

Seasonal lipid storage and dietary preferences of native European versus invasive Asian shore crabs

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ABSTRACT: The invasive Asian shore crab *Hemigrapsus sanguineus* and the native European green crab *Carcinus maenas* share intertidal habitats along European North Atlantic shores and may compete for food. We evaluated the energy-storing capacities of the 2 species and determined their dietary preferences by means of lipid analysis and fatty acid trophic marker indices. Specimens of both sexes and various sizes were sampled in the rocky intertidal of the island of Helgoland (North Sea) in April, June, August, and October 2015. Total lipids of the midgut glands were significantly higher in *H. sanguineus* than in *C. maenas* and followed a distinct seasonal cycle in both sexes (ca. 20–50% of dry mass, DM). The lower lipid contents of *C. maenas* (ca. 20% of DM) remained at a similar level throughout the seasons. The seasonal differences in the females of *H. sanguineus* may be due to higher reproductive output and, consequently, lipid turnover, but remain unexplained in males. Trophic indices for Bacillariophyceae, Chlorophyta, and especially Phaeophyceae were higher in *H. sanguineus* than in *C. maenas*, suggesting a higher degree of herbivory of the invader. In contrast, the Rhodophyta index was higher in *C. maenas*. Thus, competition for food between the 2 species will probably be low in habitats rich in macroalgae. The ability of *H. sanguineus* to utilize mainly energy-poor algae but accumulate high-energy reserves may be an advantage for successfully establishing persistent populations in new habitats.

KEY WORDS: Asian shore crab · *Hemigrapsus sanguineus* · European green crab · *Carcinus maenas* · Competition · Trophic markers · Fatty acids · North Sea

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INTRODUCTION

Biological invasions can entail severe ecological changes (Vitousek et al. 1997, Grosholz 2002, Didham et al. 2005). Non-indigenous species may alter structures of native communities and negatively influence biodiversity and ecosystem functioning. Ultimately, native species may become extinct, and ecosystem services, economic interests, and human health might be at risk (Ruiz et al. 2000, Gurevitch & Padilla 2004, Simberloff et al. 2013).

Brachyuran crabs are common invaders of marine and coastal ecosystems (Brockerhoff & McLay 2011). Among them, the European green crab *Carcinus maenas* (Linnaeus 1758) and the Asian shore crab *Hemigrapsus sanguineus* (de Haan 1835) show a very pronounced invasion potential. *C. maenas* is native to the European and northern African Atlantic coasts and invasive to many other coasts worldwide (Carlton & Cohen 2003). *H. sanguineus* originally inhabited the coastlines of Japan, Korea, and China (Fukui 1988, Stephenson et al. 2009) and was de-

tected along the US east coast in the late 1980s (Williams & McDermott 1990, Delaney et al. 2008, Epifanio 2013) as well as the European Atlantic coasts from the late 1990s onwards (Breton et al. 2002, Obert et al. 2007, Landschoff et al. 2013, Jungblut et al. 2017). Hence, *C. maenas* and *H. sanguineus* share intertidal habitats in their native and invaded habitats in Europe, the US east coast, and Asia, providing an interesting target for species invasion studies.

The factors determining the success of an invader in a new habitat are pivotal topics of ecological research (e.g. Elton 1958, Ruiz et al. 2000, Kolar & Lodge 2001, Sakai et al. 2001). Most studies are based on population structure and dynamics (e.g. Sakai et al. 2001, Russell et al. 2008, O'Connor 2014). Several ecological models and hypotheses have been established to explain invasive processes, such as the enemy release hypothesis, biotic resistance theory, or community ecology theory (e.g. Lodge 1993, Shea & Chesson 2002, Colautti et al. 2004). Only a few studies have addressed the underlying physiological mechanisms and properties, which are fundamental drivers of the invader's success (e.g. Kelley 2014).

Feeding and nutritional quality are key factors in heterotrophic organisms (Saborowski 2015). The ability to store energy reserves provides an advantage to overcome periods of food scarcity or starvation during ecdysis. In crabs, the midgut gland (hepatopancreas) is the main storage organ of dietary components such as proteins, carbohydrates, and particularly lipids (Jimenez & Kinsey 2015). Size and lipid content of the midgut gland provide a suitable indicator for the overall condition of the animal, its storage capacity, and the seasonal dynamics of energy deposition (Kyomo 1988, Griffen et al. 2015, Jimenez & Kinsey 2015). Moreover, the nutritional state is closely related to reproductive processes (Griffen et al. 2011, 2012, Zeng et al. 2014). Given the success of *H. sanguineus* in invading new areas, we expect this species to store larger amounts of lipids in their midgut glands to buffer periods of low food availability. Moreover, they have been described as 'income breeders', i.e. they use ingested energy directly for egg production, which should result in a rather constant lipid level in the midgut glands, unless food supply differs seasonally (Griffen et al. 2012). In contrast, *C. maenas* have been described as 'capital breeders', i.e. they deposit energy for the production of eggs prior to the reproductive season, which would result in a more seasonal pattern of lipid levels (Griffen et al. 2011).

Gut content analyses of *C. maenas* and *H. sanguineus* classified both species as opportunistic omnivores and thus potential competitors, although *H. sanguineus* has a higher preference for macroalgae (e.g. Ropes 1968, Tyrrell & Harris 1999, Lohrer et al. 2000, Griffen et al. 2012). Such analyses tend to overestimate recently ingested items and to underestimate easily digestible food. These biases can be eliminated by applying the fatty acid trophic marker (FATM) concept. The composition of fatty acids (FAs) integrates trophic preferences over a longer time period of days to weeks, compared to gut content analyses (Graeve et al. 2001, Dalsgaard et al. 2003, Latyshev et al. 2004).

The aims of the present study were to examine the lipid storage properties and determine feeding preferences of *C. maenas* and *H. sanguineus* by applying total lipid (TL) and FA analyses. The results were used to test the following hypotheses:

- (1) *H. sanguineus* stores larger amounts of lipids in the hepatopancreas than *C. maenas*.
- (2) The seasonal variation in TL levels is more pronounced in *C. maenas* than in *H. sanguineus*.
- (3) The FA composition differs (i) between species and within each species with (ii) sex, and (iii) seasons.
- (4) Trophic marker concentrations (i) differ between *C. maenas* and *H. sanguineus*, and differ for both species with (ii) size, (iii) sex, and (iv) season.

MATERIALS AND METHODS

Origin of samples

Males and females of *Carcinus maenas* and *Hemigrapsus sanguineus* were sampled in the intertidal at the southwestern tip ('Kringel') of the island of Helgoland (North Sea; 54° 10' 36.5" N, 7° 53' 03.3" E). Crabs were collected during low tides in April, June, August, and October 2015. Macroalgae were sampled at the same site in August 2015.

The sampling site is wave-exposed and composed of coarse sand with small to large rocks and boulders, which are covered with small to medium-sized macroalgae (see Table 3 for the prevailing macroalgae species). Further details about the habitats around Helgoland are provided by Bartsch & Tittley (2004).

Animals with carapace widths (CW) between 10 and 40 mm were collected, as these sizes are the most frequent in the intertidal of Helgoland (Jungblut et al. 2017). *H. sanguineus* do not reach sizes over 40 mm CW. To analyze animals of similar sizes, no *C. maenas* over 40 mm CW were collected.

C. maenas change their carapace color during the molt cycle from greenish to reddish, accompanied by alterations in behavior and physiology (reviewed by Styrihave et al. 2004). This red color morph was not considered, as it is rare in the intertidal of Helgoland and mostly occurs in subtidal areas. Only crabs with hard carapaces and without eggs were considered for further analysis. Freshly molted (soft carapace) and ovigerous crabs were omitted, as they usually show physiological modifications (e.g. Lewis & Haefner 1976, Naylor et al. 1997).

After sampling, crabs and algae were immediately transported to the laboratories of the Marine Station on Helgoland. Crabs were maintained in aquaria with aeration at room temperature until further processing on the same day. The CW of each crab was measured to the nearest 0.5 mm with Vernier calipers. After blotting dry with paper towels for 10 s, the fresh mass of the crabs was determined to the nearest 0.001 g. The midgut glands of the crabs were dissected and transferred into pre-weighed glass vials. The wet masses of the midgut glands were determined and the vials immediately frozen at -80°C . The hepatosomatic index (HSI) was calculated as the wet mass of the fresh midgut gland in relation to the rest of the crab's fresh mass (Kennish 1997, Griffen et al. 2011, 2012; our Table 1). The algae were briefly rinsed in distilled water, blotted dry, and frozen at -80°C .

TL extraction and FA analysis

Samples were transported on dry ice to the laboratories of the University of Bremen, Germany. The samples were lyophilized for 48 h and their dry mass was determined to the nearest 0.001 g. Lipids were extracted with dichloromethane:methanol (2:1 per volume) and an aqueous solution of KCl (0.88 %) after

Folch et al. (1957) and Hagen (2000). TL content was determined gravimetrically to the nearest 0.001 g and calculated as the percentage of lipids in relation to the dry mass of the sample ($\%_{\text{DM}}$; Table 1). For the analysis of FAs, subsamples of the lipid extracts were treated with methanol containing 3 % concentrated sulfuric acid to convert them to methyl ester derivatives (FAMES), which can be quantified by gas chromatography (GC; Kattner & Fricke 1986). The GC device (Agilent Technologies, 7890A) was equipped with a DB-FFAP column (30 m length, 0.25 mm diameter) and run with helium as carrier gas. It was operated with a programmable temperature vaporizer injector. The FAs were identified by their retention times. Menhaden fish oil and lipids of the copepod *Calanus hyperboreus* Krøyer, 1838 were used as standards (Schukat et al. 2014, Bode et al. 2015).

Free fatty alcohols and unidentified components accounted for only about 1 % of the total sample each and were not further considered. The FA dataset was evaluated according to the FATM concept. This concept is well established for marine zooplankton (reviewed by Dalsgaard et al. 2003, Lee et al. 2006) and has also been applied to benthic systems (reviewed by Kelly & Scheibling 2012). We developed new dietary indices to better distinguish between food items, i.e. carnivorous diet (I_{Ca}), Chlorophyta (I_{Ch}), Phaeophyceae (I_{p}), Rhodophyta (I_{R}), and Bacillariophyceae (I_{B}) (Table 1).

The FAs 18:1(n-7), 18:2(n-6), 18:3(n-3), and 18:4(n-3) are major components of Chlorophyta (green macroalgae). Additionally, 16:4(n-3) is a dominating FA in the order Ulvales (Kelly & Scheibling 2012 and references therein). Because 18:4(n-3) is the only FA that could be used as an indicator for Phaeophyceae (brown macroalgae) (Kelly & Scheibling 2012), it was omitted as a green algae indicator. Thus, $I_{\text{Ch}} = 16:4(n-3) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3)$ was used

Table 1. Equations for the calculation of condition parameters and fatty acid-based trophic marker indices

Parameter	Equation
Hepatosomatic index	$\text{HSI} = \text{Wet mass}_{\text{hepatopancreas}} / (\text{Fresh mass}_{\text{crab}} - \text{Wet mass}_{\text{hepatopancreas}})$
Total lipid content ($\%_{\text{DM}}$)	$\text{TL} = \text{Mass}_{\text{extracted lipids}} / \text{Dry mass}_{\text{sample}} \times 100$
Carnivory index	$I_{\text{Ca}} = 18:1(n-9) / [16:1(n-7) + 16:4(n-3) + 16:4(n-1) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3) + 18:4(n-3) + 20:4(n-6) + 20:5(n-3)]$
Chlorophyta index	$I_{\text{Ch}} = 16:4(n-3) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3)$
Phaeophyceae index	$I_{\text{p}} = 18:4(n-3)$
Rhodophyta index	$I_{\text{R}} = 20:5(n-3) / [16:0 + 18:0 + 22:6(n-3)]$
Bacillariophyceae index	$I_{\text{B}} = 16:1(n-7) + 16:4(n-1)$

as the dietary index for Chlorophyta, and $I_p = 18:4(n-3)$ as the indicator for Phaeophyceae. Rhodophyta (red macroalgae) are particularly rich in 20:5(n-3), which is also an important biomembrane FA in marine animals (Kelly & Scheibling 2012). The red algae diet index $I_R = 20:5(n-3) / [16:0 + 18:0 + 22:6(n-3)]$ sets 20:5(n-3) in relation to the 3 other FAs generally known as membrane FAs (Lee et al. 2006, Boissonnot et al. 2016). A high index might, thus, indicate consumption of red algae. The sum of $I_B = 16:1(n-7) + 16:4(n-1)$ was used as an index for diatom ingestion. 18:1(n-7) is also a Bacillariophyceae marker (Dalsgaard et al. 2003). However, the latter is also abundant in green algae and was thus omitted in the diatom index (Kelly & Scheibling 2012). The FA 18:4(n-3) is commonly used as dinoflagellate marker (Dalsgaard et al. 2003). It is also prevalent in green and brown algae (Kelly & Scheibling 2012) and thus cannot contribute to a dinoflagellate index. However, crabs probably do not distinguish between diatoms and dinoflagellates, and a high diatom index might thus generally indicate ingestion of benthic microalgae. To assess the carnivory of the crabs, we developed a carnivory index similar to that of zooplankton (Schukat et al. 2014, Bode et al. 2015). The carnivory marker FA 18:1(n-9) was used versus all FAs that are abundant or are marker FAs for algae: $I_{Ca} = 18:1(n-9) / [16:1(n-7) + 16:4(n-3) + 16:4(n-1) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3) + 18:4(n-3) + 20:4(n-6) + 20:5(n-3)]$. For benthic animals, I_{Ca} has to be interpreted with caution, as 18:1(n-9) is also an abundant FA in Phaeophyceae. This has to be considered when evaluating high I_{Ca} levels to avoid misinterpretations.

Statistical analysis

We used R version 3.2.3 (R Development Core Team 2015) to develop 7 individual linear models to test for the effects of the fully crossed factors 'species', 'sex', 'month', and 'fresh mass' on HSI, TL, and the 5 trophic marker indices. If needed, data were $\log(\text{data} + 1)$ -transformed to meet the assumptions of normal distribution and homogeneous variances of the residuals. Model stability was checked using Cook's distance, leverage, and dffits. All models were fitted using the generic function 'lm'. Significances of main factors and interaction terms were established with likelihood ratio tests (LRTs) using the function 'anova' with the argument 'test' set to 'F'. Non-significant terms/factors were excluded, unless they were relevant for significant interactions of

higher orders. Graphs were produced with the software GraphPad Prism (version 5.03).

Principal component analyses (PCAs) were conducted using the whole set of FA components (see Table 3). The percentages of each sample were transformed to proportions and arcsine-square-root transformed to achieve normality and homogeneity of variances. First, samples were checked for clustering by sex within one species and season. Subsequently, each species was checked for seasonal differences, not considering sexes any more. Finally, a PCA was conducted using all samples, including the algae. PCAs were conducted and graphs were produced with Primer v6 software (Clarke & Warwick 1994).

For more details, see 'Statistical analyses' in Supplement 1 at www.int-res.com/articles/suppl/m602p169_supp.pdf.

RESULTS

Mean values, averaged over fresh mass, of HSI, TL, and the dietary indices I_{Ca} , I_{Ch} , I_p , I_R , and I_B are presented in Table 2. Detailed figures for the dietary indices in relation to fresh mass are shown in Figs. S2–S6 of Supplement 1. Statistical results are summarized in Table 3. Here, we focus on the results of interactions that include the factor 'species'. Interactions excluding this factor (e.g. Sex \times Month \times Fresh mass) were not meaningful in view of the aims and questions of our study.

HSI

Carcinus maenas and *Hemigrapsus sanguineus* showed different seasonal patterns in HSI. *C. maenas* exhibited the highest average HSIs in April and June. The values decreased in August and October (Table 2, Fig. 1A,B). In contrast, *H. sanguineus* showed no significant differences in the average HSI between seasons (Table 2, Fig. 1C,D). Furthermore, differently sized *C. maenas* showed the same HSI, and a negative relationship between HSI and fresh mass was only found in *H. sanguineus* from June and August.

TL content

The midgut glands of *H. sanguineus* had higher TL contents than those of *C. maenas* (Table 2, Fig. 2).

Table 2. Mean (\pm SD) of the condition parameters hepatosomatic index (HSI) and total lipid content (TL; % of dry mass or %_{DM}), and the trophic marker indices for carnivory (I_{Ca}), Chlorophyta (I_{Ch}), Phaeophyceae (I_p), Rhodophyta (I_R), and Bacillariophyceae (I_B), of male and female *Carcinus maenas* (Cm) and *Hemigrapsus sanguineus* (Hs) between April and October 2015 from Helgoland. M: male; F: female

Sex	HSI		TL (% _{DM})		I_{Ca}		I_{Ch}		I_p		I_R		I_B	
	Cm	Hs	Cm	Hs	Cm	Hs	Cm	Hs	Cm	Hs	Cm	Hs	Cm	Hs
Apr														
M	0.096 \pm 0.027	0.093 \pm 0.021	20.5 \pm 6.0	41.2 \pm 8.6	0.30 \pm 0.11	0.28 \pm 0.08	12.8 \pm 1.4	17.0 \pm 3.0	1.1 \pm 0.8	5.3 \pm 1.6	0.7 \pm 0.2	0.4 \pm 0.1	8.6 \pm 4.2	12.2 \pm 2.7
F	0.098 \pm 0.020	0.094 \pm 0.010	18.5 \pm 8.8	35.0 \pm 8.5	0.28 \pm 0.07	0.27 \pm 0.08	13.6 \pm 1.5	17.8 \pm 2.4	1.4 \pm 1.2	5.5 \pm 1.7	0.8 \pm 0.2	0.4 \pm 0.1	8.5 \pm 2.6	11.3 \pm 2.6
Jun														
M	0.089 \pm 0.018	0.085 \pm 0.015	16.4 \pm 5.4	49.2 \pm 9.5	0.18 \pm 0.05	0.31 \pm 0.08	13.7 \pm 1.8	16.6 \pm 1.6	1.3 \pm 0.7	4.7 \pm 1.3	0.7 \pm 0.1	0.4 \pm 0.2	6.3 \pm 2.0	13.5 \pm 2.6
F	0.103 \pm 0.020	0.086 \pm 0.014	18.9 \pm 5.4	45.5 \pm 8.8	0.19 \pm 0.06	0.27 \pm 0.05	13.8 \pm 2.3	18.5 \pm 2.2	1.2 \pm 0.6	5.6 \pm 1.3	0.6 \pm 0.1	0.3 \pm 0.1	8.1 \pm 3.8	11.9 \pm 1.7
Aug														
M	0.072 \pm 0.017	0.074 \pm 0.019	19.3 \pm 5.5	28.3 \pm 12.5	0.26 \pm 0.07	0.26 \pm 0.05	12.4 \pm 1.8	17.4 \pm 3.3	0.6 \pm 0.4	2.7 \pm 1.1	0.6 \pm 0.1	0.3 \pm 0.1	4.3 \pm 1.2	6.4 \pm 1.4
F	0.084 \pm 0.040	0.081 \pm 0.010	22.8 \pm 5.7	27.3 \pm 12.5	0.26 \pm 0.07	0.34 \pm 0.07	13.2 \pm 1.5	16.9 \pm 2.2	0.8 \pm 0.4	2.2 \pm 0.8	0.5 \pm 0.1	0.3 \pm 0.1	5.4 \pm 3.5	7.8 \pm 2.3
Oct														
M	0.063 \pm 0.018	0.085 \pm 0.010	19.2 \pm 8.8	34.7 \pm 12.9	0.26 \pm 0.06	0.35 \pm 0.05	12.4 \pm 2.4	15.8 \pm 2.9	0.5 \pm 0.4	2.9 \pm 1.1	0.6 \pm 0.2	0.3 \pm 0.1	6.5 \pm 3.0	10.6 \pm 3.2
F	0.067 \pm 0.020	0.102 \pm 0.011	19.6 \pm 9.5	34.2 \pm 13.3	0.27 \pm 0.10	0.36 \pm 0.06	12.5 \pm 2.2	16.9 \pm 2.7	0.5 \pm 0.3	2.8 \pm 0.8	0.5 \pm 0.2	0.2 \pm 0.1	6.1 \pm 2.8	9.9 \pm 2.9
Total	0.085 \pm 0.028	0.087 \pm 0.016	19.4 \pm 7.0	38.0 \pm 13.0	0.25 \pm 0.08	0.30 \pm 0.08	13.1 \pm 1.9	17.2 \pm 2.6	0.9 \pm 0.7	4.1 \pm 1.8	0.6 \pm 0.2	0.3 \pm 0.1	6.7 \pm 3.3	10.7 \pm 3.3

When sexes were combined, the species showed different seasonal relationships between TL and fresh mass. TL levels did not change with fresh mass in *C. maenas*, but showed a significant negative correlation in August and October for *H. sanguineus*. Pooled over fresh mass, *C. maenas* showed quite similar average TL levels in all seasons, but for *H. sanguineus*, values differed remarkably between seasons. When pooled by sex, *C. maenas* males showed slightly lower TL values than females (pooled over all other factors: 18.8 \pm 6.6%_{DM} vs. 20.0 \pm 7.4%_{DM}, respectively), whereas in *H. sanguineus*, the males had higher TL values than the females (39.3 \pm 13.3%_{DM} vs. 36.7 \pm 12.5%_{DM}, respectively).

FA composition

Mean FA compositions of the midgut glands of both crab species and the macroalgae are presented in Table 4, and the corresponding raw data are presented in Table S1 (see Supplement 2 at www.int-res.com/articles/suppl/m602p169_supp.xlsx).

The main FAs in the midgut glands of *C. maenas* and *H. sanguineus* were 16:0, 20:5(n-3), 18:1(n-9), and 16:1(n-7) (Table 4). Summarized over all months and fresh masses, *C. maenas* showed lower values of 16:0, 16:4(n-3), 18:1(n-9), and 18:3(n-3) than *H. sanguineus* (Table 4). In contrast, the FAs 18:0, 20:1(n-11), 20:1(n-7), and especially 20:5(n-3) were higher in *C. maenas*.

The macroalgae showed group-specific patterns as described by Kelly & Scheibling (2012) (our Table 4). The Chlorophyta *Ulva* sp. was richest in 16:0. Other prevalent FAs were 18:3(n-3), 16:4(n-3), and 18:1(n-7). The 3 species of Phaeophyceae were richest in 16:0, 18:1(n-9), 20:4(n-6), 18:2(n-6), 14:0, and 20:5(n-3). In *Fucus serratus*, 18:1(n-9) dominated with about 32.0%, whereas it comprised only about 7.7% and 18.4% in *Sargassum muticum* and *Desmarestia aculeata*, respectively. The 4 species of Rhodophyta were rich in 20:5(n-3), 16:0, 20:4(n-6), and 18:1(n-9). Within this group, *Ceramium virgatum* and *Corallina officinalis* showed comparably low values of 20:4(n-6), but high values of 20:5(n-3).

PCAs of the FA composition were conducted separately for males and females of either species in each of the 4 months. They did not show sex-specific differences within species. Furthermore, the PCAs conducted separately for the 2 crab species and pooling the sexes did not show season-specific differences. Only the PCA for *H. sanguineus* showed an arrangement of samples separating April and June

Table 3. Likelihood ratio tests (LRTs) of 7 individual linear models (only those that include the factor 'species') comparing midgut gland samples of *Carcinus maenas* and *Hemigrapsus sanguineus* with regard to the condition parameters hepatosomatic index (HSI) and total lipid content (TL) and the trophic marker indices for carnivory (I_{Ca}), Chlorophyta (I_{Ch}), Phaeophyceae (I_p), Rhodophyta (I_R), and Bacillariophyceae (I_B). Samples were collected in April, June, August, and October 2015 from Heigoland. Significant p-values are in **bold** (alpha = 0.05)

HSI		TL		I_{Ca}		I_{Ch}		I_p		I_R		I_B			
df	F	df	p	df	F	df	p	df	F	df	p	df	F	df	p
LRT _{Species × Sex × Month × Fresh mass}															
375,3	0.009	375,3	0.955	376,3	0.892	376,3	1.173	376,3	0.790	376,3	0.665	376,3	0.666	376,3	0.573
LRT _{Species × Sex × Month}															
378,3	1.547	378,3	0.267	386,3	3.815	382,3	3.657	385,3	2.970	379,3	0.621	379,3	2.303	379,3	0.077
LRT _{Species × Sex × Fresh mass}															
378,1	1.348	378,1	0.087	379,1	1.177	382,1	16.185	385,1	6.023	388,1	11.805	379,1	0.776	379,1	0.379
LRT _{Species × Month × Fresh mass}															
390,3	4.670	378,3	2.710	379,3	1.118	382,3	4.787	379,3	1.618	379,3	2.525	379,3	0.247	379,3	0.863
LRT _{Species × Month}															
393,3	19.979	388,3	31.301	389,3	15.437	389,3	0.335	392,3	8.974	395,3	5.785	396,3	6.609	396,3	<0.001
LRT _{Species × Fresh mass}															
388,1	3.057	388,1	4.438	389,1	0.030	389,1	6.216	392,1	2.177	395,1	4.172	396,1	4.460	396,1	0.035
LRT _{Species × Sex}															
388,1	0.387	388,1	11.264	389,1	1.188	389,1	4.458	392,1	0.050	389,1	3.112	396,1	6.375	396,1	0.012
LRT _{Species}															
400,1	3.514	400,1	324.56	401,1	75.038	401,1	329.15	401,1	1044.5	401,1	761.75	401,1	183.59	401,1	<0.001

from August and October (Fig. S1 in Supplement 1).

The PCA of the FA compositions of all crab and macroalgae tissues revealed distinct clusters for *C. maenas* and *H. sanguineus*. Likewise, the FA of the macroalgae formed distinct groups and clearly separated from the crab species as well as from each other (Fig. 3). The first 3 principal components (PCs) accounted for about 68% of the variance, while the first 2 together explained about 58%. The main contributing eigenvectors of PC1 were, in decreasing order, 18:4(n-3), 18:3(n-3), 16:2(n-4), and 16:1(n-7) with positive values, as well as 20:5(n-3), 18:0, 20:1(n-11), and 20:1(n-7) with negative values. PC2 was mostly characterized by positive values of 20:4(n-6) and 20:5(n-3), as well as by negative values of 16:1(n-7), 22:5(n-3), 18:1(n-7), and 20:1(n-7), again in decreasing order.

Carnivory index (I_{Ca})

Levels of I_{Ca} were higher in *H. sanguineus* than in *C. maenas* and showed significantly differing seasonal patterns between the 2 species (Table 2, Fig. S2). Males and females of *C. maenas* showed similar seasonal patterns. In June, the average I_{Ca} was lower than in the other months. In *H. sanguineus*, males showed similar average I_{Ca} values in April, June, and August, but an increase in October. In female *H. sanguineus*, I_{Ca} levels were similar in April and June, increased in August, and remained at this level in October.

Chlorophyta index (I_{Ch})

H. sanguineus showed higher I_{Ch} levels than *C. maenas* (Table 2, Fig. S3). Only *H. sanguineus* females showed a positive correlation between I_{Ch} and fresh mass. When pooled over sexes, the 2 species also differed in their seasonal relationship of I_{Ch} with fresh mass. Then, only *H. sanguineus* individuals from October showed a positive I_{Ch} correlation to fresh mass, in contrast to all other *H. sanguineus* and all *C. maenas* individuals. When all different sizes were

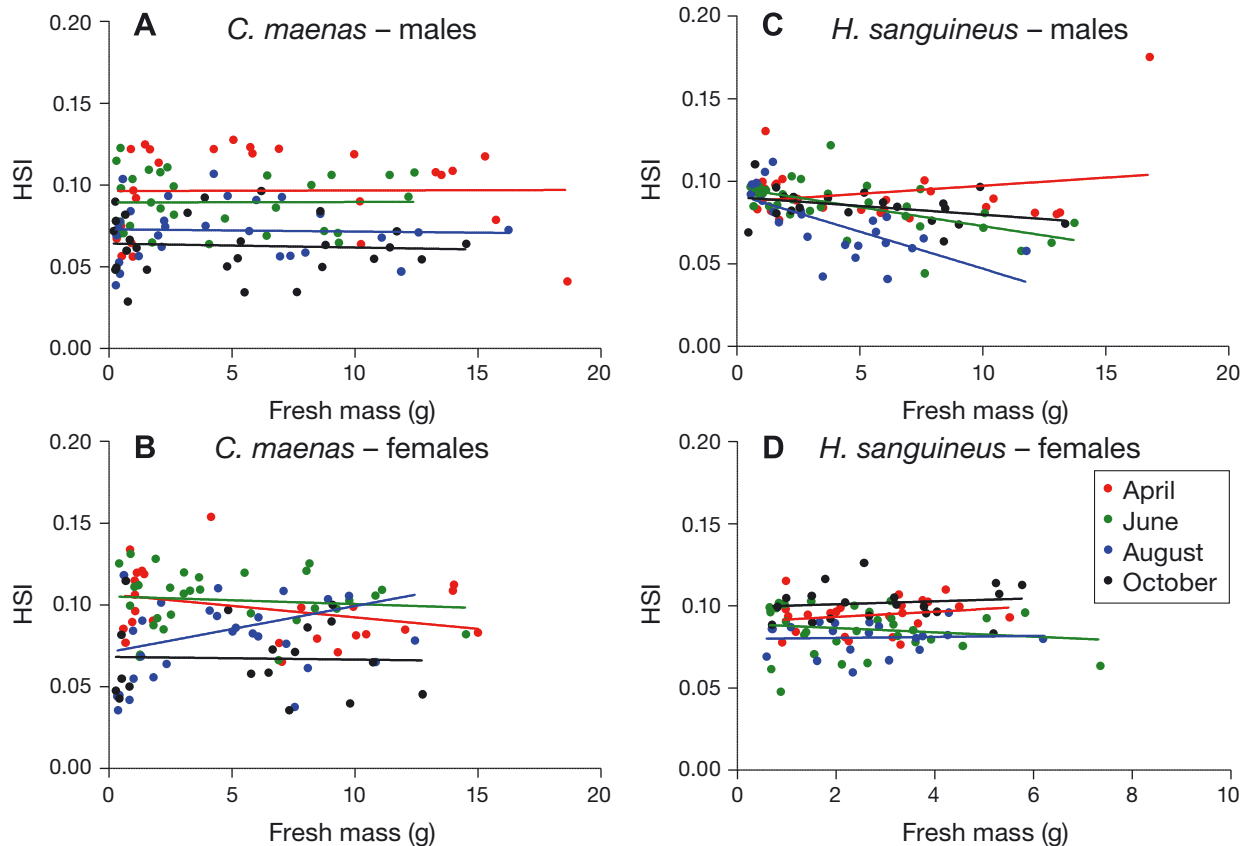


Fig. 1. Hepatosomatic indices (HSI) of *Carcinus maenas* (A) males and (B) females and *Hemigrapsus sanguineus* (C) males and (D) females sampled at Helgoland between April and October 2015. Note the differing x-axis of (D). For the calculation of HSI, see Table 1

combined, the 2 species differed in their seasonal pattern between species and sex. Pooling the different months and sexes revealed that I_{Ch} levels of *H. sanguineus* specimens were correlated to fresh mass, whereas this was not the case in *C. maenas*.

Phaeophyceae index (I_p)

Levels of I_p were higher in *H. sanguineus* than in *C. maenas* (Table 2, Fig. S4). For males, a similar pattern was detected in both species: similar levels in April and June, a decrease towards August, followed by a similar level in October. The seasonal patterns in female *H. sanguineus* were similar to that of males. Females of *C. maenas* showed a decrease from August to October. Additionally, when both sexes and fresh masses were pooled, the 2 species differed in their seasonal I_p patterns. Only females of *H. sanguineus* showed a positive correlation between I_p and fresh mass. No correlation occurred in *H. sanguineus* males and in both sexes of *C. maenas*.

Rhodophyta index (I_R)

Overall, values of I_R were lower in *H. sanguineus* than in *C. maenas* (Table 2, Fig. S5). Only the I_R values of *H. sanguineus* females showed a negative correlation with fresh mass. When the data were pooled over months and sexes, a negative relationship of I_R and fresh mass was found for *H. sanguineus* but not for *C. maenas*. Values of *C. maenas* were similar in April and June, decreased in August, and remained at the same level in October. In *H. sanguineus*, I_R decreased from April to June and August and again to October.

Bacillariophyceae index (I_B)

Values of I_B were higher in *H. sanguineus* than in *C. maenas* (Table 2, Fig. S6). *C. maenas* showed decreasing values from April to August and an increase in October. In contrast, *H. sanguineus* had similar I_B values in April and June, decreasing values in

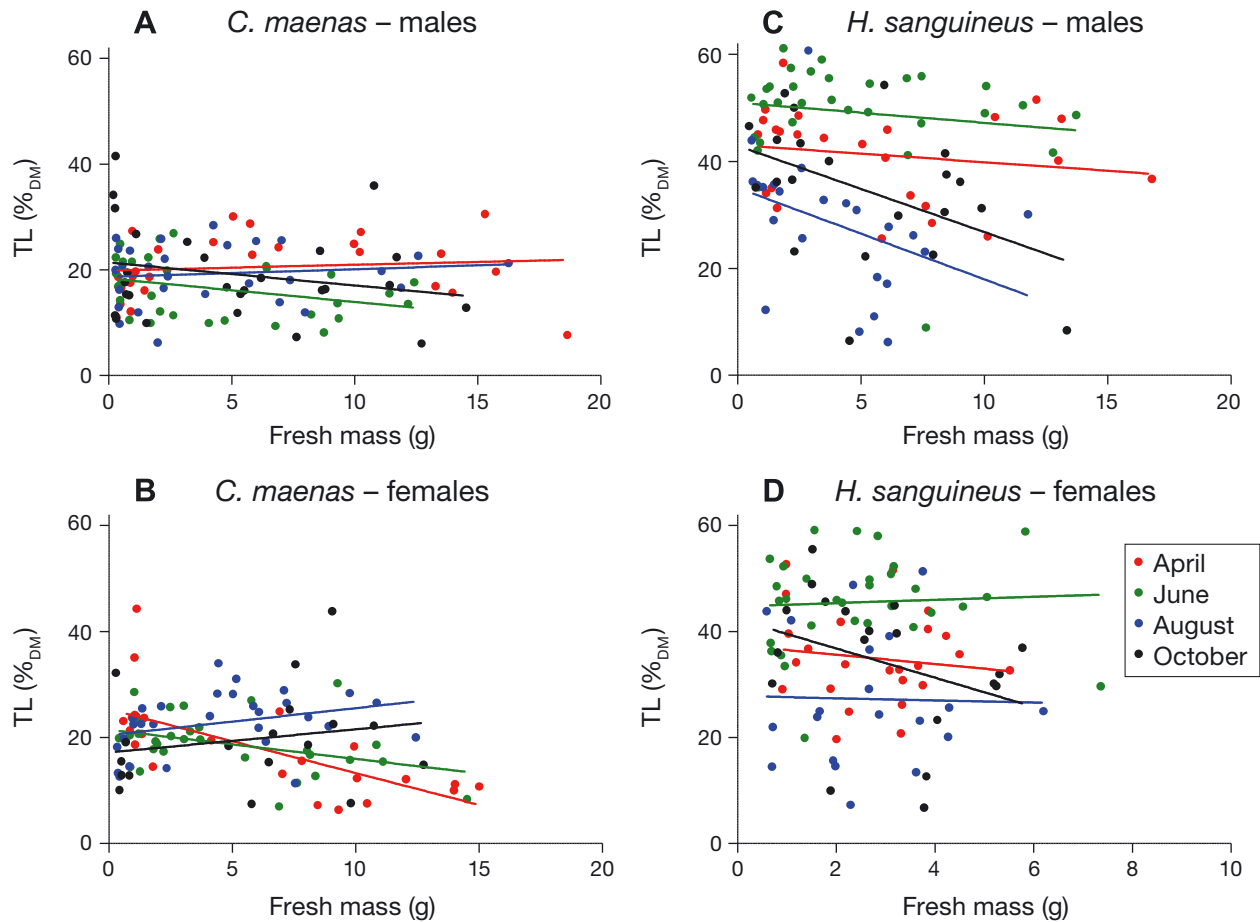


Fig. 2. Total lipid content (TL; % of dry mass or %_{DM}) of *Carcinus maenas* (A) males and (B) females and *Hemigrapsus sanguineus* (C) males and (D) females sampled at Helgoland between April and October 2015. Note the differing x-axis of (D). For the calculation of TL, see Table 1

August, followed by an increase to October. When pooled over month and fresh mass, *C. maenas* males had lower I_B values than females (6.4 ± 3.1 and 7.1 ± 3.5 , respectively). The opposite occurred in *H. sanguineus* males and females (11.0 ± 3.7 and 8.6 ± 4.9 , respectively). Positive correlations between I_B and fresh mass were present in both sexes of *C. maenas*, but not in *H. sanguineus*.

DISCUSSION

Energy storage

We used HSI and TL as measures for the energy storage capacities of *Carcinus maenas* and *Hemigrapsus sanguineus*. HSI values were in the same range as reported previously for these 2 species from the east coast of North America (0.02–0.13 for both species; Griffen et al. 2011, 2012). TL values were in a range reported previously for *C. maenas* (Aagaard

1996, Styrrishave & Andersen 2000) and *H. sanguineus* (Griffen et al. 2015). The TL levels for *H. sanguineus* showed a strong seasonal pattern, whereas TL values of *C. maenas* did not differ between seasons around Helgoland, which is in contrast to observations from Denmark (Styrrishave & Andersen 2000).

Energy stores are required when animals need to overcome periods of food scarcity, e.g. low-production seasons, or when energy stores are used for reproduction (Kyomo 1988, Kucharski & Da Silva 1991, Kennish 1997, Yamaguchi 2004, Lee et al. 2006, Alava et al. 2007, Barrento et al. 2009). The difference in the annual TL patterns cannot be explained by the differing reproductive strategies described for *C. maenas* ('capital breeder') and *H. sanguineus* ('income breeder') (Griffen et al. 2011, 2012). These strategies would result in TL levels with and without seasonality, respectively; however we detected the opposite.

Our results may be explained by the huge difference in lipid turnover between both species. Around Helgoland, the reproductive period of *H. sanguineus*

Table 4. Number of samples (n), fresh mass, hepatosomatic index (HSI), total lipid content (TL; % of dry mass or %_{DM}), fatty acid composition (% of total fatty acids or %_{TFA}), and the trophic indices for carnivory (I_{Ca}), Chlorophyta (I_{Ch}), Phaeophyceae (I_P), Rhodophyta (I_R), and Bacillariophyceae (I_B) of *Carcinus maenas* (Linnaeus 1758), *Hemigrapsus sanguineus* (De Haan 1835), and several macroalgae species (*Ulva* sp. Linnaeus 1753, *Fucus serratus* Linnaeus 1753, *Sargassum muticum* (Yendo) Fen-sholt 1955, *Desmarestia aculeata* (Linnaeus) J.V.Lamouroux 1813, *Mastocarpus stellatus* (Stackhouse) Guiry 1984, *Chondrus crispus* Stackhouse 1797, *Ceramium vir-gatum* Roth 1797, and *Corallina officinalis* Linnaeus 1758) from Helgoland. Values of *C. maenas* and *H. sanguineus* are averages from April, June, August, and October. Macroalgae were sampled in August 2015. All values are mean ± SD; ‘-’: not measured/not detected; ‘+’: traces (<0.1%). For the calculation of HSI, TL, and the dietary indices, see Table 1. The corresponding raw data are in Table S1 in Supplement 2

	Brachyura		Chlorophyta		Phaeophyceae			Rhodophyta				
	<i>C. maenas</i>	<i>H. sanguineus</i>	<i>Ulva</i> sp.	<i>F. serratus</i>	<i>S. muticum</i>	<i>D. aculeata</i>	<i>M. stellatus</i>	<i>C. crispus</i>	<i>C. virgatum</i>	<i>C. officinalis</i>		
n	214	195	5	5	5	5	5	4	5	5		
Fresh mass (g)	5.0 ± 4.4	3.8 ± 3.1	-	-	-	-	-	-	-	-		
HSI	0.09 ± 0.05	0.09 ± 0.02	-	-	-	-	-	-	-	-		
TL (% _{DM})	19.4 ± 7.0	38.7 ± 15.8	0.6 ± 0.2	2.3 ± 0.8	2.2 ± 0.2	3.5 ± 1.1	0.2 ± 0.1	0.1 ± 0.0	2.6 ± 0.3	0.2 ± 0.1		
Fatty acids (%_{TFA})												
14:0	2.3 ± 1.0	2.6 ± 0.7	0.9 ± 0.2	10.0 ± 0.5	3.7 ± 0.3	7.1 ± 0.3	3.1 ± 0.2	3.5 ± 0.2	5.3 ± 0.1	2.8 ± 1.1		
16:0	16.6 ± 2.3	20.0 ± 2.9	18.8 ± 0.6	16.6 ± 0.8	22.6 ± 0.6	19.0 ± 0.2	25.5 ± 1.2	24.7 ± 1.7	26.8 ± 0.6	21.3 ± 0.3		
16:1(n-7)	6.7 ± 3.3	10.5 ± 3.1	1.8 ± 0.3	1.1 ± 0.6	7.3 ± 0.7	0.2 ± 0.3	4.0 ± 1.3	5.1 ± 1.7	5.7 ± 0.6	6.4 ± 3.1		
16:2(n-4)	0.6 ± 0.4	3.3 ± 1.8	-	-	0.7 ± 0.1	-	0.6 ± 1.0	0.7 ± 0.4	0.4 ± 0.1	0.8 ± 0.4		
16:4(n-3)	2.1 ± 1.3	4.0 ± 1.6	17.3 ± 1.0	0.7 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	1.0 ± 0.2	0.7 ± 0.5	0.9 ± 0.2	1.4 ± 0.2		
16:4(n-1)	+	0.2 ± 0.4	-	-	0.7 ± 0.2	-	0.2 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	1.1 ± 0.8		
Iso 17:0	1.3 ± 0.6	0.9 ± 0.2	1.7 ± 0.1	0.2 ± 0.2	0.6 ± 0.1	0.4 ± 0.0	0.3 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.1		
18:0	5.2 ± 1.6	2.8 ± 0.7	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.3	3.0 ± 0.2	1.7 ± 0.5	1.7 ± 0.5	1.0 ± 0.1	0.8 ± 0.1		
18:1(n-9)	10.4 ± 3.3	13.4 ± 2.9	1.5 ± 0.4	32.0 ± 5.4	7.7 ± 0.2	18.4 ± 1.3	8.9 ± 1.2	7.6 ± 0.7	5.2 ± 0.3	2.4 ± 0.1		
18:1(n-7)	7.0 ± 1.2	5.7 ± 1.0	9.8 ± 0.9	-	0.5 ± 0.2	+	4.2 ± 1.4	4.0 ± 1.0	2.5 ± 0.3	3.0 ± 0.4		
18:2(n-6)	2.0 ± 0.6	3.2 ± 0.5	7.3 ± 0.7	7.1 ± 0.5	5.8 ± 0.4	11.7 ± 0.3	1.5 ± 0.3	2.3 ± 0.7	0.9 ± 0.1	2.6 ± 0.4		
18:3(n-6)	+	0.3 ± 0.1	1.7 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	1.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.0	0.7 ± 0.2	0.5 ± 0.2		
18:3(n-3)	2.0 ± 1.1	4.2 ± 1.2	16.8 ± 1.5	4.0 ± 0.7	8.5 ± 0.9	4.6 ± 0.1	0.8 ± 0.4	1.9 ± 1.0	0.1 ± 0.2	1.3 ± 0.3		
18:4(n-3)	0.9 ± 0.7	4.1 ± 1.8	13.5 ± 0.6	4.4 ± 1.2	6.9 ± 0.6	9.7 ± 0.5	0.6 ± 0.4	1.6 ± 0.9	0.6 ± 0.1	1.6 ± 0.3		
20:1(n-11)	2.9 ± 1.1	0.9 ± 0.3	-	0.3 ± 0.0	1.0 ± 0.1	-	0.5 ± 0.8	0.2 ± 0.3	-	-		
20:1(n-9)	1.0 ± 0.3	0.9 ± 0.2	-	-	-	-	0.7 ± 0.1	+	-	0.5 ± 0.1		
20:1(n-7)	2.7 ± 1.2	0.9 ± 0.3	-	-	-	-	0.1 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	+		
20:2(n-6)	1.5 ± 0.4	0.6 ± 0.2	-	+	-	-	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.2	0.5 ± 0.1		
20:3(n-3)	1.0 ± 0.3	0.5 ± 0.2	-	+	-	-	-	-	-	-		
20:4(n-6)	6.3 ± 2.9	4.5 ± 1.7	2.8 ± 0.2	12.8 ± 1.7	17.4 ± 0.3	12.8 ± 0.6	22.5 ± 2.8	20.5 ± 6.0	9.2 ± 1.6	16.3 ± 3.5		
20:5(n-3)	15.9 ± 3.8	8.4 ± 2.3	1.9 ± 0.2	6.3 ± 1.5	11.4 ± 0.9	7.8 ± 0.3	18.6 ± 1.6	19.6 ± 1.1	36.6 ± 1.8	31.6 ± 2.3		
22:5(n-3)	3.1 ± 1.2	1.5 ± 0.5	1.8 ± 0.1	-	-	-	-	-	-	-		
22:6(n-3)	3.6 ± 2.1	2.9 ± 1.6	-	-	-	-	1.4 ± 1.3	1.1 ± 1.0	-	1.1 ± 0.6		
∑Saturated	26.3 ± 3.2	27.1 ± 3.8	22.3 ± 0.6	27.9 ± 0.8	27.8 ± 0.9	29.8 ± 0.6	31.0 ± 0.7	30.8 ± 1.1	33.9 ± 0.6	25.8 ± 1.2		
∑Monounsaturated	30.7 ± 6.2	32.3 ± 5.3	13.1 ± 1.5	33.3 ± 5.6	16.5 ± 0.8	18.6 ± 1.4	18.4 ± 2.1	17.1 ± 3.6	13.6 ± 0.8	12.3 ± 2.9		
∑Polyunsaturated	38.1 ± 6.9	37.3 ± 6.1	62.9 ± 2.3	35.8 ± 5.2	53.0 ± 1.6	48.8 ± 1.4	48.0 ± 2.2	49.8 ± 3.7	50.0 ± 1.1	58.9 ± 3.9		
∑Others/undetermined	4.9 ± 1.1	3.4 ± 0.6	1.7 ± 0.6	2.9 ± 0.7	2.7 ± 0.2	2.8 ± 0.3	2.5 ± 0.8	2.3 ± 0.7	2.5 ± 0.3	3.0 ± 0.4		
Dietary indices												
I _{Ca}	0.2 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.9 ± 0.3	0.1 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0		
I _{Ch}	13.1 ± 1.9	17.2 ± 2.6	51.2 ± 1.2	11.8 ± 1.0	15.6 ± 1.0	17.1 ± 0.5	7.4 ± 1.7	8.9 ± 2.4	4.3 ± 0.3	8.4 ± 1.1		
I _P	0.9 ± 0.7	4.1 ± 1.8	13.5 ± 0.6	4.4 ± 1.2	6.9 ± 0.6	9.7 ± 0.5	0.6 ± 0.4	1.6 ± 0.9	0.6 ± 0.1	1.6 ± 0.3		
I _R	0.6 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	1.3 ± 0.1	1.4 ± 0.1		
I _B	6.7 ± 3.3	10.7 ± 3.3	1.8 ± 0.3	1.1 ± 0.6	8.0 ± 0.8	0.2 ± 0.3	4.2 ± 1.5	5.7 ± 1.9	6.2 ± 0.8	7.5 ± 3.9		

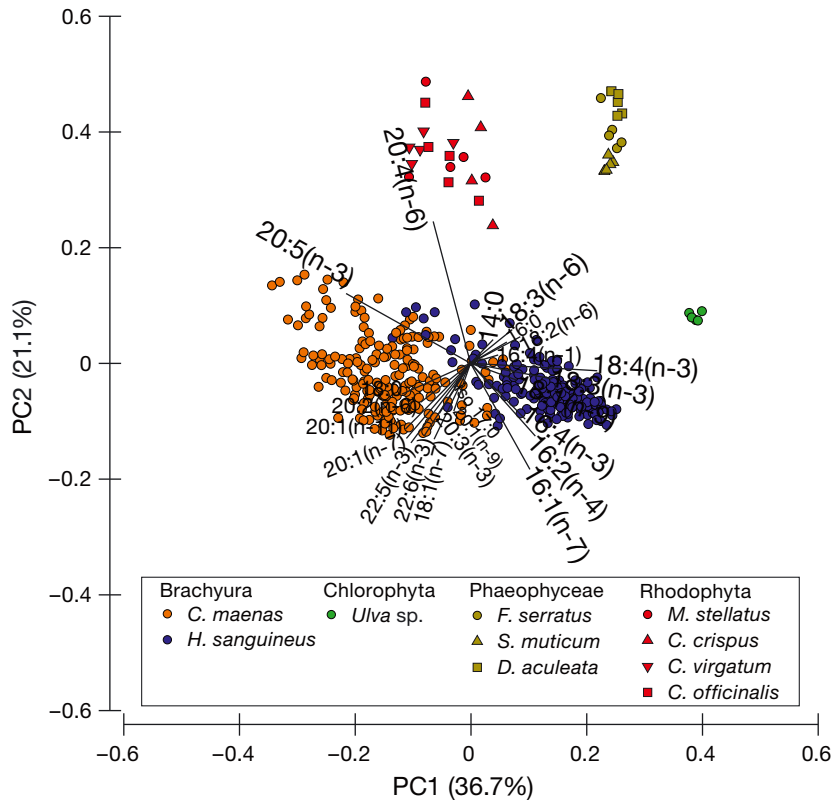


Fig. 3. Principal component analysis of fatty acid composition of hepatopancreas samples of *Carcinus maenas* and *Hemigrapsus sanguineus*, and of different macroalgae species (*Ulva* sp., *Fucus serratus*, *Sargassum muticum*, *Desmarestia aculeata*, *Mastocarpus stellatus*, *Chondrus crispus*, *Ceramium virgatum*, and *Corallina officinalis*) sampled at Helgoland in 2015

covers at least 5 mo (June to October), when they produce up to 5 lipid-rich (28%_{DM}) egg clutches, together comprising 42% of their body mass (Fukui 1988, M. L. McCarthy & S. Jungblut unpubl. data). In contrast, *C. maenas* produce only 1 egg clutch (less lipid-rich, at around 23%_{DM}) in their shorter reproductive period (April to June) (Klassen & Locke 2007, M. L. McCarthy & S. Jungblut unpubl. data). Thus, *H. sanguineus* females invest in higher egg quantity and quality, leading to seasonally increased lipid reserves, usually accumulated as triacylglycerols (S. Jungblut unpubl. data).

The cause of the seasonal TL pattern in male *H. sanguineus* remains unknown. There is no evidence of, e.g., energy-demanding mating behavior like in *C. maenas* (Styrishave & Andersen 2000) or interrupted feeding during the mating season.

Energy storage levels likely depend on the amount and quality of the ingested diet. Higher HSI and TL levels occurred in both species when fed with animal diet instead of algae (Griffen et al. 2011, 2012, Griffen 2017). To store larger amounts of lipids, e.g. for

reproductive efforts, *H. sanguineus* might ingest more food or increase carnivory to compensate for the low energy content of the usually preferred algae material.

FA composition of midgut glands

No distinct differences in FA composition in relation to sex or seasons were detected in each of the 2 species. Other studies, however, have reported variable FA compositions, which were attributed to ovarian maturation, sex, seasons, temperature, and, in case of *C. maenas*, color morph (Chapelle 1978, Styrishave & Andersen 2000, Alava et al. 2007, Barrento et al. 2009).

The FA composition of the midgut glands of *C. maenas* and *H. sanguineus* formed 2 well-separated clusters in the PCA, which may partly be due to the large differences in TL levels. The cluster of *C. maenas* is oriented towards the direction of 3 membrane FAs, 18:0, 20:5(n-3), and 22:6(n-3), because the TL levels were rather low and hence biomembrane FAs prevail. The carnivory-indicating FA 18:1(n-9) is directed towards the samples of *H. sanguineus*. At first glance, this could indicate a higher degree of carnivory; however, Phaeophyceae were also rich in 18:1(n-9), especially *Fucus serratus* and *Desmarestia aculeata*. This illustrates that I_{Ca} cannot be used for dietary interpretation alone (see 'Materials and methods').

Dietary preferences

The example of the FA 18:1(n-9) emphasizes the need for closer examination of the dietary preferences of *C. maenas* and *H. sanguineus* as well as benthic animals in general. Each of the 5 taxon-specific FA trophic marker indices clearly showed differences between the 2 species. For some indices, differences were also found between seasons, between sexes, and among different sizes of crabs. These differences, however, did not follow consistent trends. Similarly, the few studies on the stomach contents of differently sized *C. maenas* showed inconsistent results (Ropes 1968, 1988, Elnor 1981, Baeta et

al. 2006). Therefore, we encourage FATM analyses as the intermediate way between biased short-term gut content studies and rather unspecific long-term stable isotope analyses, to evaluate complex dietary preferences and to explore competition for food in co-occurring crabs (Graeve et al. 2001, Latyshev et al. 2004, Griffen 2014).

Except for Rhodophyta, all dietary indices of algae were higher in *H. sanguineus*, indicating a higher level of herbivory. The intense consumption of Phaeophyceae, rich in 18:1(n-9), is most likely the reason for the high—yet misleading—values of the carnivory index in *H. sanguineus*. These dietary preferences are all corroborated by laboratory experiments, gut content analyses, and studies on gut morphology (e.g. Eriksson & Edlund 1977, Birch 1979, Pihl 1985, Tyrrell & Harris 1999, Brousseau & Goldberg 2007, Griffen et al. 2008, 2011, Griffen & Mosblack 2011).

Competition for food between *C. maenas* and *H. sanguineus* is probably low in macroalgae-rich habitats. So far, the trophic impact of *H. sanguineus* has mostly been evaluated for potential animal prey organisms like barnacles, mytilid mussels, or littorinid snails (e.g. Lohrer et al. 2000, Lohrer & Whitlatch 2002, Brousseau & Baglivo 2005, Tyrrell et al. 2006, Brousseau & Goldberg 2007, Brousseau et al. 2014). Only a few studies have considered the more likely case: the impact of *H. sanguineus* on the algal community (Tyrrell & Harris 1999). Unlike *C. maenas*, the European individuals of *H. sanguineus* do not retreat to subtidal areas or bury in the sediment in winter (Janke 1986, Aagaard et al. 1995). *H. sanguineus* is present and forages in the intertidal area during the whole year (R. Saborowski unpubl. data). This foraging pressure may reduce the winter recruitment success of, e.g., Phaeophyceae in the invaded habitats. Given the macroalgae richness, this effect may be weak around Helgoland. For macroalgae-poor habitats like the Wadden Sea, however, the competition for food between *C. maenas* and *H. sanguineus* and the effect of *H. sanguineus* on Phaeophyceae might be significant.

CONCLUSIONS

We demonstrated that *Hemigrapsus sanguineus* stored much higher amounts of lipids as energy reserves than *Carcinus maenas* (hypothesis 1 supported). In contrast to *C. maenas*, *H. sanguineus* showed a pronounced seasonality in lipid deposition and depletion (hypothesis 2 rejected). These differences may be due to the higher lipid turnover in *H. sanguineus*. The

FA composition of *C. maenas* and *H. sanguineus* differed distinctly from one another (hypothesis 3i supported), but within each species, no differences with respect to sex or seasons became apparent (hypotheses 3ii and 3iii rejected). *H. sanguineus* was able to accumulate high lipid quantities in relatively short periods, apparently exploiting macroalgae as a primary resource. The dietary indices for Chlorophyta, Bacillariophyceae, and especially Phaeophyceae were higher for *H. sanguineus* than for *C. maenas* (hypothesis 4i supported). Only the Rhodophyta index suggested a higher consumption of red algae by *C. maenas*. There were no species-specific patterns in dietary preferences with regard to crab size, sex, and season (hypotheses 4ii, 4iii, and 4iv undecided). The competition for food between *H. sanguineus* and *C. maenas* may be low in macroalgae-rich habitats. Overall, our data suggest that *H. sanguineus* is clearly more herbivorous, apparently occupying quite a different trophic niche than *C. maenas*. Even though it consumes energy-poor macroalgae, *H. sanguineus* is able to accumulate large lipid reserves. These deposits buffer periods of food paucity, facilitates reproductive output, and thus contributes to the success of this invasive crab in new areas.

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