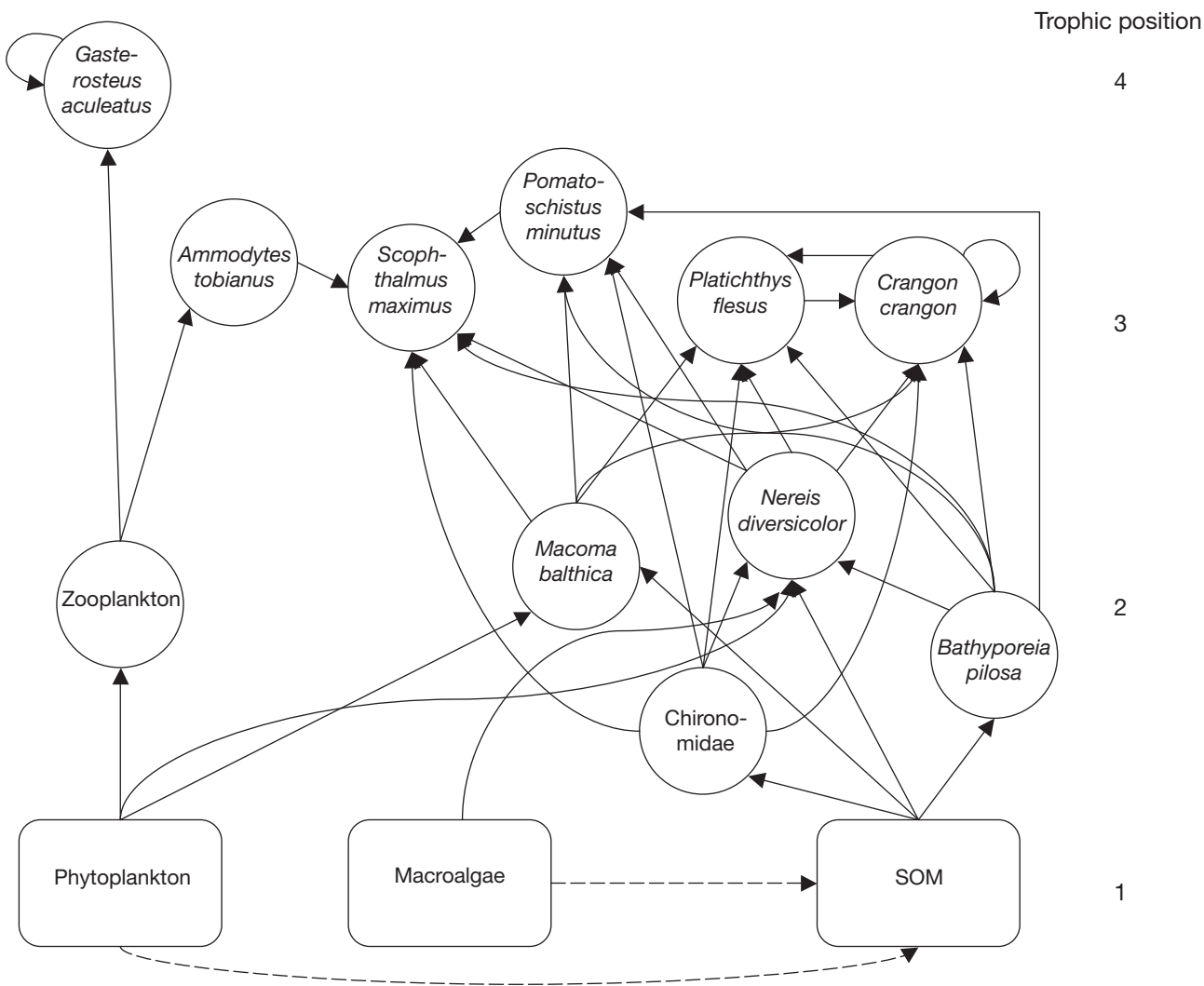

Fig. 6. Trophic links and trophic positions of organisms in a simplified sandy substrate food web (based on this study and other published studies). Solid lines indicate trophic transfer; dashed lines show primary production contribution to detritus pool. Arrows returning to species/groups indicate cannibalism or feeding on roe

indicate isotopic processes connected to the seasonality of the abiotic factors driving production (Gu et al. 2006). Changes in isotope ratios of higher-level organisms are probably due to changes occurring at the base of the food web as well as changes in consumer diet. Benthic invertebrates showed a collective utilization of one abundant food source, but inter-specific resource partitioning seems to occur otherwise (Fig. 4). Resource partitioning was indicated also for epibenthic predators. Generalist feeding is, however, characteristic for these species. Thus, there is a possibility for overlaps in diet and trophic niche among benthic macrofauna of the northern Baltic Sea, but the true width of feeding or functions may have remained hidden during the conditions of this study.

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