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Trophic niche partitioning of littoral fish species from the rocky intertidal of Helgoland, Germany

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Abstract During a 3-year field study, interspecific and interannual differences in the trophic ecology of littoral fish species were investigated in the rocky intertidal of Helgoland island (North Sea). We investigated trophic niche partitioning of common coexisting littoral fish species based on a multi-tracer approach using stable isotope and fatty acids in order to show differences and similarities in resource use and feeding modes. The results of the dual-tracer approach showed clear trophic niche partitioning of the five target fish species, the goldsinny wrasse *Ctenolabrus rupestris*, the sand goby *Pomatoschistus minutus*, the painted goby *Pomatoschistus pictus*, the short-spined sea scorpion *Myoxocephalus scorpius* and the long-spined sea scorpion *Taurulus bubalis*. Both stable isotopes and fatty acids showed distinct differences in the trophic ecology of the studied fish species. However, the combined use of the two techniques added an additional resolution on the interannual scale. The sand goby *P. minutus* showed the largest trophic plasticity with a pronounced variability between years. The present data analysis provides valuable information on trophic niche partitioning of fish species in

the littoral zones of Helgoland and on complex benthic food webs in general.

Introduction

Understanding the trophic interactions in complex and diverse marine ecosystems is a significant challenge. In order to obtain a profound knowledge on complex ecosystems, it is important to address trophodynamic interactions and to account for trophic niche partitioning of coexisting species in individual systems.

While stable carbon isotopes ($^{13}C/^{12}C$) provide a measure of the carbon sources used by consumers, nitrogen isotopes ($^{15}N/^{14}N$) serve as an indicator of the relative trophic position of the organism within the food web (DeNiro and Epstein 1978; Minagawa and Wada 1984; Peterson and Fry 1987). In contrast to e.g. quantitative gut content analyses which provide only 'snapshots' of ingested prey items used by a specific consumer, stable isotopes (SI) provide long-term, time-integrated information on the food sources, thus allowing estimates on the trophic ecology of individual species (Polacik et al. 2014).

The use of $\delta^{13}C$ and $\delta^{15}N$ in food web studies is considered as a reliable tool for estimates on community structure and niche width (Layman et al. 2007; Jackson et al. 2011), as well as for intra- and interspecific variability and feeding modes (Bearhop et al. 2004; Aberle et al. 2005; Newsome et al. 2007; Polacik et al. 2014) in complex ecosystems. To avoid misinterpretations of SI data, it is

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beneficial to combine it with additional approaches, e.g. fatty acid (FA) analysis—providing supplementary dietary information such as a clear indication of the food sources used (Herman et al. 2005; Perga et al. 2006; Inger and Bearhop 2008). Herman et al. (2005) showed that, based on SI data, killer whales *Orcinus orca* predominantly prey marine mammals, while FA profiles pointed at a more fish-based diet. Here, the combination of different tracer methods widened the range of dietary information in this specific field study. Consequently, the combined use of SI and FA analyses is a promising approach to characterize complex food web interactions and to define specific trophic niches in a given ecosystem (Peterson and Fry 1987; Fry et al. 1999).

In the present study, we analysed the trophic ecology of the common littoral marine fish species goldsinny wrasse, sand goby, painted goby, long-spined and short-spined scorpion due to their wide distribution ranges in a variety of habitats along the European coastline (i.e. Wheeler and Du Heaume 1969; Hureau et al. 1986). The study focussed on the rocky intertidal fish community of Helgoland, a small and isolated island in the German Bight, North Sea. The aim was to disentangle food web interactions of our target fish species and to define their trophic niches and position of coexisting species over a period of three consecutive years. Due to the ecological relevance of Gobiidae in rocky and soft-bottom habitats (Wennhage and Pihl 2002), the specific role of the sand goby *P. minutus* was considered in detail. The crucial role of *P. minutus* in marine and estuarine ecosystems has been stressed in the literature since this species acts both as predator as well as prey (e.g. Leitao et al. 2006 and references therein), serving as a preferred prey item of coexisting fish species e.g. *T. bubalis* and *M. scorpius* (Ehrenbaum 1936; Wheeler and Du Heaume 1969). Therefore, we considered this goby species more specifically and tried to define its trophic role among other littoral fish species within the same littoral community.

We hypothesized that we would find (1) interspecific variations in trophic niches of coexisting littoral fish species and (2) annual patterns in trophic niche partitioning (e.g. changes in prey availability).

Materials and methods

Study area and sample collection

A 3-year sampling campaign of littoral fish species was conducted (2009–2011) around the island of Helgoland (54°11′N, 7°53′O), situated in the North Sea 50 km off the German west coast (Fig. 1) to investigate the trophic ecology and trophic niche partitioning of littoral marine fish species.

Target fish species were: goldsinny wrasse *Ctenolabrus rupestris* (L.), sand goby *Pomatoschistus minutus* (Pallas), painted goby *Pomatoschistus pictus* (Malm), short-spined sea scorpion *Myoxocephalus scorpius* (L.) and long-spined sea scorpion *Taurulus bubalis* (Euphrasen) (Table 1). Specimen were collected from late spring to autumn (June to November) over a period of three consecutive years (2009–2011) using beach seining (20 m × 1.20 m, 6-mm codend), fyke nets and hand netting with suitable mesh sizes as fishing techniques. Furthermore, bivalve (*Mytilus edulis*) and gastropod (*Littorina littorea*) samples were collected each year at monthly intervals from June to November to provide a seasonal baseline for SI values of fish species. In general, 10–20 individuals of each species (incl. *M. edulis* and *L. littorea*) were collected based on the availability at each sampling season. Subsamples were frozen immediately after processing and stored at −80 °C until analyses.

Fatty acid (FA) analysis

The extraction of FAs was carried out on each individual fish applying a modification of the methods following the analytical procedure by Malzahn et al. (2007). For all fish species, we used dorsal muscle samples dissected directly from posterior to the head. Fatty acids were analysed by gas chromatography (Varian CP 8400 gas chromatograph equipped with a DB-225 column [J&W Scientific, 30-m length, 0.25-mm inner-diameter (ID), 0.25-μm film]. Quantification of the fatty acid methyl esters (FAME, i.e. a type of fatty acid ester that are derived by transesterification of fats with methanol) was done by using GALAXIE Workstation software (Galaxie 1.9 SP2b, Varian, Inc., USA), and each chromatogram was manually assessed for correct peak identification (and also reintegrated when it was necessary). A known amount of C23:0 as internal standard was added additionally before starting the FAME extraction.

All FA data were reported in μg FA/μg C referring to the carbon (C) amount of defatted muscle tissue from the SI measurements (Table 2). The trophic tracers of dietary fatty acids used in this study are DHA/EPA, 18:1n9/18:1n7 and PUFA/SFA (El-Sabaawi et al. 2009).

Samples showing a total FA content higher than 0.15 μg FA/μg C were not included into the analysis since analytical errors were expected.

Stable isotope (SI) analysis

SI analysis was performed using solely muscle tissue of fish due to discrepancies in synthesis and replacement processes in different animal tissues (Pinnegar and Polunin 1999; Bearhop et al. 2002). Each tissue sample was freeze-dried and defatted with a dichloromethane–methanol (2:1
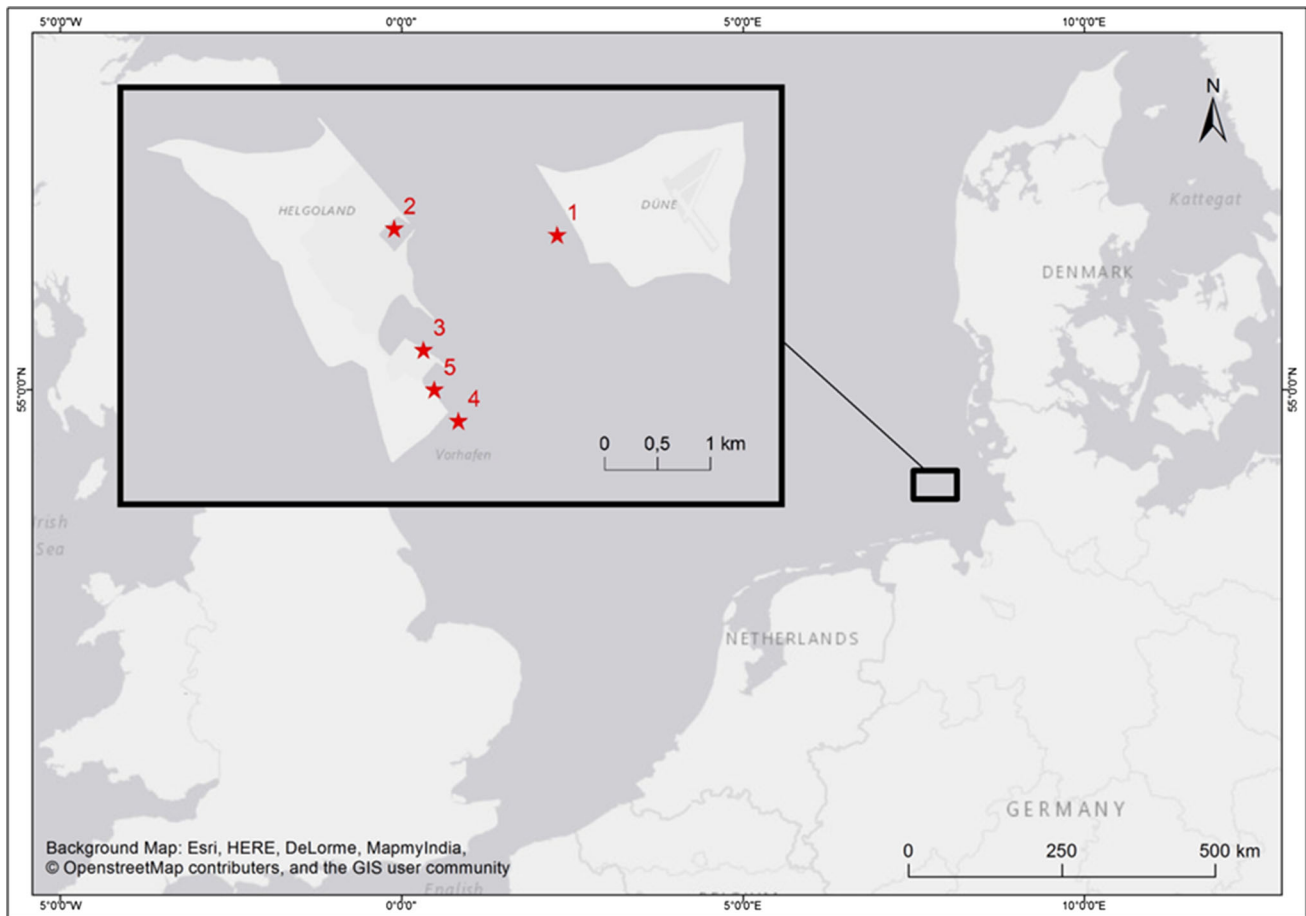
Fig. 1 Map of study area around the island Helgoland (Germany) showing positions of sampling catches taken for biochemical analyses between 2009 and 2011 within a sampling radius of approx. 2 km.

*Location of sampling sites is represented by stars: [1]Dune harbour, [2]North-east harbour, [3]Pier of institute, [4]South harbour, [5]West pier

vol/vol) mixture, treated in an ultrasound bath for 30 min and dried again before ±1 mg of sample was weighed into a tin capsule (HEKAtech GmbH) for further SI analysis ($\delta^{15}N$ and $\delta^{13}C$). While lipid extraction can alter the $\delta^{13}C$ of consumers (Kiljunen et al. 2006), $\delta^{15}N$ usually remains unaffected by the defatting procedure (Ingram et al. 2007).

Freeze-dried bivalve and gastropod tissue was scraped from the shells prior to grinding. An adequate amount of ground tissue was defatted for 24 h before preparing subsamples for SI analysis.

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were determined using continuous flow isotope ratio mass spectrometry (PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer, Sercon Ltd., Cheshire, UK) with an analytical precision of 0.2 ‰ for $\delta^{13}C$ and 0.3 ‰ for $\delta^{15}N$ carried out by UC Davis Stable Isotope Facility, University of California, USA. The ratios (R) of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ are expressed in conventional delta notation (δ) as parts per thousand

relative to the international carbon standard Pee Dee Belemnite and atmospheric N_2 for nitrogen:

$$\delta X\ (‰) = \left[\left(R_{sample} - R_{standard} \right) / R_{standard} \right] \times 10^3$$

where X: equal to ^{13}C or ^{15}N and R: corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Calculations on the trophic position (TP) of our fish species relied in general on the baseline SI of our benthic grazer *L. littorea*. Only *P. minutus* from 2010 showed a stronger dependency on ^{13}C derived from pelagic production, and therefore, *M. edulis* served as baseline organism in this specific case (Table 3).

In order to estimate the trophic position (TP) of each individual, the following equation was used (Post 2002):

$$TP = [(\delta^{15}N_{consumer} - \delta^{15}N_{reference})/2.9] + 2$$

assuming that 2.9 ‰ describes the averaged ^{15}N trophic enrichment factor after McCutchan et al. (2003). 2 represents the trophic position of the baseline organisms and

Table 1 Sample information on number of individuals (N), mean $\pm$ standard deviation (SD) of standard length (SL) and mass (M) as well as specific sampling locations of the key fish species collected around Helgoland from 2009 to 2011

Species (common name)	Sampling year	N	SL (mm) mean $\pm$ SD	M (g) mean $\pm$ SD	Sampling location (i.e. fishing grounds)
Ctenolabrus rupestris (goldsinny wrasse)	2009	19	116 $\pm$ 12	39.04 $\pm$ 11.88	Pier of institute, South harbour (2011)
	2010	17	107 $\pm$ 17	29.39 $\pm$ 13.13	
	2011	10	95 $\pm$ 10	16.94 $\pm$ 4.43	
Pomatoschistus minutus (sand goby)	2009	20	63 $\pm$ 6	3.56 $\pm$ 1.19	Dune harbour
	2010	19	59 $\pm$ 5	2.19 $\pm$ 0.57	
	2011	10	52 $\pm$ 3	1.83 $\pm$ 0.39	
Pomatoschistus pictus (painted goby)	2009	17	27 $\pm$ 5	0.41 $\pm$ 0.26	North-east harbour
	2010	9	32 $\pm$ 7	0.22 $\pm$ 0.07	
	2011	7	23 $\pm$ 3	0.17 $\pm$ 0.08	
Myoxocephalus scorpius (short-spined sea scorpion)	2009	11	161 $\pm$ 19	106.41 $\pm$ 42.67	Pier of institute, South harbour, West pier
	2010	3	154 $\pm$ 32	98.44 $\pm$ 48.72	
	2011	9	147 $\pm$ 37	87.56 $\pm$ 62.36	
Taurulus bubalis (long-spined sea scorpion)	2009	20	111 $\pm$ 15	41.94 $\pm$ 17.18	Pier of institute, South harbour, West pier
	2010	18	119 $\pm$ 13	53.61 $\pm$ 23.03	
	2011	10	124 $\pm$ 19	63.74 $\pm$ 28.10	

enables a legitimate tool for comparability with other trophic level calculations from literature.

Length–weight relationship of *P. minutus*

One of the most frequently used morphometric indices is *Fulton's condition factor K* that can be used to describe interannual variations in *P. minutus* condition (Nash et al. 2006). Due to its conflicting results in preceding studies (i.e. Trippel and Hubert 1989; Davidson and Marshall 2010; Mozsar et al. 2015), we used the relative condition factor K_n, with:

$$K_n = W/a\mathrm{SL}^n$$

where W: weight (g), SL: standard length (mm), a and n are the exponential form of the intercept and slope, respectively, of the logarithmic (log) length–weight equation.

Statistical analyses

To assess the trophic ecology and interactions between fish species, we analysed the degree of similarity between FAs and SI compositions.

Patterns in FA composition of individual fish were analysed using principal component analysis (PCA) with PRIMER 6. We selected 20 FAs that were the most abundant across all species, including 16:0, 16:1n7, 18:0, 18:1n7, 18:1n9, 18:2n6, 18:3n6, 18:3n4, 20:0, 20:1n9, 20:4n6, 20:4n3, 20:5n3 (EPA), 22:0, 22:1n9, 22:2n6, 22:5n3 (DPA), 22:6n3 (DHA), 24:0 and 24:1n9. Prior to analysis, each FA content was ln-transformed in order to reduce the relationship between the mean and the variance and to linearize the relationship between variables. A standardized PCA based on the correlation matrix was performed on the transformed values, and the sample scores were shown on the first factorial plane (PC1 vs. PC2). In order to further test for interspecific and interannual FA patterns, a redundancy analysis (RDA; Legendre and Legendre 1998) was performed. The significance of the relationship between FA and the two factors as well as their interactive effects was investigated by Monte Carlo permutation tests. First, a general test was applied to test the null hypothesis of independence between datasets, the response and the explanatory variables. Second, a forward selection process was performed in order to identify the most important variables. Here, the adjusted R^2-function is maximized, similar to the selection process in stepwise multiple regression. RDA analyses were performed with the software R 3.0.2 (R Core Team 2013) for statistical computing and the related package vegan (Oksanen et al. 2013).

To test for the suitability of carnivory markers as well as the capacity of FA indices as indicators for trophic position, we performed linear regressions between the baseline-corrected δ^{15}N of each fish with the three FA markers for carnivory (PUFA/SFA, DHA/EPA and 18:1n9/18:1n7).

To account for interspecific/interannual differences and potential interactive effects in SI, analysis of variance

Table 2 Summary of fatty acid (FA) and stable isotope (SI) dataset of fish species *Ctenolabrus rupestris* (*N* = 46), *Pomatoschistus minutus* (*N* = 49), *Pomatoschistus pictus* (*N* = 33), *Myoxocephalus scorpius* (*N* = 23) and *Taurulus bubalis* (*N* = 48)—collected from 2009 to 2011

Fatty acid	Ctenolabrus rupestris			Pomatoschistus minutus			Pomatoschistus pictus		
	2009	2010	2011	2009	2010	2011	2009	2010	2011
16:0	0.0099 ± 0.0030	0.0189 ± 0.0037	0.0216 ± 0.0038	0.0072 ± 0.0012	0.0154 ± 0.0030	0.013 ± 0.0041	0.0123 ± 0.0020	0.0195 ± 0.0023	0.0199 ± 0.0066
16:1n7	0.0019 ± 0.0011	0.0023 ± 0.0011	0.0030 ± 0.0019	0.0014 ± 0.0003	0.0022 ± 0.0007	0.0020 ± 0.0007	0.0017 ± 0.0004	0.0020 ± 0.0007	0.0030 ± 0.0015
18:0	0.0038 ± 0.0014	0.0072 ± 0.0018	0.0064 ± 0.0019	0.0028 ± 0.0006	0.0073 ± 0.0019	0.0056 ± 0.0016	0.0060 ± 0.0015	0.0107 ± 0.0029	0.0083 ± 0.0024
18:1n7	0.0009 ± 0.0020	0.0008 ± 0.0001	0.0008 ± 0.0001	0.0008 ± 0.0002	0.0007 ± 0.0001	0.0007 ± 0.0002	0.0011 ± 0.0002	0.0009 ± 0.0001	0.0011 ± 0.0001
18:1n9	0.0066 ± 0.0026	0.0111 ± 0.0036	0.0096 ± 0.0029	0.0042 ± 0.0009	0.0089 ± 0.0019	0.0039 ± 0.0012	0.0075 ± 0.0014	0.0119 ± 0.0036	0.0096 ± 0.0032
18:2n6	0.0013 ± 0.0003	0.0021 ± 0.0004	0.0012 ± 0.0002	0.0012 ± 0.0003	0.0045 ± 0.0015	0.0009 ± 0.0003	0.0024 ± 0.0004	0.0028 ± 0.0004	0.0024 ± 0.0005
18:3n6	0.0055 ± 0.012	0.0005 ± 0.0001	0.0005 ± 0.0001	0.0056 ± 0.0015	0.0006 ± 0.0001	0.0004 ± 0.0001	0.0075 ± 0.0014	0.0006 ± 0.0001	0.0007 ± 0.0002
18:3n4	0.0050 ± 0.0012	0.0004 ± 0.0001	0.0004 ± 0.0001	0.0048 ± 0.0014	0.0004 ± 0.0001	0.0003 ± 0.0001	0.0063 ± 0.0014	0.0004 ± 0.0001	0.0005 ± 0.0001
20:0	0.0014 ± 0.0003	0.0013 ± 0.0002	0.0012 ± 0.0002	0.0013 ± 0.0004	0.0011 ± 0.0002	0.0010 ± 0.0003	0.0018 ± 0.0004	0.0015 ± 0.0002	0.0016 ± 0.0002
20:1n9	0.0012 ± 0.0003	0.0017 ± 0.0005	0.0010 ± 0.0002	0.0007 ± 0.0002	0.0007 ± 0.0001	0.0005 ± 0.0002	0.0011 ± 0.0002	0.0009 ± 0.0001	0.0009 ± 0.0001
20:4n6	0.0024 ± 0.0007	0.0038 ± 0.0011	0.0033 ± 0.0005	0.0022 ± 0.0004	0.0034 ± 0.0009	0.0017 ± 0.0005	0.0029 ± 0.0005	0.0031 ± 0.0006	0.0030 ± 0.0007
20:4n3	0.0010 ± 0.0002	0.0010 ± 0.0002	0.0007 ± 0.0001	0.0009 ± 0.0002	0.0008 ± 0.0001	0.0005 ± 0.0002	0.0012 ± 0.0002	0.0011 ± 0.0001	0.0009 ± 0.0001
20:5n3 (EPA)	0.0056 ± 0.0016	0.0075 ± 0.0015	0.0073 ± 0.0020	0.0073 ± 0.0008	0.0087 ± 0.0024	0.0064 ± 0.0015	0.0111 ± 0.0019	0.0139 ± 0.0025	0.0098 ± 0.0024
22:0	0.0018 ± 0.0004	0.0016 ± 0.0003	0.0018 ± 0.0003	0.0033 ± 0.0013	0.0014 ± 0.0002	0.0016 ± 0.0005	0.0040 ± 0.0015	0.0020 ± 0.0003	0.0025 ± 0.0003
22:1n9	0.0010 ± 0.0002	0.0009 ± 0.0002	0.0009 ± 0.0002	0.0009 ± 0.0003	0.0009 ± 0.0002	0.0008 ± 0.0003	0.0012 ± 0.0003	0.0009 ± 0.0001	0.0013 ± 0.0002
22:2n6	0.0009 ± 0.0002	0.0008 ± 0.0002	0.0008 ± 0.0002	0.0009 ± 0.0003	0.0007 ± 0.0001	0.0008 ± 0.0002	0.0012 ± 0.0003	0.0009 ± 0.0001	0.0011 ± 0.0001
22:5n3 (DPA)	0.0030 ± 0.0008	0.0037 ± 0.0011	0.0055 ± 0.0057	0.0036 ± 0.0005	0.0031 ± 0.0020	0.0036 ± 0.0010	0.0033 ± 0.0005	0.0021 ± 0.0003	0.0021 ± 0.0005
22:6n3 (DHA)	0.0130 ± 0.0039	0.0284 ± 0.0066	0.0127 ± 0.0054	0.0081 ± 0.0033	0.0223 ± 0.0036	0.0074 ± 0.0024	0.0212 ± 0.0066	0.0348 ± 0.0055	0.0166 ± 0.0057
24:0	0.0026 ± 0.0006	0.0022 ± 0.0004	0.0030 ± 0.0005	0.0024 ± 0.0008	0.0019 ± 0.0003	0.0028 ± 0.0009	0.0034 ± 0.0007	0.0027 ± 0.0005	0.0043 ± 0.0006
24:1n9	0.0015 ± 0.0003	0.0014 ± 0.0003	0.0015 ± 0.0003	0.0013 ± 0.0004	0.0011 ± 0.0002	0.0013 ± 0.0004	0.0018 ± 0.0004	0.0015 ± 0.0003	0.0020 ± 0.0002
18:1n9/18:1n7	7.5810 ± 3.1188	13.9199 ± 5.0587	11.5795 ± 2.6632	5.2422 ± 0.8699	12.6419 ± 2.7442	5.4259 ± 0.8333	6.7921 ± 1.5793	13.8548 ± 4.2034	9.0624 ± 2.8988
DHA/EPA	2.3893 ± 0.5801	3.8234 ± 0.7062	1.8906 ± 1.0612	1.1046 ± 0.4352	2.6660 ± 0.5604	1.1544 ± 0.2163	1.8895 ± 0.4146	2.5258 ± 0.2595	1.7403 ± 0.5298
PUFA/SFA	2.1544 ± 0.2571	1.6743 ± 0.1575	1.0603 ± 0.1107	2.2391 ± 0.1710	1.8057 ± 0.1785	1.0386 ± 0.1171	2.2616 ± 0.1725	1.7394 ± 0.1727	1.1444 ± 0.1865
Stable isotope									
$\delta^{13}C$	−16.8 ± 0.4	−16.7 ± 0.3	−16.0 ± 0.3	−14.9 ± 1.0	−17.6 ± 1.4	−15.5 ± 0.6	−16.4 ± 0.5	−15.3 ± 0.2	−17.3 ± 0.5
$\delta^{15}N$	18.2 ± 0.4	18.9 ± 0.5	18.6 ± 0.5	17.8 ± 0.7	14.5 ± 1.5	17.3 ± 0.5	16.5 ± 0.4	17.0 ± 0.5	16.2 ± 0.6
Trophic position (TP)	3.7 ± 0.2	3.6 ± 0.2	3.8 ± 0.1	3.5 ± 0.2	3.4 ± 0.5	3.3 ± 0.2	3.1 ± 0.1	3.0 ± 0.2	3.0 ± 0.2

Table 2 continued

Fatty acid	Myoxocephalus scorpius			Taurulus bubalis		
	2009	2010	2011	2009	2010	2011
16:0	0.0051 ± 0.0013	0.0125 ± 0.0044	0.0140 ± 0.0064	0.0053 ± 0.0014	0.0135 ± 0.0026	0.0147 ± 0.0014
16:1n7	0.0007 ± 0.0003	0.0014 ± 0.0006	0.0020 ± 0.0120	0.0007 ± 0.0002	0.0016 ± 0.0006	0.0018 ± 0.0005
18:0	0.0019 ± 0.0004	0.0054 ± 0.0005	0.0054 ± 0.0039	0.0022 ± 0.0006	0.0075 ± 0.0029	0.0056 ± 0.0012
18:1n7	0.0005 ± 0.0001	0.0008 ± 0.0002	0.0009 ± 0.0003	0.0006 ± 0.0001	0.0013 ± 0.0005	0.0009 ± 0.0001
18:1n9	0.0034 ± 0.0012	0.0072 ± 0.0028	0.0068 ± 0.0030	0.0035 ± 0.0010	0.0086 ± 0.0025	0.0064 ± 0.0012
18:2n6	0.0010 ± 0.0002	0.0017 ± 0.0003	0.0012 ± 0.0004	0.0010 ± 0.0003	0.0023 ± 0.0006	0.0012 ± 0.0002
18:3n6	0.0034 ± 0.0005	0.0005 ± 0.0001	0.0005 ± 0.0002	0.0035 ± 0.0008	0.0008 ± 0.0003	0.0005 ± 0.0001
18:3n4	0.0032 ± 0.0005	0.0004 ± 0.0001	0.0004 ± 0.0002	0.0038 ± 0.0011	0.0007 ± 0.0003	0.0004 ± 0.0001
20:0	0.0008 ± 0.0001	0.0011 ± 0.0002	0.0012 ± 0.0005	0.0009 ± 0.0002	0.0018 ± 0.0007	0.0012 ± 0.0002
20:1n9	0.0005 ± 0.0001	0.0008 ± 0.0002	0.0007 ± 0.0003	0.0005 ± 0.0001	0.0011 ± 0.0003	0.0007 ± 0.0001
20:4n6	0.0019 ± 0.0005	0.0032 ± 0.0013	0.0025 ± 0.0008	0.0020 ± 0.0004	0.0039 ± 0.0009	0.0026 ± 0.0006
20:4n3	0.0006 ± 0.0001	0.0009 ± 0.0002	0.0007 ± 0.0002	0.0006 ± 0.0001	0.0013 ± 0.0004	0.0006 ± 0.0001
20:5n3 (EPA)	0.0052 ± 0.0013	0.0092 ± 0.0038	0.0077 ± 0.0031	0.0047 ± 0.0008	0.0086 ± 0.0025	0.0059 ± 0.0007
22:0	0.0011 ± 0.0002	0.0015 ± 0.0002	0.0020 ± 0.0007	0.0012 ± 0.0003	0.0025 ± 0.0010	0.0020 ± 0.0003
22:1n9	0.0006 ± 0.0001	0.0009 ± 0.0002	0.0011 ± 0.0004	0.0006 ± 0.0002	0.0014 ± 0.0005	0.0010 ± 0.0002
22:2n6	0.0006 ± 0.0001	0.0008 ± 0.0001	0.0010 ± 0.0003	0.0006 ± 0.0002	0.0013 ± 0.0005	0.0009 ± 0.0002
22:5n3 (DPA)	0.0018 ± 0.0003	0.0026 ± 0.0008	0.0020 ± 0.0006	0.0016 ± 0.0004	0.0033 ± 0.011	0.0025 ± 0.0006
22:6n3 (DHA)	0.0074 ± 0.0026	0.0136 ± 0.0061	0.0080 ± 0.0026	0.0066 ± 0.0017	0.0094 ± 0.0032	0.0058 ± 0.0011
24:0	0.0015 ± 0.0003	0.0021 ± 0.0003	0.0035 ± 0.0013	0.0017 ± 0.0004	0.0035 ± 0.0014	0.0034 ± 0.0006
24:1n9	0.0009 ± 0.0002	0.0012 ± 0.0003	0.0017 ± 0.0006	0.0010 ± 0.0002	0.0019 ± 0.0007	0.0016 ± 0.0003
18:1n9/18:1n7	6.5561 ± 2.0621	9.0001 ± 2.1672	7.2086 ± 1.9430	6.1707 ± 2.2665	7.1961 ± 2.0555	7.2564 ± 1.1873
DHA/EPA	1.4233 ± 0.2481	1.4524 ± 0.0746	1.0895 ± 0.1644	1.4319 ± 0.3312	1.0967 ± 0.2355	1.0023 ± 0.2009
PUFA/SFA	2.7136 ± 0.2063	1.5701 ± 0.2394	1.1039 ± 0.1970	2.5302 ± 0.3173	1.3094 ± 0.1906	0.8725 ± 0.0568
Stable isotope						
$\delta^{13}C$	−16.0 ± 0.5	−15.5 ± 0.2	−15.1 ± 0.4	−16.8 ± 0.5	−16.4 ± 0.4	−15.2 ± 0.4
$\delta^{15}N$	18.7 ± 0.4	19.9 ± 0.4	18.5 ± 0.7	18.6 ± 0.5	19.4 ± 0.7	17.9 ± 0.9
Trophic position (TP)	3.9 ± 0.1	4.0 ± 0.1	3.7 ± 0.2	3.8 ± 0.2	3.8 ± 0.2	3.6 ± 0.3

Data are expressed as mean ± standard deviation (SD) in [µgFA/µgC] and (‰), respectively

EPA is eicosapentaenoic acid (20:5n-3) and DHA is docosahexaenoic acid (22:6n-3)

PUFA is the sum of all unsaturated fatty acids

SFA is the sum of all saturated fatty acids

(ANOVA) were performed with Statistica 9.1, followed by Tukey's honest significant difference test (HSD) for unequal sample size (N). Interannual changes in the condition of *P. minutus* were investigated with analysis of covariance (ANCOVA) by comparing the logarithmic length–weight relationships between sampling years. Prior to these analyses, data were tested for the analytical assumptions of variance homogeneity and normality.

Results

Fatty acids (FA)

Applying a principal component analysis (PCA), the most important 20 FAs of all samples resulted in 69.1 %

Table 3 Summary of stable isotope analyses (SIA) dataset of baseline organisms under study expressed as mean values ± standard deviation (SD) in (‰): *Mytilus edulis* (collected each year at monthly intervals from June to November from North-east harbour and South harbour) and *Littorina* littorea (collected each year at monthly intervals from June to November from South beach)

	N	Mean $\delta^{13}C \pm$ SD	Mean $\delta^{15}N \pm$ SD
Mytilus edulis			
2009	100	-17.9 ± 1.3	11.1 ± 1.1
2010	180	-17.6 ± 1.0	10.5 ± 1.2
2011	90	-19.4 ± 0.9	10.1 ± 0.8
Littorina littorea			
2009	100	-17.3 ± 0.9	13.3 ± 0.9
2010	120	-15.7 ± 1.4	14.2 ± 1.6
2011	60	-16.8 ± 0.7	13.4 ± 0.8

explained variability on the first two axes (PC1 = 47 %, PC2 = 22.1 %), and only little further information was added by subsequent PCs (e.g. PC3 = 8.6 %, Fig. 2). Most of the variability in the FA composition of the five littoral fish species was caused by FAs listed in Table 4. Among these FAs, the trophic markers 18:1n7, 18:1n9 and 22:6n3 (DHA) displayed high loadings on the first two axes contributing to the clustering effects in Fig. 2a, b. We found distinct annual FA compositions with a slight overlap between the samples collected in 2010 and 2011 (Fig. 2a). The interannual differences were mainly described by the second axis (PC2) with negative scores for 2010 and 2011 and positive scores for 2009. High positive correlations with the second PC were found e.g. for the FAs 18:3n6 and 18:3n4 and a negative correlation with 16:0 (see Table 4), meaning that the first two FAs were found in above-average concentrations in 2009 and in below-average concentrations in 2010 and 2011. In contrast to this, interspecific differences were less pronounced and species-specific samples overlapped to a high degree on the first factorial plane (Fig. 2b). However, the highest variability between species was found on the first PC, to which all FAs displayed a negative correlation (Table 4). This means that samples with positive PC scores had below-average concentrations for most of these FAs, whereas samples with negative PC scores showed above-average FA concentrations. Highest scores were found for *P. minutus*, lowest for *P. pictus*. The other three species (*C. rupestris*, *M. scorpius* and *T. bubalis*) were not clearly separated on the first two axes.

To elucidate the differences among individual FA patterns, redundancy analysis (RDA) was used in order to
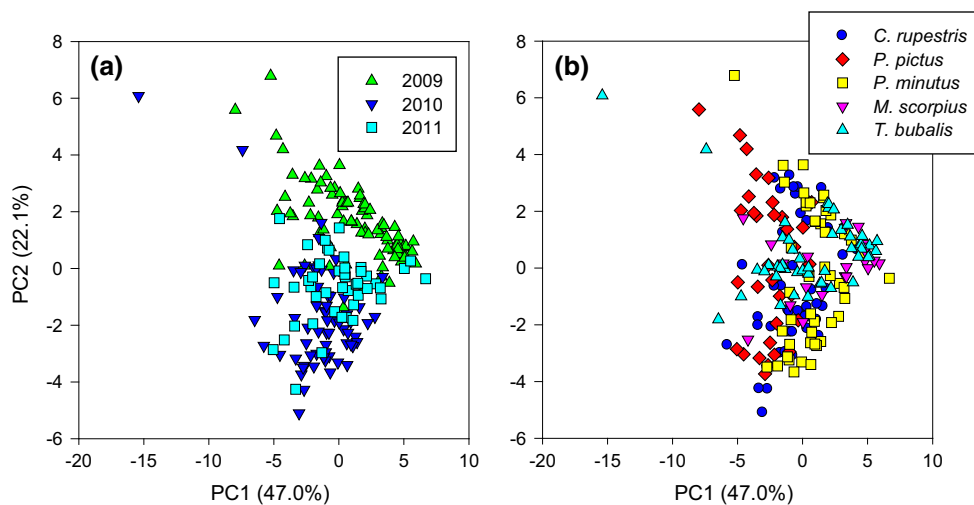

Fig. 2 Principal component analysis plots each based on the correlation matrix of 20 ln(x + 1)-transformed fatty acids (FA) measured of the fish species *Ctenolabrus rupestris*, *Pomatoschistus minutus*, *Pomatoschistus pictus*, *Myoxocephalus scorpius* and *Taurulus bubalis* collected from 2009 to 2011. The first two axes explain 69.1 % of the total variability. **a** Samples are marked according to their sampling year; **b** samples are marked according to species

test for species- and annual-specific effects. Both factors explained a significant amount of the variability in the FA contents ($p < 0.005$) with a total of 58.3 % variability explained on all axes and 42.3 % on the first factorial plane (Table 5). The interaction between both factors, species and sampling year, was significant on the $p < 0.01$ level. When accounting for factors separately as well as sequentially, all effects turned out to be significant with a slightly more variation explained by sampling year than by species (Table 5). In order to assess the importance of each factor level, we additionally repeated the analysis with binary-coded variables for each species and sampling year. In the selection process, the year 2010 and the species *P. pictus* were revealed to be the variables explaining most of the variation in the FA patterns. This suggests that the gobies caught in 2010 showed strong deviations from the general FA pattern in comparison with all other species.

Stable isotopes (SI)

Compared to FA profiles, SI showed less pronounced interannual differences in fish species (Fig. 3a; Table 2). Significant interannual differences were observed in δ^{13}C values with a smaller difference observed between 2009 and 2010 (ANOVA, $p < 0.05$) and a bigger difference between 2010 and 2011 (ANOVA, $p < 0.01$). In contrast to this, annual mean δ^{15}N values showed no clear difference between years, but δ^{15}N were extremely variable in 2010, ranging from 14.5 ‰ for *P. minutus* to 19.9 ‰ for *M. scorpius*. This variability was mainly caused by *P. minutus* that moreover showed significant differences in 2010 compared to *P. minutus* sampled in 2009 and 2011 with 17.8 ‰ and 17.3 ‰, respectively, (2009–2010, 2010–2011 both with an ANOVA p value of <0.01, Fig. 3). Hence, some species also experienced differences in SI throughout the sampling years (i.e. *T. bubalis* showed a different SI in 2010), but *P. minutus* displayed the strongest variability in calculated SI ± standard deviation (δ^{15}N: 16 ± 2 ‰ and δ^{13}C: −16 ± 2 ‰).

With regard to δ^{15}N, the two gobiid species *P. minutus* and *P. pictus* were clearly separated from the remaining species (ANOVA, $p < 0.01$), with distinct δ^{15}N values of ~ 16 ‰ for *P. minutus* and ~ 18 ‰ for *P. pictus*. Differences in δ^{13}C were less pronounced and only the δ^{13}C of *C. rupestris* and *M. scorpius* differed significantly from each other (ANOVA, $p < 0.01$). A notable distinction in the ^{13}C-enrichment was found between *M. scorpius* and the remaining species *C. rupestris*, *P. pictus* and *T. bubalis*. (−15.6 ‰ for *M. scorpius* vs. ~ −17 ‰, respectively). The δ^{13}C values of *P. minutus* varied strongly between years. The 2010 samples showed significantly depleted δ^{13}C values compared to samples from 2009 and 2011 (−18, −15 and −15 ‰, respectively, ANOVA, $p < 0.05$). The mean trophic position (TP) among the two gobiid species did not differ significantly (TP of 3.0–3.5), but the gobies differed from all other species (TP 4) resulting in a lower trophic position than *C. rupestris*, *M. scorpius* and *T.*

Table 4 Summary of top 10 PCA loadings based on $\ln(x + 1)$-transformed and standardized fatty acid (FA) data of the fish species *Ctenolabrus rupestris*, *Pomatoschistus minutus*, *Pomatoschistus pictus*, *Myoxocephalus scorpius* and *Taurulus bubalis* collected from 2009 to 2011, expressed in [µgFA/µgC] explaining 69.1 % of variability on the first two axes (see Fig. 2)

Variable	PC1	Variable	PC2
20:0	−0.297	18:3n4	0.376
18:1n7	−0.293	18:3n6	0.366
22:1n9	−0.293	16:0	−0.307
24:1n9	−0.287	18:1n9	−0.290
20:4n3	−0.284	22:0	0.278
22:2n6	−0.278	22:6n3 (DHA)	−0.241
24:0	−0.260	18:0	−0.238
18:0	−0.235	16:1n7	−0.231
18:1n9	−0.235	22:2n6	0.218
16:0	−0.231	20:4n6	−0.203

Table 5 Results of RDA analyses constraining individual FA compositions with the factors species, sampling year as well as their interaction

Source	df	Variance	Proportion explained	F	P
Full model					
Constrained	14	11.6604	0.58302	18.376	0.005
Unconstrained	184	8.3396	0.41698		
Total	198	20	1		
Effects (terms sequentially added)					
Species	4	3.7142	0.18571	20.487	0.01
Sampling year	2	4.91	0.2455	54.1659	0.01
Species: sampling year	8	3.0362	0.15181	8.3737	0.01
Residual	184	8.3396	0.41698		

Fig. 3 Stable isotope biplots of nitrogen stable isotopes (δ^{15}N) versus carbon stable isotopes (δ^{13}C) of the fish species *Ctenolabrus rupestris*, *Pomatoschistus minutus*, *Pomatoschistus pictus*, *Myoxocephalus scorpius* and *Taurulus bubalis* collected from 2009 to 2011. Baseline organisms *Littorina littorea* and *Mytilus edulis* are represented as mean values. **a** Samples are marked according to their sampling year; **b** samples are marked according to species
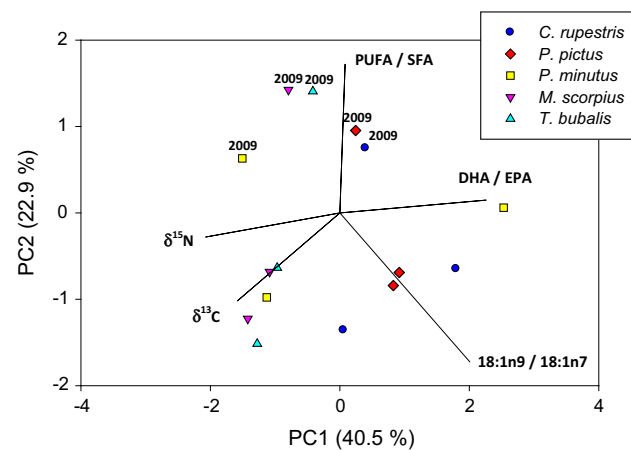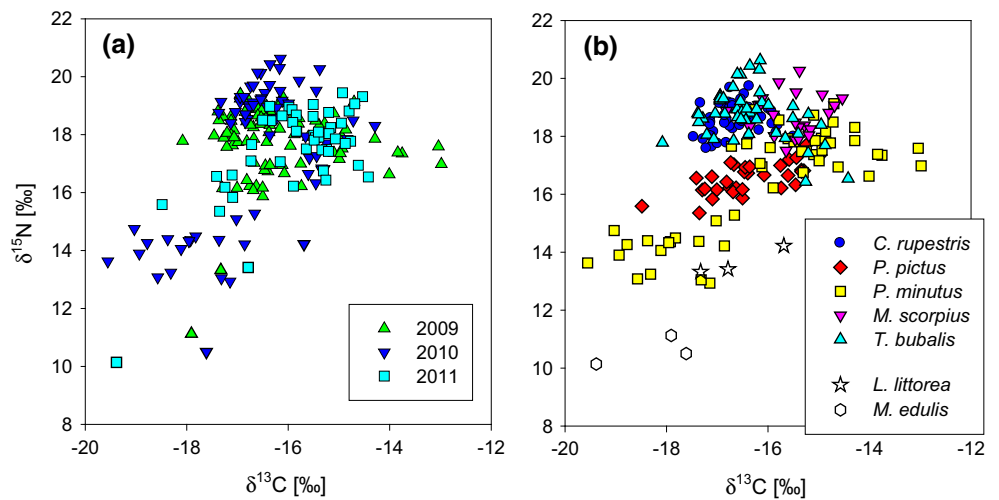
Fig. 4 Biplot of the principal component analysis based on the correlation matrix of the combined trophic markers, i.e. three fatty acid (FA) ratios as well as carbon stable isotope (δ^{13}C) and nitrogen stable isotope (δ^{15}N). 63.4 % of the total variability is explained on the first two axes. Mean PC1 and PC2 scores are visualized for each year and each of the five fish species (*Ctenolabrus rupestris*, *Pomatoschistus minutus*, *Pomatoschistus pictus*, *Myoxocephalus scorpius*, *Taurulus bubalis*). Dotted lines indicate the time trajectory from 2009 to 2011

Table 6 Summary on pivotal PCA loadings based on standardized trophic markers incl. fatty acid (FA) ratios and stable isotope (SI) data of fish species *Ctenolabrus rupestris*, *Pomatoschistus minutus*, *Pomatoschistus pictus*, *Myoxocephalus scorpius* and *Taurulus bubalis* collected from 2009 to 2011 explaining 63.4 % of variability on the first two axes (see Fig. 4)

Variable	PC1	Variable	PC2
DHA/EPA	0.566	PUFA/SFA	0.862
δ^{13}C	−0.519	18:1n9/18:1n7	−0.431
18:1n9/18:1n7	0.502	δ^{13}C	−0.255
δ^{15}N	−0.396	δ^{15}N	−0.070
PUFA/SFA	0.041	DHA/EPA	0.037

bubalis (Table 2). However, TP of *P. minutus* did not differ significantly among sampling years when baseline corrections were applied for specimen sampled in 2010.

Multi-tracer approach

We used a PCA to combine the results of SI and FA analyses (Fig. 4; Table 6). Here, 63.4 % of the total variation was explained by the first two principal components (PC1: 40.5 %, PC2: 22.9 %). Moreover, PC3 explained 17.2 % of the variance and δ^{15}N showed the highest loading therein. For clarity, only the species-specific annual mean PC scores were visualized in Fig. 4. PC2 mainly represents the FA trophic marker PUFA/SFA ratio and accounts for the differences that were found between 2009 and the other two sampling years (2010 and 2011). These were, with the exception of samples from *P. minutus* in 2010, similar according to their PC2 score. Species scores are separated on the first axis, with FAs (i.e. DHA/EPA and 18:1n9/18:1n7) loading positively, SIs loading negatively on PC1. *C. rupestris*, *P. pictus* and the 2010 population of *P. minutus* have above-average values for FA biomarkers and below-average values for SIs. In contrast, samples of *M. scorpius*, *T. bubalis* and *P. minutus* (2009 + 2010) are characterized by negative PC1 values. Thus, lower levels of 18:1n9/18:1n7 and DHA/EPA and higher levels of SI biomarkers were observed. Furthermore, species-specific scores of *T. bubalis* resembled those of *M. scorpius*, over all sampling years. *P. minutus* is the only species where interannual differences were pronounced not only on the second, but also on the first PC forming a separate cluster of the 2010 population in contrast to 2009 and 2011. The latter are presented by an opposing trend caused by a significant lower DHA/EPA ratios and

remarkably higher $\delta^{13}C$ as well as $\delta^{15}N$ values (Fig. 4). None of the three FA markers—suggested being representative for carnivory (El-Sabaawi et al. 2009)—correlated significantly with the baseline-corrected $\delta^{15}N$ of fish. However, we still decided to use these markers in our analysis in order to separate fish species from each other.

Length–weight relationship of *P. minutus*

Due to its distinct role, we compared variations in *P. minutus* condition by logarithmic length-weight relationships among sampling years (Fig. 5). No significant interactions between log length and sampling year were found, meaning that the slope of the regression line remained the same over the entire investigation period. However, when comparing the intercepts and calculating marginal means, the *P. minutus* population of 2010 showed considerably lower weights at the same length compared to the populations from 2009 and 2011 ($p < 0.001$, ANCOVA), indicating overall a lower condition.

Discussion

The present study aimed at analysing trophic variation and trophic niche partitioning among littoral fish species within the rocky intertidal community off Helgoland (German Bight).

The fauna around Helgoland is characterized by a high diversity and complex trophic relations (Janke 1990). The habitat structure is of considerable relevance when the ranges of trophic positions within a given food web are addressed (Bearhop et al. 2004; Layman et al. 2007). In general, trophic niche partitioning is considered as a major

strategy for fish assemblages which might be of even greater relevance than temporal and spatial separation (Ross 1986). In this study, we used FA and SI analyses since these tracers allow an integration of diet preferences over a longer period of time prior to sampling. SIs and FAs analyses allowed us to characterize the trophic ecology of the five fish species and provided valuable information on the trophic positions as well as interannual and interspecific variations in our local fish community.

Fatty acid composition of target fish species

Fatty acid (FA) analyses are considered as a reliable tool to trace food sources within a given food web by identifying characteristic, taxa-specific FA pattern at the base of the food web, e.g. aquatic primary producers which are in turn transferred to higher trophic levels (Dalsgaard et al. 2003). However, this approach has recently been criticized pointing at specific uncertainties in using FA as trophic markers when studying the trophic ecology of consumers especially in benthic communities (Kelly and Scheibling 2012; McLeod et al. 2013). Despite potential flaws, FAs are still considered to provide useful dietary information especially with regard to higher-order consumers, e.g. vertebrates, mammals (Kelly and Scheibling 2012).

FA profiles of the examined fish species provided insights into the trophic ecology within the Helgoland rocky intertidal food web, suggesting that FA patterns are driven by differences in dietary preferences, associated with interannual variability. The unsaturated FAs 18:1n7, 18:1n9 and 22:6n3 (DHA) were identified as the most important drivers characterizing trophic relations. These FAs are considered as essential components in the diets of fish (Sargent et al. 1999b) acting as reliable indicators, i.e. functioning as dietary markers by providing valuable information on trophic niche partitioning of fish species. The conservative character of these FAs leads to a valid detection of the transfer from ingested dietary sources to higher trophic levels (Bell et al. 1986; Dalsgaard and St John 2004). The occurrence of FAs with other functions (i.e. metabolic processes and/or storage function in organisms) like 16:0 further provide information on species-specific markers that are of considerable relevance when describing complex food webs. Overall, the present results support the assumption that diet variation results in broad FA spectra of consumers (Budge et al. 2002).

In this study, interannual differences in FA signatures were slightly more pronounced than differences between species, but both factors were highly significant. In addition, we found some evidence for intraspecific differences in FA signatures, but interspecific variations played a more pronounced role. This is in line with observations from other fish assemblages at e.g. the Scotian Shelf, Georges
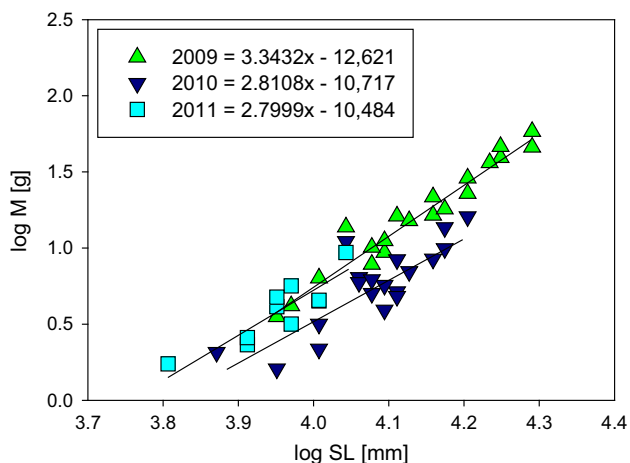

Fig. 5 Logarithmic length–weight relationship of *Pomatoschistus minutus* as an index of relative body condition for each sampling year 2009–2011

Bank, Gulf of Lawrence or the North-western Hawaiian Islands where strong interspecific variations in FA were reported (Budge et al. 2002; Piche et al. 2010). Overall, the factors sampling year and species explained most of the FA variation of our five fish species with slightly more variation explained by interannual changes in FA profiles.

The observed interannual variations might be explained by changes in the foraging strategy of our fish species. We sampled all specimens with regard to availability, and therefore, spatial and temporal differences between sampling years and seasonal changes in prey availability and/or consumers' preferences have to be taken into consideration (e.g. El-Sabaawi et al. 2009). Further, intrapopulation variations in terms of resource use and habitat need to be addressed when evaluating food web relations (Bolnick et al. 2003). The observed intraspecific and interannual variability in this study can most likely be explained by a strong flexibility in the foraging behaviour, since changes in feeding modes are a common pattern in fish and diet switches resulting from changes in prey availability and quality commonly occur (Hart 1993).

Stable isotope composition of target fish species

The δ^{15}N and δ^{13}C signatures of our fish species showed distinct niche separation patterns. While δ^{15}N reflects the 'stepwise enrichment with trophic transfers' (Fry 2006; Inger and Bearhop 2008), δ^{13}C is an indicator for the diet preferences of consumers (Peterson and Fry 1987; Post 2002). Strong overlaps in δ^{13}C indicate a similar pool of carbon sources used, while species with a similar δ^{15}N are considered to occupy similar trophic positions. In our study, we found a close relationship between the two gobiid species *P. pictus* and *P. minutus* resulting in similar trophic positions (TP) and diet preferences (δ^{13}C) with a tendency towards a more generalistic feeding mode of *P. minutus*. Leitao et al. (2006) characterized *P. minutus* as an opportunistic carnivorous species, with a feeding mode which is confirmed by the wide δ^{13}C ranges found in our field study. This is in line with studies reporting on similar feeding preferences of both species with only slightly different dietary sources (Wheeler and Du Heaume 1969; Hureau et al. 1986) and minor differences in habitat preference in terms of microhabitat utilization: semi-hyperbenthic (*P. pictus*) versus epibenthic (*P. minutus*) guilds identified by Wilkins and Myers (1992). We are aware that the different sampling sites in this study might have been influenced by differences in microhabitat utilization. Due to the close vicinity of the sampling sites, we are convinced that our study still provides representative data of the entire rocky intertidal fish community. Moreover, differences in mouth gape opening between similar-sized specimens of both species could furthermore explain the observed

moderate differences in prey preference/consumption between *P. pictus* and *P. minutus*.

The wide range for δ^{13}C in *M. scorpius* implies a generalistic feeding mode which is in good agreement with other field studies (Norderhaug et al. 2005), while *C. rupestris*, commonly considered as a generalist (Fjosne and Gjosaeter 1996), showed a more specialized feeding mode in our study. The closely related Cottidae *T. bubalis* and *M. scorpius* are not only characterized by a strong morphological similarity, but also by a specific overlap in their preferred diets with a varying degree of similarity across rocky- and soft-bottom habitats (Wennhage and Pihl 2002). In our field study, distinct prey-derived carbon sources were observed assuming different preferences in their prevalent prey taxa. With regard to carbon isotopes, planktonic consumers in marine coastal environments such as *M. edulis* are in general considered to show a lower ^{13}C/^{12}C ratio (i.e. reflected by more negative δ^{13}C values) relative to benthic consumers (France 1995) such as *L. littorea*.

The five fish species analysed in this study were ^{13}C enriched relative to the mussels assuming the use of carbon sources derived from benthic production (with the exception of *P. minutus* 2010). This observation is in line with trophic information given by, e.g. Wennhage and Pihl (2002) for benthic fish communities in the rocky intertidal along the Swedish West coast as well as Norderhaug et al. (2005) for a Norwegian kelp forest habitat. However, *M. scorpius* showed a slightly stronger enrichment in ^{13}C relative to *C. rupestris*, *P. pictus* and *T. bubalis* pointing at strictly benthic-derived carbon sources, whereas *P. minutus* populations from 2010 as appeared to be the only component which was influenced by pelagic-derived carbon sources.

Moreover, the SI of *C. rupestris*, *M. scorpius*, *T. bubalis* point at a higher trophic position when compared to the gobiid species. Generally, the TP estimates of our fish species are in a good agreement with the predicted ones published in the literature (see Froese and Pauly 2000). In this context, especially the role of *P. minutus* needs further consideration due to its role as a key component of littoral fish assemblages and its relevance in marine and estuarine food webs. It has been stressed that *P. minutus* acts both as an intermediate predator and a preferred prey organism for other fish species (Ehrenbaum 1936; Wheeler and Du Heaume 1969; Leitao et al. 2006). Throughout our study, *P. minutus* and *P. pictus* showed lower TPs compared with *C. rupestris*, *M. scorpius* and *T. bubalis*. The lower TP in our gobiids might support previous observations pointing at a strong predation on gobiids by *C. rupestris*, *M. scorpius* and *T. bubalis* as reported by previous studies (Hildén 1978; Wennhage and Pihl 2002; Norderhaug et al. 2005). With regard to the trophic ecology of *P. minutus*, Dolbeth

et al. ([2008](#)) reported a more specialized feeding strategy which indicates that the diet ranges in *P. minutus* can also be restricted depending on the life history variations and habitat-specific differences of a given population. The above-mentioned conclusion could explain the wide range of $\delta^{13}C$ and $\delta^{15}N$ values measured from *P. minutus* samples between the 3 years observed in this study. Furthermore, the observed distinction among years could point to a more plankton-derived C influence in 2010 (i.e. caused by a massive decay of jellyfish blooms, pers. observation) which could have affected the local food web.

Multi-tracer approach

Various publications have suggested the combined use of different biomarkers in order to gain more reliable results on trophodynamic interactions in food web studies (Alfaro et al. [2006](#); Fry [2006](#); El-Sabaawi et al. [2009](#); Kelly and Scheibling [2012](#)). In this study, we used a tracer approach where we combined SI and FA biomarkers. On the one hand, we reduced our dataset of all measured FA up to a specific group of relevant diet-specific FA, the so-called fatty acid trophic markers (FATM after El-Sabaawi et al. ([2009](#)), assuming that these dietary components act as reliable indicators for a trophic niche partitioning of our fish species. We are aware that our FATM selection might have caused a simplification of the actual trophic interaction patterns. On the other hand, differences in FA might be obscured either by FATM composition of prey and/or by consumer's metabolic activities, and therefore, food sources and their differences in quality could modify the observed trophic interactions within the investigated community. Furthermore, we added the SI markers $\delta^{15}N$ and $\delta^{13}C$ to our PCA as response variables. It is known that not only fatty acid but also isotopic variation is not simply related to dietary differences among individuals in a consumer population, but likely to be a function of a number of processes such as nutrient availability, starvation or nutrient-specific assimilation efficiency (Aberle and Malzahn [2007](#); Inger and Bearhop [2008](#)). Given that SI analysis has limitations too, the use of a combined tracer approach minimizes susceptibility that emerges from single tracer techniques (Kelly and Scheibling [2012](#)). Thus, the proximity in PCA of *T. bubalis* and *M. scorpius* uncovered a distinct mutuality in feeding guilds (Wennhage and Pihl [2002](#)) showing similar patterns in feeding strategies and life histories of these two, closely related fish species (e.g. Ehrenbaum [1936](#); Wheeler and Du Heaume [1969](#)).

In general, the DHA/EPA ratio is used as an index for carnivory (Dalsgaard et al. [2003](#); Piche et al. [2010](#)). With regard to *C. rupestris*, a preference for crustaceans and mussels described by Hilldén ([1978](#)) and Wennhage and Pihl ([2002](#)) is supported by the high values in both FA

18:1n9/18:1n7 and DHA/EPA, indicating carnivorous feeding modes. In contrast, *M. scorpius* and *T. bubalis* commonly considered as carnivores in coastal food webs (Wheeler and Du Heaume [1969](#)), showed comparatively low DHA/EPA values in our study. Using SI as an additional trophic marker allowed us to confirm carnivory in *M. scorpius* and *T. bubalis* by displaying the highest $\delta^{15}N$ in the present study.

Although FAs were reduced to only three dietary biomarkers, we were still able to confirm interannual variability patterns which were mainly driven by consistently higher PUFA/SFA ratios in 2009. Moreover, the interannual variability of *P. minutus*, characterized by a high DHA/EPA and low $\delta^{15}N$ as well as $\delta^{13}C$ in 2010, was even more pronounced than in previous analyses. The combination of FA and SI markers allowed us to describe clear trophic patterns: one cluster of *P. minutus* (except 2010), *M. scorpius* and *T. bubalis* feeding on prey relying exclusively on benthic-derived carbon sources and another cluster of *C. rupestris* and *P. pictus*, which are characterized by the consumption of prey items using carbon sources derived from planktonic production. Furthermore, SI biomarkers rather than FA biomarkers enabled a clear segregation of *P. minutus*, *M. scorpius* and *T. bubalis* from the remaining species thus allowing a detailed view on trophic relations within our fish community. While FA signatures of consumers are known to become harder to differentiate with increasing trophic levels due to a wide variety in their dietary sources (Dalsgaard et al. [2003](#)), the additional use of SI filled this gap and provided valuable insights into our local food web. It has to be noted that FA measures like DHA/EPA, PUFA/SFA and 18:1n9/18:1n7, which were suggested being representative for carnivory in the literature, did not correlate with the trophic position of the fish in our study. Initially, these markers were reported as a measure for trophic relations among copepods (El-Sabaawi et al. [2009](#)) as well as other crustaceans, e.g. amphipods and krill (Stevens et al. [2004](#); Nyssen et al. [2005](#); Schmidt et al. [2006](#); Hagen et al. [2007](#)). It can be speculated that taxonomic differences and differences in fatty acid metabolism between benthic versus pelagic consumers as well as vertebrate versus invertebrates might be responsible for the contrasting results.

Identifying substructures in apparently homogeneous populations is another advantage of multi-tracer approaches. In particular, the trophodynamic patterns in *P. minutus* were significantly different in 2010—suggesting that FA and SI reflect differences in feeding modes and/or prey availability/quality. In general, a high interannual heterogeneity can be expected in gobiids, as these small fish are well known for their "great ecological diversity" (Bouchereau and Guelorget [1998](#)). Furthermore, habitat selection in gobiids implies that their distribution ranging from

estuaries, coastal areas to lagoons (Wheeler and Du Heaume 1969; Jaquet and Raffaelli 1989; Leitao et al. 2006) is wider than the one from other more stationary fishes. In this context, the low $\delta^{13}C$ and $\delta^{15}N$ of *P. minutus* in 2010 could result from differences in feeding habits or shifts in prey type and abundance. The pronounced inter-annual variability is supported by FA biomarkers which showed a consistently high PUFA/SFA ratio in 2009 and a distinct DHA/EPA ratio in 2010. Since we did not find any significant shifts in the baseline, such interannual variations in trophic markers can most likely be related to dietary shifts or changes in metabolic processes/activities (Sargent et al. 1999a).

In order to elucidate the differences among years for *P. minutus*, the body condition in terms of the interannual length–weight relationship as morphometric condition index for estimating the magnitude of energy reserve (Davidson and Marshall 2010) was further examined. The 2010 population turned out to have a significantly lower condition compared with the other two sampling years, and hence ranked the 2010 population to a lower nutritional condition. It can be assumed that the relative contribution of benthic and pelagic carbon sources caused the observed alterations in length–weight relationship in *P. minutus*. With regard to the gobies, a carbon source derived from pelagic production seems to be less beneficial for the condition of *P. minutus*.

This aspect would need further consideration in the future when addressing the pros and cons of differently derived carbon sources on the growth and condition of littoral fish species.

Conclusion

Trophic markers enable the characterization of diets, predator–prey relationships and trophic relations in food webs, especially when higher trophic levels are involved.

The observed differences in our target fish species were based on differences in SI as well as FA profiles, and hence the trophic position of each species within the local food web was highlighted. Furthermore, the present data enabled insights into the functional diversity of the Helgoland intertidal food web in the North Sea, thus allowing a characterization of this specific community. Furthermore, we were able to highlight the advantage of using a multi-dimensional trophic marker approach, thus strengthening the evaluation in the light of trophic niche partitioning. This permitted a detailed description of trophic structures in our local food web. Further, among- and within-species interactions and the advantages of single trophic markers were investigated in detail. Here, especially the focus on intraspecific interactions within *P. minutus* elucidated a high ecological diversity in this community thus pointing at the great advantage of multi-tracer approaches in food web studies.

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