

Seasonal lipid dynamics of the shrimps *Crangon crangon* and *Pandalus montagui* in the German Bight (North Sea)

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ABSTRACT: Environmental fluctuations can impose energetic constraints on organisms in terms of food shortage or compensation for metabolic stress. To better understand the biochemical strategies that support adaptive physiological processes in variable environments, we studied the lipid dynamics of the brown shrimp *Crangon crangon* and the pink shrimp *Pandalus montagui* by analysing their midgut glands during an annual cycle. Both species have an overlapping distribution range in the southern North Sea, but differ in their habitat preferences, reproductive strategies, and life-history traits. *C. crangon* showed minor total lipid accumulation in their midgut glands, ranging between 14 and 17% of dry mass (DM), dominated by phospholipids. In contrast, *P. montagui* stored significantly larger amounts of total lipid (47–70% DM, mainly triacylglycerols) and showed a distinct seasonal cycle in lipid accumulation with a maximum in summer. Fatty acid trophic markers indicated a wide food spectrum for both species, with higher preferences of *P. montagui* for microalgae. In *C. crangon*, feeding preferences were less distinct due the low total lipid levels in the midgut gland. PCA based on fatty acid compositions of both species suggested that *C. crangon* has a broader dietary spectrum than *P. montagui*. *C. crangon* seems to have the capacity to use sufficient energy directly from ingested food to fuel all metabolic requirements, including multiple spawnings, without building up large lipid reserves in the midgut gland. *P. montagui*, in contrast, relies more on the energy storage function of the midgut gland to overcome food scarcity and to allocate lipids for reproduction.

KEY WORDS: Lipids · Fatty acids · Trophic markers · Life-history traits · Adaptation · Variable environment

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1. INTRODUCTION

Species that inhabit a highly variable environment such as the North Sea must have evolved physiological and behavioural adaptations to overcome challenging conditions. Adaptations are species-specific and depend on different external and internal factors such as food availability and feeding habits, metabolic properties, and reproductive processes. High flexibility in the digestive physiology of crustaceans is essential for their ability to grow and reproduce, given the strong variation of food supply across sea-

sons (Sánchez-Paz et al. 2006, Martínez-Alarcón et al. 2018).

The brown shrimp *Crangon crangon* (Linnaeus, 1758) is highly abundant and plays a key role in the North Sea ecosystem. It is the most important target of coastal fisheries in the southern North Sea, yielding €128 million in 2014 (STECF 2016). *C. crangon* is exposed to a pronounced seasonal temperature cycle (Campos et al. 2012), with winter temperatures from below 0 to 4°C, summer temperatures of 18 to 20°C, and occasionally even 30°C in tide pools of the Wadden Sea (Reiser et al. 2014). Growth rates are highly

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variable and depend, among other factors, on size and sex (Hufnagl & Temming 2011). *C. crangon* is a multiple spawner. Egg-carrying females are observed during a long reproductive season starting in November. Maximum numbers of gravid females occur in February/March followed by the main hatching period in April/May (Siegel et al. 2008). The brown shrimp can act as a structuring force of benthic shallow-water communities (Campos et al. 2012). It is omnivorous and feeds opportunistically on various small invertebrates, algae, and carrion (Plagmann 1939, Pihl & Rosenberg 1984, Siegenthaler et al. 2019).

The pink shrimp *Pandalus montagui* (Leach, 1814) has a narrower thermal tolerance window than the brown shrimp. Typical features of its distribution areas are lower temperatures (usually below 10°C) and constant salinities between 32 and 34. *P. montagui* appears in shallow coastal waters in spring and summer, but recedes to deeper waters in autumn and winter (Simpson et al. 1967, Stevenson & Pierce 1985). Mature males change sex to become secondary females after the second or third year (Simpson et al. 1967). *P. montagui* is a single spawner with a short spawning period between November and February. It feeds mainly on polychaetes, but crustaceans, foraminifers, hydroids, and fish remains are also frequently found in their stomachs (Simpson et al. 1967). Though abundant and ecologically relevant, the economic importance of *P. montagui* in the North Sea is negligible.

In shrimps, lipids are a major energy source usually deposited in the midgut gland. Lipids are involved in various key processes, including growth, moulting, and reproduction. Lipid droplets may also be accumulated in the cells of other tissues such as ovaries, and serve as energy stores (Lee & Walker 1995). These lipid reserves are mobilized during periods of food deprivation (Sánchez-Paz et al. 2006) and gonad maturation. During starvation, crustaceans preferentially catabolize neutral lipids, e.g. triacylglycerols, while polar lipids, mainly phospholipids, are conserved due to their important function as structural components of cell membranes (Heath & Barnes 1970, Stuck et al. 1996, Hervant et al. 1999). However, the relative importance of metabolic reserves and the intensity of their utilization vary among species (Hervant et al. 1999). *C. crangon* and *P. montagui* from the North Sea have been analysed for proteins and enzyme activities (Teschke & Saborowski 2005, Saborowski et al. 2012), as well as for lipid compositions of muscle of *C. crangon* (Mika et al. 2014) from the Gulf of Gdansk and of whole *P. mon-*

tagui (Clarke 1979a). According to Mika et al. (2014), the lipids in the abdominal muscle of *C. crangon* were mainly composed of neutral lipids, while Clarke (1979a) reported that the dominant lipid classes in *P. montagui* were polar lipids and triacylglycerols. In order to better understand the biochemical and physiological aspects of storage capacities, nutritional requirements, and finally, life strategies of these North Sea shrimps, it is necessary to analyse the lipid composition of their midgut gland, which is the main organ of lipid storage in decapod crustaceans.

Complementary to the investigations of Clarke (1979a) and Mika et al. (2014), we determined total lipid contents, lipid classes, and fatty acid (FA) compositions of the midgut glands of *C. crangon* and *P. montagui* from the North Sea. Feeding preferences were also determined by applying the FA trophic marker concept (Dalsgaard et al. 2003). Samples were collected in February, April, July, and October 2016 for seasonal coverage.

We hypothesized that the importance of lipid depots in the midgut gland differs between both species, and that lipid levels are linked to the reproductive cycle and the reproductive effort of either species. We also hypothesized that feeding preferences will vary between species among seasons.

2. MATERIALS AND METHODS

2.1. Collection of shrimp samples

Specimens of *Crangon crangon* and *Pandalus montagui* were collected by bottom trawling with the RV 'Uthörn' during 4 sampling campaigns. The sampling site was located in the southern North Sea near the island of Helgoland. Sampling was carried out in February, April, July, and October 2016 (Table 1). Directly after collection, adult specimens of similar size were sorted from the catch. Total body length (rostrum to tip of telson) and sex of the animals were determined on board. The midgut glands of the animals were dissected, shock-frozen in liquid nitrogen, transported to the Marine Zoology laboratory at the University of Bremen, and stored at -80°C until further analysis.

2.2. Total lipid, lipid class, and FA analyses

Dry mass (DM) of the midgut glands was determined after lyophilisation for 48 h (CHRIST Alpha 1-4 LD plus). Total lipids were extracted from the

Table 1. Sampling time, location, and biological data of *Crangon crangon* and *Pandalus montagui*. No specimens of *P. montagui* were available in February. SND: sex not determined

Date (2016)	Location	No. ind. analysed	Total length (mm)	Male (n)	Female (n)	Females without eggs (n)	Females with eggs (n)	SND (n)
<i>C. crangon</i>								
February 19	54°08'N, 07°52'E	20	56–68	0	19	3	16	1
April 18	53°44'N, 08°15'E	29	55–80	6	23	0	23	0
July 19–21	53°44'N, 08°15'E	14	55–68	7	7	0	7	0
October 24–26	54°08'N, 07°52'E	14	63–78	3	11	10	1	0
<i>P. montagui</i>								
April 18	53°44'N, 08°15'E	20	56–82	5	11	10	1	4
July 19–21	53°44'N, 08°15'E	15	59–72	6	8	8	0	1
October 24–26	54°08'N, 07°52'E	13	41–68	0	0	0	0	13

midgut gland samples after Folch et al. (1957) with dichloromethane:methanol (2:1 v/v) and an aqueous solution of KCl (0.88%). The amount of total lipids was determined gravimetrically after Hagen (2000) and expressed as the percentage of lipids in relation to the DM of the sample (total lipid in %DM).

Lipid class compositions of the midgut gland from individuals sampled in February, April, and October were analysed in triplicate and quantified by thin-layer chromatography–flame-ionisation detection (TLC-FID) on an Iatroscan Mark V device after Fraser et al. (1985). Calibration was done with single compound standards as listed in Table 2.

FAs were first converted to their methyl ester derivatives (FAMES) by transesterification for 4 h at 80°C in hexane and methanol containing 3% concentrated sulphuric acid (Kattner & Fricke 1986). FAMES were extracted with aqua bidest. (DDW) and hexane. After centrifugation, the upper lipid phase was transferred into a clean sample vial and the vial placed in an evaporator (N-EVAP model 112, Organomation) to dry the sample with a gentle stream of nitrogen. Subsequently, the samples were analysed by gas chromatography (Agilent Technologies, GC model 7890A). The device was equipped

with a DB-FFAP column (30 m length, 0.25 mm inner diameter) and a programmable temperature vaporizer injector, operating with helium as the carrier gas. FAs were identified by their retention times in comparison to known FA standard compositions (FAMES of the copepod *Calanus hyperboreus* and menhaden fish oil) (Schukat et al. 2014, Bode et al. 2015).

The FA compositions were evaluated according to the FA trophic marker concept of Dalsgaard et al. (2003), where 16:1(n-7), 16:4(n-1), and 18:1(n-7) are indicators of diatom-dominated food sources, and 18:4(n-3) is used as a dinoflagellate marker. Also, according to St. John & Lund (1996), Dalsgaard et al. (2003), and Bode et al. (2015), the ratio 16:1(n-7)/16:0 can be used as another index of diatom feeding (values >1 indicate significant feeding on diatoms). The carnivory:herbivory index (CHI) was determined after Schukat et al. (2014), as modified by Bode et al. (2015), by dividing the 18:1(n-9) content by the sum of all herbivorous biomarkers and 18:1(n-9), i.e. $CHI = 18:1(n-9)/(16:1(n-7)+18:1(n-7)+18:1(n-9))$. Free fatty alcohols and unidentified components with <1% of total FA concentrations were not considered for analysis.

Table 2. Standard compounds for lipid class calibration

Lipid class	Abbreviation	Standard	Sigma Aldrich art. no.
Phospholipid	PL	L- α -phosphatidylcholine	P3556
Sterol	ST	Cholesterol	C8667
Free fatty acid	FFA	Oleic acid	O1008
Diacylglycerol	DAG	Distearoylglycerol	D9019
Triacylglycerol	TAG	Glyceryltriolate	T7140
Wax ester	WE	Laurylpalmitoleate	P1642

2.3. Statistical analysis

The FA data sets were arcsine-square-root transformed to establish normal distributions and homogeneity of variance. To test for differences in the total lipid contents and the FA concentrations between seasons, a 1-way ANOVA was used. Differences among groups were identified by

pairwise comparison with Tukey's post hoc test. The level for statistical significance was set at $\alpha = 0.05$. In order to identify species-specific differences in the FA compositions of *C. crangon* and *P. montagui*, a PCA was conducted. Males and females were pooled for the seasonal analyses after a Student's *t*-test showed no significant sex-related differences. Differences in the amount of total lipid (%DM) between species were also tested with Student's *t*-test. All statistical tests and graphic presentations were carried out with the RStudio software, version 0.99.491.

3. RESULTS

3.1. Total lipid contents and lipid class compositions

Throughout the year, the amount of total lipid (%DM) was significantly lower in the midgut glands of *Crangon crangon* than in those of *Pandalus montagui* ($p < 0.01$). The brown shrimps had mean total lipid levels between 14% DM in July and February and 17% DM in October. Total lipids in the midgut glands of the pink shrimp ranged from 47% DM in April to 70% DM in July (Fig. 1). In *C. crangon* midgut glands, the amount of total lipid (%DM) changed significantly from July to October and from October to February (Fig. 1a). In *P. montagui* midgut glands, the total lipid amount (% DM) increased significantly from April to July, and decreased significantly again towards October (Fig. 1b). No specimens of *P. montagui* were available in February, due to its seasonal migration to deeper waters. No statistical differences were detected between males and females of either species. The mean amount (\pm SD) of

total lipid in the midgut glands of *C. crangon* was $14.7 \pm 6.8\%$ DM in females and $15.4 \pm 5.6\%$ DM in males ($p = 0.68$). In *P. montagui*, the mean amount of total lipid was $57.0 \pm 14.6\%$ DM in females and $56.9 \pm 16.1\%$ DM in males ($p = 0.99$).

The lipid class composition (% of total lipid, TL) of the midgut glands of *C. crangon* was dominated by phospholipids (PL: 75% TL), followed by triacylglycerols (TAGs: 17% TL) and cholesterol (Chol+DAG: 7% TL). In *P. montagui*, TAGs prevailed (73% TL), followed by PL (26% TL) and Chol+DAG (1% TL) (Table 3). No free FAs were detected, indicating that no autolytic degradation processes occurred during collection, dissection, and storage.

3.2. FA compositions

FA compositions of the midgut glands of both species are presented in Table 4. In *C. crangon*, principal FAs were 20:5(n-3), 22:6(n-3), 16:0, 18:1(n-9), and 16:1(n-7). The portion of polyunsaturated FAs (PUFAs) was higher (38–47% of total FAs) than that of monounsaturated (MUFAs, 26–31%) and saturated

Table 3. Lipid class compositions (mean \pm SD % total lipid) in the midgut glands of *Crangon crangon* ($n = 10$) and *Pandalus montagui* ($n = 8$). Data sets include females and males collected in February, April, and October. TAG: triacylglycerols; PL: phospholipids; Chol: sterols; DAG: diacylglycerol; FFA: free fatty acids

Species	TAG	PL	Chol+DAG	FFA
<i>C. crangon</i>	17 \pm 11	75 \pm 14	7 \pm 1	Below detection limit
<i>P. montagui</i>	73 \pm 10	26 \pm 12	1 \pm 1	<1%

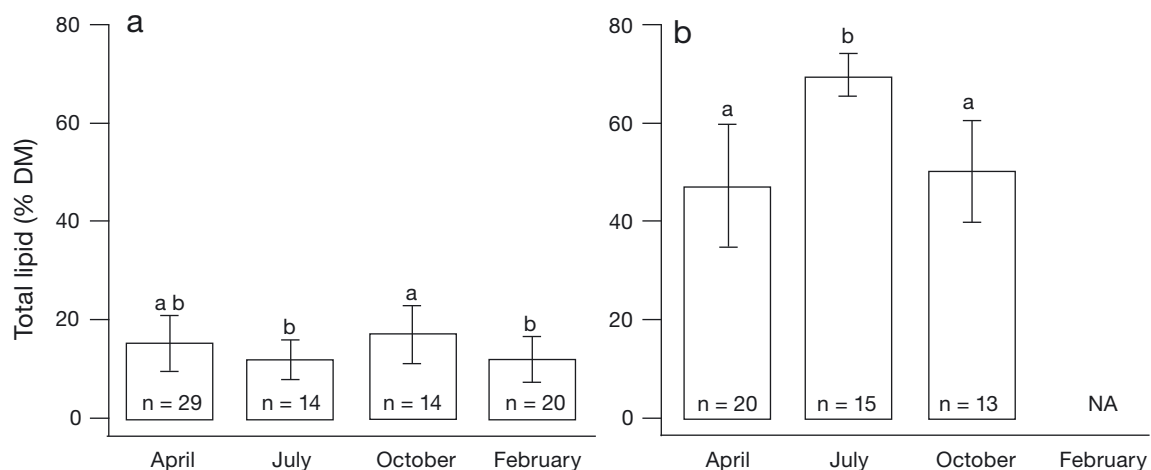


Fig. 1. Total lipid contents (% of dry mass, DM) of the midgut glands of (a) *Crangon crangon* and (b) *Pandalus montagui* from different seasons in 2016 (mean \pm SD). Different letters above bars indicate significantly different values. NA: not available

FAs (SFAs, 18–23%). Palmitic acid (16:0) dominated within the SFAs. 16:1(n-7) and 18:1(n-9) prevailed in the MUFAs. Eicosapentaenoic acid (EPA, 20:5(n-3)) and docosahexaenoic acid (DHA, 22:6(n-3)) were the dominant components of the PUFAs.

In *P. montagui*, the dominant MUFAs were 16:1(n-7) and 18:1(n-9), while 20:5(n-3) and 22:6(n-3) prevailed in the PUFA fraction. In April, percentages of MUFAs were higher than those of the PUFAs. In July and October, the portion of PUFAs exceeded that of MUFAs. In all cases, SFAs comprised the smallest fraction.

PCA based on FA compositions of the midgut gland of *C. crangon* grouped the different months in unspecific overlapping clusters (Fig. 2a). Two principal components (PCs) explained 58% of the variance. The first PC was mainly represented by positive values of the FAs 18:1(n-9) and 20:4(n-6), and negative values of 14:0 and 20:1(n-11). The second PC was mostly characterized by positive values of the FAs 20:1(n-7) and 16:3(n-4), and negative values of 18:0 and 18:1(n-7). In contrast, PCA on the FA compositions of the midgut gland of *P. montagui* grouped the different months in specific clusters (Fig. 2b). Two main PCs explained 67% of the variance. The main contributing vectors of PC1 were 18:1(n-7) and 18:0

with positive values, and 14:0 with a negative value. PC2 was mostly defined by 20:1(n-7) and negative values of 20:4(n-6).

Combined PCA of both species in April (Fig. 3a), July (Fig. 3b), and October (Fig. 3c) showed more clearly defined clusters for *P. montagui* than for *C. crangon*. The clusters overlapped in July, but not in April and only very little in October. Two PCs explained 55% of the variance in April, 69% in July, and 58% in October. In the 3 PCAs, the vector of 20:4(n-6) appeared to have an impact on the *C. crangon* clusters, while the 14:0 vector was more directed towards *P. montagui*. The vector 18:1(n-9) was directed towards *P. montagui* in April, but towards *C. crangon* in July and October. Finally, the vector 16:1(n-7) contributed to PC1 with negative values in April and July in the direction of the *P. montagui* clusters.

4. DISCUSSION

The southern North Sea at 54° N is subjected to distinct seasonal changes in irradiation and temperature (Otto et al. 1990, van Aken 2008, Sündermann & Pohlmann 2011, Neumann et al. 2017). Productivity and biomass of the system vary concordantly (Beu-

Table 4. Fatty acid compositions of *Crangon crangon* and *Pandalus montagui* expressed as percentage of total fatty acids (% TFA). Sum of herbivory markers (%) (16:1(n-7) and 18:1(n-7)), diatom ratio (16:1(n-7)/16:0), and carnivory:herbivory index (18:1(n-9)/ herb.+18:1(n-9)) are also presented. Values are given as means \pm SD. n: number of samples analysed. Concentrations <1% TFA are not presented

Species	<i>C. crangon</i>				<i>P. montagui</i>		
	April (n = 15)	July (n = 14)	October (n = 12)	February (n = 20)	April (n = 19)	July (n = 15)	October (n = 13)
Fatty acids (%)							
14:0	2.5 \pm 0.9	1.9 \pm 1.2	3.2 \pm 1.3	1.1 \pm 0.6	3.1 \pm 0.7	3.9 \pm 1.3	3.9 \pm 0.4
16:0	14.6 \pm 1.4	14.7 \pm 3.2	14.1 \pm 2.7	12.9 \pm 1.5	14.1 \pm 1.4	14.1 \pm 0.8	14.5 \pm 1.5
16:1(n-7)	9.7 \pm 3.2	6.8 \pm 3.7	10.4 \pm 4.0	6.3 \pm 2.5	14.1 \pm 2.3	12.2 \pm 4.0	11.3 \pm 1.8
iso 17:0	1.4 \pm 0.3	1.9 \pm 1.5	1.6 \pm 0.7	1.4 \pm 0.4	1.6 \pm 0.5	1.4 \pm 0.2	1.0 \pm 0.2
16:3(n-4)	0.9 \pm 0.1	1.1 \pm 0.4	1.3 \pm 0.3	1.8 \pm 1.5	1.1 \pm 0.1	0.9 \pm 0.2	1.0 \pm 0.1
18:0	3.9 \pm 1.0	5.1 \pm 1.4	3.6 \pm 0.6	3.1 \pm 0.5	3.8 \pm 0.3	4.3 \pm 1.2	3.6 \pm 0.3
18:1(n-9)	8.8 \pm 1.7	11.2 \pm 2.9	11.7 \pm 1.0	12.2 \pm 1.6	10.1 \pm 1.1	8.9 \pm 1.7	9.5 \pm 0.6
18:1(n-7)	5.0 \pm 0.8	5.5 \pm 0.7	5.4 \pm 0.8	6.6 \pm 0.9	5.9 \pm 0.5	6.3 \pm 0.9	5.3 \pm 0.6
20:1(n-11)	0.9 \pm 0.4	1.5 \pm 1.5	1.6 \pm 0.8	1.5 \pm 0.9	2.5 \pm 0.8	2.3 \pm 0.8	1.2 \pm 0.6
20:1(n-7)	2.3 \pm 0.9	0.9 \pm 0.8	2.2 \pm 1.1	1.4 \pm 1.0	2.0 \pm 0.5	1.8 \pm 0.3	1.3 \pm 0.3
20:4(n-6)	3.1 \pm 0.7	4.0 \pm 1.4	3.6 \pm 1.0	4.5 \pm 1.2	1.8 \pm 0.3	2.5 \pm 1.2	2.1 \pm 0.2
20:5(n-3)	20.3 \pm 3.8	19.1 \pm 4.4	16.2 \pm 4.3	19.9 \pm 2.8	15.6 \pm 2.6	18.1 \pm 2.3	18.3 \pm 1.5
22:5(n-3)	2.6 \pm 0.9	3.3 \pm 2.7	2.1 \pm 1.3	2.7 \pm 1.5	2.6 \pm 1.2	2.8 \pm 0.6	1.5 \pm 0.3
22:6(n-3)	15.7 \pm 3.1	17.0 \pm 4.7	14.9 \pm 4.5	18.7 \pm 3.2	11.1 \pm 1.4	10.4 \pm 2.9	13.4 \pm 1.2
Σ saturated	22.4 \pm 1.1	23.5 \pm 0.5	22.5 \pm 0.8	18.4 \pm 1.3	22.6 \pm 1.4	23.7 \pm 1.1	23.0 \pm 1.7
Σ monounsaturated	26.7 \pm 0.7	25.9 \pm 0.5	31.4 \pm 0.6	28.0 \pm 0.7	34.6 \pm 1.0	31.5 \pm 0.7	28.6 \pm 1.2
Σ polyunsaturated	42.6 \pm 0.6	44.5 \pm 0.4	38.0 \pm 0.4	47.6 \pm 0.5	32.2 \pm 0.9	34.7 \pm 0.7	36.3 \pm 1.5
Sum of herbivory markers (%)	14.7 \pm 3.1	12.3 \pm 3.9	15.8 \pm 4.2	12.9 \pm 2.6	19.9 \pm 2.3	18.4 \pm 3.9	16.6 \pm 1.8
Diatom ratio	0.7 \pm 0.2	0.5 \pm 0.3	0.7 \pm 0.3	0.5 \pm 0.2	1.0 \pm 0.1	0.9 \pm 0.3	0.8 \pm 0.1
Carnivory:herbivory index	0.4 \pm 0.1	0.5 \pm 0.1	0.4 \pm 0.1	0.5 \pm 0.1	0.3 \pm 0.0	0.3 \pm 0.1	0.4 \pm 0.0

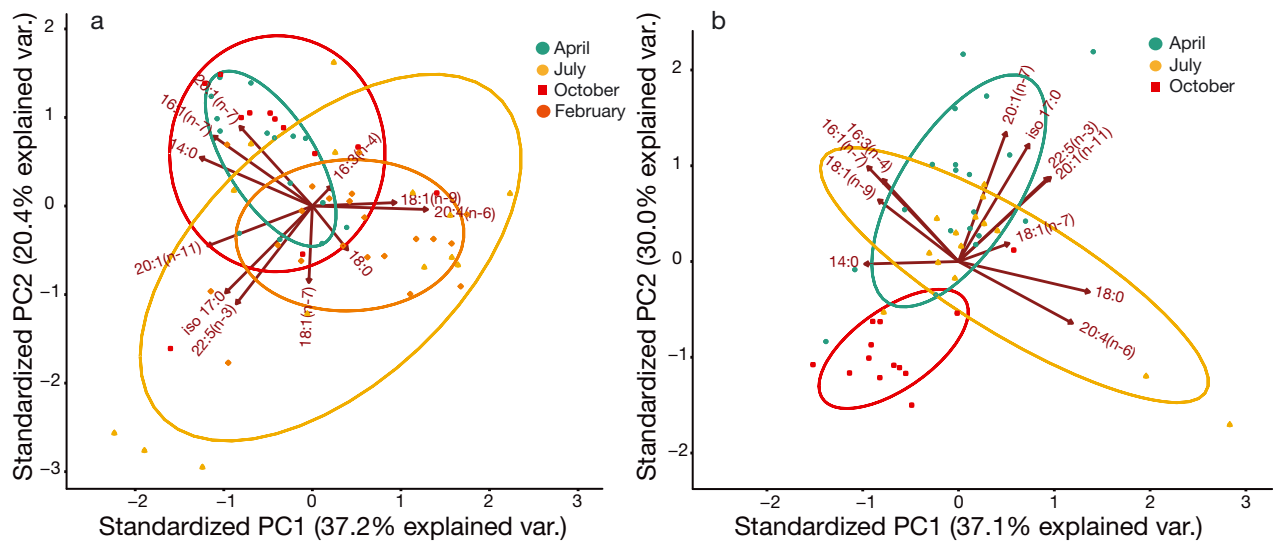


Fig. 2. PCA based on seasonal fatty acid compositions of the midgut glands of (a) *Crangon crangon* and (b) *Pandalus montagui*. The ellipses group data at a confidence level of 95 %

kema 1974, Reiss & Kröncke 2004). The roughly bimodal phytoplankton bloom occurs from March to June and ends in autumn (Wiltshire et al. 2015), with secondary production rising in between (Roff et al. 1988). Therefore, it can be expected that lipid dynamics follow a seasonal pattern in both shrimp species, in accordance with seasonal environmental factors and to buffer food availability.

Although both species share the same habitat, they show pronounced differences in their lipid storage strategies. *Pandalus montagui* accumulated significantly higher amounts of total lipids in the midgut gland, and showed a more distinct seasonal lipid variation than *Crangon crangon*. Differences between species were also reflected in their lipid class compositions. *P. montagui* accumulated TAGs in the midgut gland, which are typical storage lipids (Lee et al. 2006), whereas the midgut glands of *C. crangon* contained very little TAG. Due to this low lipid level, *C. crangon* had high percentages of phospholipids, which are essential structural components of biomembranes rather than storage lipids. Hence, lipid accumulation in *C. crangon* was low during summer, and increased only slightly in spring and autumn. These results are consistent with other previous measurements in our laboratory: total lipid contents of midgut glands of *C. crangon* from the southern North Sea ranged between 13 % DM in March and 20 % DM in July (C. Sahlmann unpubl. data) and between 10 % DM in May and 32 % DM in September (K. Pöhlmann unpubl. data), which indicates some interannual variability. In contrast, lipid levels in the midgut glands of

P. montagui increased significantly during the productive summer season, from 47 % to a maximum of 70 % DM, indicating intensive lipid storage activity.

4.1. Lipid deposition

The energy density of lipids is twice as high as that of carbohydrates and proteins. Therefore, accumulation of energy reserves via lipid stores, predominantly TAGs and wax esters (WEs), is the most efficient and most common means of energy storage in marine invertebrates, especially herbivorous zooplankton. The stores are used to overcome periods of food paucity or for the transfer of energy towards reproductive processes (Lee et al. 2006). The midgut gland of crustaceans is generally accepted as the central metabolic organ and the principal lipid storage site (O'Connor & Gilbert 1968). Other arthropods, e.g. insects, possess a lipid storage organ called the fat body (Arrese & Soulagés 2010), which is considered to be unique to this taxon of arthropods (Law & Wells 1989). Several studies, however, reported the involvement of a fat body in the vitellogenesis of crustaceans such as isopods (Picaud 1980, Souty & Picaud 1981) and euphausiids (Cuzin-Roudy 1993). However, the presence of an explicit fat body has not been confirmed for decapods.

In addition to the midgut gland, muscle tissue may also act as a lipid storage organ. Mika et al. (2014) reported that the muscle of *C. crangon* contained 32.2 mg total lipid g⁻¹ wet mass in spring and 7.7 mg

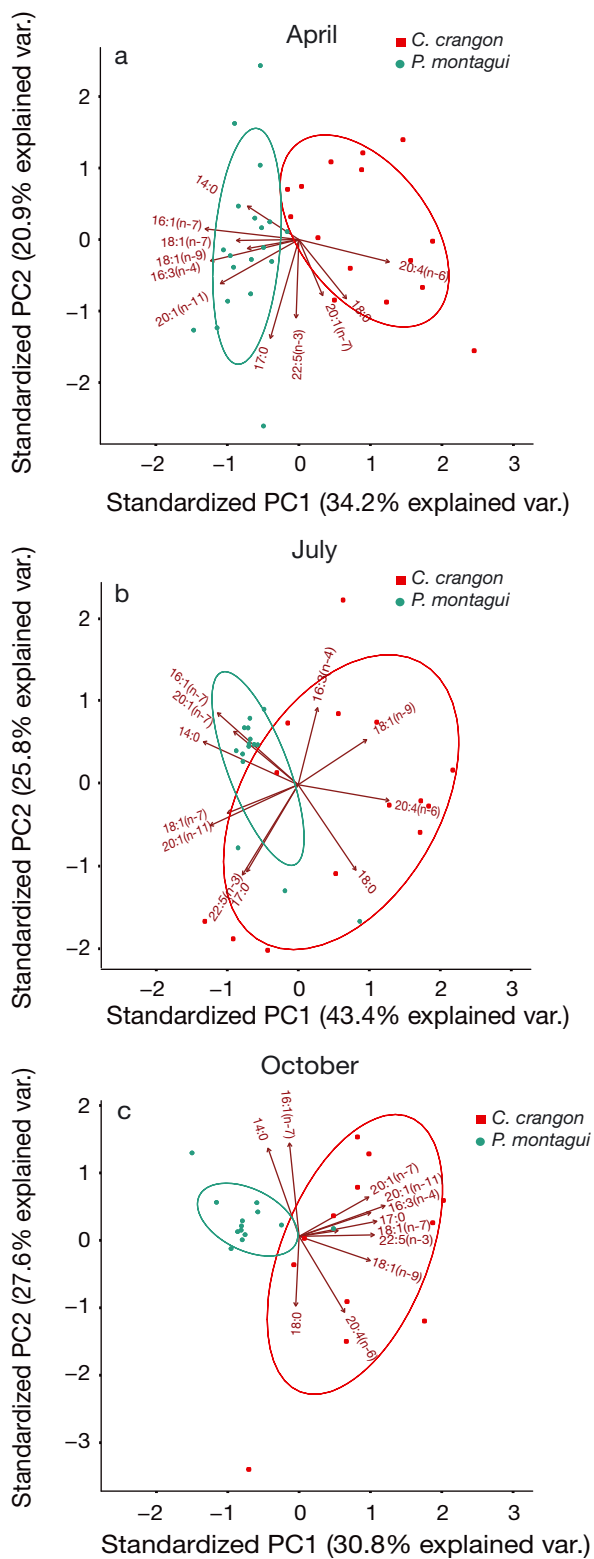


Fig. 3. PCA based on the fatty acid compositions of midgut glands of *Crangon crangon* and *Pandalus montagui* in (a) April, (b) July, and (c) October. The ellipses group data at a confidence level of 95 %

total lipid g^{-1} wet mass in summer. This corresponds to 128.8 mg (12.9 % DM) and 30.8 mg (3.1 % DM) on a dry mass basis assuming a water content of 75 %. These values are in the same range or clearly below the lipid contents we determined in the midgut gland. Therefore, it is unlikely that muscle tissue represents an important storage organ for lipids in *C. crangon*.

4.2. Food and trophic markers

C. crangon is an opportunistic omnivorous predator on micro-, meso-, and macrofauna. Smaller specimens feed on ostracods and harpacticoid copepods, while larger shrimps prefer various species of mussels, polychaetes, and small crustaceans (Plagmann 1939, Pihl & Rosenberg 1984, Feller 2006). Cannibalism is also common (Pihl & Rosenberg 1984). Microphytoplankton, such as diatoms and dinoflagellates, are ingested when they become abundant during the seasonal plankton blooms. Additionally, macroalgae of the genus *Ulva* and *Enteromorpha* were found in the stomachs of *C. crangon*. During the course of the year the shrimps show a pronounced trophic flexibility, and change their dietary spectrum according to the availability of the food organisms (Plagmann 1939). The preferred food of *P. montagui* covers a similar spectrum to that of *C. crangon*. It consists of polychaetes, crustaceans, and other small pelagic and benthic organisms (Simpson et al. 1967).

According to the FA trophic marker concept, ingested marker FAs are incorporated unmodified, usually in lipid depots, and can provide information about the trophic preferences of species (Dalsgaard et al. 2003). *C. crangon* showed low total lipid levels and, consequently, a higher portion of polar lipids. Moreover, PUFAs are more frequent as important components of biomembranes in the polar lipid fraction (Jezyk & Penicnak 1966), which explains the larger fraction of PUFAs in the midgut gland of *C. crangon* compared to *P. montagui* (Table 4).

Diatoms and dinoflagellates differ in their FA compositions. Typical FAs of diatoms are 16:1(n-7), 18:1(n-7), 20:5(n-3), and C16 PUFAs. Dinoflagellates are rich in 18:4(n-3) and 22:6(n-3) (Graeve et al. 1994). Due to the prevalence of neutral lipids in the pink shrimp, the concentrations of those FAs and the higher ratio 16:1(n-7)/16:0 strongly suggest that *P. montagui* feed on diatoms and dinoflagellates, but in different seasons. The carnivory marker 18:1(n-9) was higher in April than in the other months, while the CHI remained at the same level among seasons,

indicating a wide food spectrum and an omnivorous feeding behaviour.

For *C. crangon*, such assumptions are less validated due to the low total lipid content, and thus high phospholipid fraction. The concentrations of 16:1(n-7) indicate that *C. crangon* feed on diatoms, and high levels of 16:1(n-7) in April match with the phytoplankton spring bloom (Wiltshire et al. 2015). The variation in the 16:1(n-7)/16:0 ratio, which also indicates diatom feeding, followed the same pattern as the total lipid amount in the midgut gland of *C. crangon*. Therefore, it may be possible that the low lipid level in the midgut gland of *C. crangon* partly originates from diatoms. Elevated levels of 22:6(n-3) in July coincide with a high dinoflagellate abundance (Löder et al. 2012). However, there is not enough evidence to state that they feed significantly on dinoflagellates, because the FA 18:4(n-3), another relevant dinoflagellate marker, accounts for <1% of total FAs. As the FA 22:6(n-3) is one of the principal (non-dietary) components of the lipid membrane, the inverse relationship with 16:1(n-7) could also point to a higher portion of this FA in the membrane lipids. Accordingly, at relatively high lipid levels in April and October, the percentage of 22:6(n-3) decreased, but increased in July and February, when lipid levels were low.

PCA based on the FA composition of *C. crangon* was significantly affected by the membrane FAs. Therefore, we excluded those FAs from this analysis to emphasize the trophic markers. No clustering of FAs was evident between months in *C. crangon*, which indicates that this species did not display different seasonal feeding preferences in our study. *P. montagui* showed 3 distinct clusters corresponding to the 3 sampling months, which suggests a distinct seasonal change of food preferences by *P. montagui* (Fig. 2). Diatom markers mainly affected the clusters of April and July but not October (Fig. 3), which indicates that in April and July, *P. montagui* fed preferably on diatoms. The very well defined PCA clusters of *P. montagui*, especially in July and October, compared to the broader clusters of *C. crangon*, suggest more specific feeding preferences of *P. montagui* and a broader dietary spectrum of *C. crangon* (Fig. 3).

4.3. Lipids and reproduction

Both species, *C. crangon* and *P. montagui*, are r-strategists showing fast growth, short longevity, high fecundity, and small but numerous eggs. They exhibit a higher individual annual reproductive ef-

fort than K-strategists (slow growth, deferred maturity, greater longevity, low fecundity, large yolky eggs) (Clarke 1979b). When food availability for planktonic offspring is patchy or unpredictable, it is less risky and more efficient to produce many small eggs (Vance 1973, Clarke 1979b). Due to the variable environmental conditions of the North Sea, species following the r-strategy will probably be more successful.

C. crangon show intensive reproductive activities in the southern North Sea. Egg-bearing females of *C. crangon* are present almost year-round, but mainly from November to September, with the number of ovigerous females peaking in spring and early summer (Boddeke & Becker 1979, Siegel et al. 2008, Campos et al. 2010). Females can repeatedly produce eggs, and the clutch size varies from 1000 to 14 000 eggs (Clarke 1979b). In the German Bight, they may spawn 2 to 3 times during the season (Ehrenbaum 1890, Havinga 1930). Meixner (1966) even reported up to 5 spawnings in an aquarium at 14°C water temperature.

Egg-carrying females of *P. montagui* occur largely between November and March, and planktonic larvae are released only once per year (Allen 1963), mainly during spring (Simpson et al. 1967). During our study, no females with eggs were caught, since sampling took place outside the spawning season. According to Clarke (1979a), ovigerous females of *P. montagui* have low lipid contents in the midgut glands, whereas midgut glands of females with maturing ovaries have high lipid levels. Therefore, the elevated lipid contents in the midgut glands found in July may reflect the presence of females in the early phase of maturing ovaries (Fig. 1). Our results agree with the observations of Warren (1973) who stated that ovary development in *P. montagui* is paralleled by a decrease in total lipids in the midgut gland. Apparently, from July to October the shrimps transfer lipids from the midgut gland to the maturing ovaries to prepare for the upcoming spawning season between November and March.

The eggs of *C. crangon* contain almost 60% DM protein and 33% DM lipid (Pandian 1967). Since their eggs are smaller than those of *P. montagui*, they contain less lipid (5.3 µg vs. 23.2 µg egg⁻¹). However, the clutch size is much higher in *C. crangon* (1000–14 000 vs. 150–4000 in *P. montagui*; Clarke 1979b). Still, the total amount of lipid would be lower for an average egg clutch of *C. crangon* (39.8 mg) compared to that of *P. montagui* (48.1 mg).

C. crangon, the multiple spawner, exhibited low lipid-storage capacities, whereas *P. montagui*, the

single spawner, deposited much higher amounts of lipid in the midgut gland. The opposite was observed in the brachyuran crabs *Carcinus maenas* and *Hemigrapsus sanguineus* (Jungblut et al. 2018). *C. maenas*, a single spawner, has low lipid reserves, whereas *H. sanguineus*, which spawn several times a year, have high lipid levels and a strong seasonal variation in lipid levels (Fig. 4). These contrasting results suggest that lipid levels may not be related to the frequency of spawning. Apparently, the differences in lipid deposition in *C. crangon* and *P. montagui* may be explained by their deviating strategies to use the midgut gland for metabolic activities and for storage (Fig. 5). Similar to *C. crangon*, the midgut gland of the Antarctic shrimp *Chorismus antarcticus* acts as an active metabolic centre rather than a lipid storage organ (Clarke 1982). The metabolic activity and efficiency of the midgut gland allow immediate processing of dietary lipids, and reduce the need for large lipid stores in the midgut gland. This energetic strategy, however, demands continuous food supply and specific physiological and biochemical adaptations (Martínez-Alarcón et al. 2019), at least during the extended period of gonad maturation. Apparently, *C. crangon* is usually not confronted with prolonged periods of food deprivation, but may be adapted to cope at least with shorter starvation periods, e.g. by reducing metabolic rates (T. Werner pers. comm.).

This study also showed no statistically significant differences in total lipid contents of the midgut

glands between sexes in both species. Similar observations were reported for the crabs *Armases cinereum* and *Sesarma nr. reticulatum* (Hasek & Felder 2005), *Aegla platensis* (Oliveira et al. 2007), the crayfish *Parastacus defossus* (Buckup et al. 2008), the green shore crab *Carcinus maenas*, and the Asian shore crab *Hemigrapsus sanguineus* (Jungblut et al.

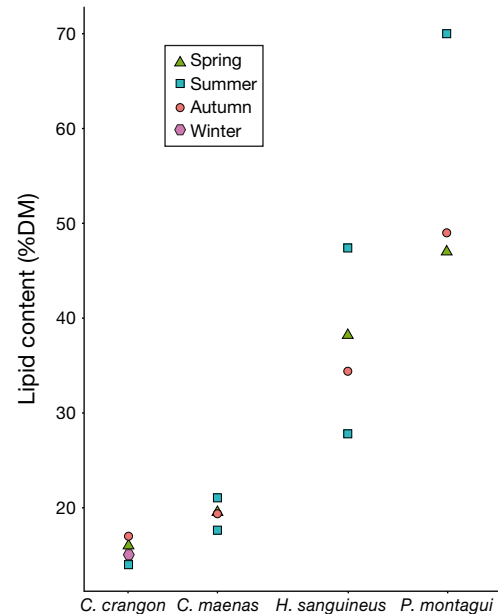


Fig. 4. Seasonal variation in mean lipid content in the midgut glands of *Carcinus maenas*, *Crangon crangon*, *Hemigrapsus sanguineus*, and *Pandalus montagui*. Data for *C. maenas* and *H. sanguineus* from Jungblut et al. (2018)

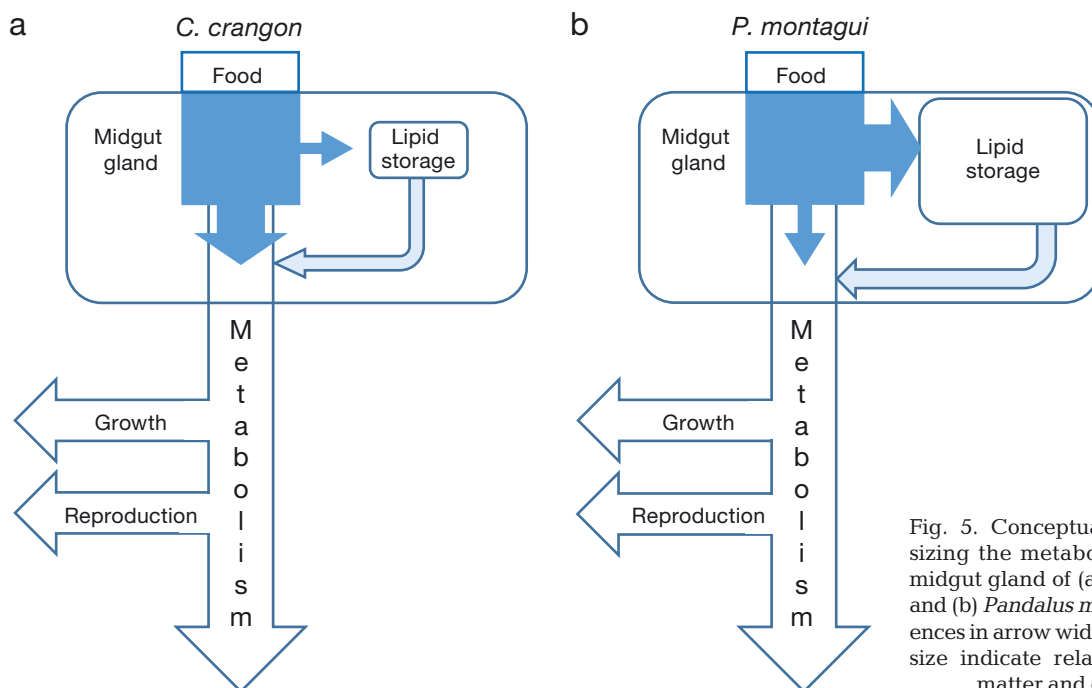


Fig. 5. Conceptual scheme emphasizing the metabolic function of the midgut gland of (a) *Crangon crangon* and (b) *Pandalus montagui*. The differences in arrow widths and lipid storage size indicate relative differences in matter and energy flows

2018). This is surprising, since a significant transfer of lipids from the midgut gland to the ovaries should result in pronounced differences between the sexes, as the energetic costs for the production of sperm is much lower compared to the egg production of females (Hayward & Gillooly 2011). Our findings also indicate that the ability of lipid storage is an intrinsic, probably genetically determined trait, at least in the species listed here. Further studies are required to elucidate the underlying biochemical processes in lipid metabolism and lipid-storage capacities in decapod crustaceans, also with regard to the different sexes.

We conclude that *C. crangon* and *P. montagui* follow very different energetic strategies in the southern North Sea, possibly related to the more northern distribution centre of the latter, which also tends to inhabit deeper water layers. In *P. montagui*, the variable but usually high lipid levels of the midgut gland are, to a certain extent, linked to the reproductive cycle. Lipid levels change according to reproductive seasons, but they are not sex-specific. Feeding preferences in the omnivorous *P. montagui* also vary with the seasons. In the omnivorous *C. crangon*, the low lipid content indicates that the midgut gland does not function primarily as an energy depot to support reproductive processes. Apparently, the midgut gland in *C. crangon* rather serves as a dynamic metabolic centre with high turnover rates. Hence, in spite of the limited lipid-storage capacity, the brown shrimp is well adapted to cope with a highly variable environment with periods of food paucity, and it has successfully established large stocks in the North Sea and surrounding waters.

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