Sacculina carcini impact on energy content of the shore crab Carcinus maenas L.

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Abstract

The impact of *Sacculina carcini* infection on the nutritional status of the shore crab *Carcinus maenas* was investigated in the western Dutch Wadden Sea for a period of 20 months. About 3.3% of the population was sacculinized, i.e. externally infected with *S. carcini* and only 0.7% presented scars of previous infection. The results of mixed linear models showed that sacculinized and non-sacculinized crabs had similar morphometric condition, while the energy density of parasitized crabs (externa excluded) was significantly reduced by about 4.3% overall, and by up to 5.8% in crabs under 40 mm carapace width. However, when *Sacculina* externa was included in the energy determinations, the difference in energy density decreased to 1.2%, while total energy content of the pair infected crab-parasite including externa was 30.8% higher than non-sacculinized crabs of similar size. The total energy content of ovigerous females (eggs included) was even higher, near doubling the energy of similar-sized crabs. The same way, total energy content of *Sacculina* externa was about 4 times lower than total energy of egg mass. The results suggest that the rhizocephalan parasite is efficient in consuming the energy that the host may allocate for growth and maintenance, but require future studies to disentangle the impact of the degree of internal infection and the implications for the dynamics of the population.

Introduction

The shore crab *Carcinus maenas* (L.) is an abundant epibenthic decapod crustacean species within its native range in the northeastern Atlantic hard and soft shallow coastal areas (Roman and Palumbi, 2004), and has a worldwide invasive character (Lowe *et al.*, 2000; Darling *et al.*, 2008). It is considered an 'ecosystem engineer' because of its ability to modify entire ecosystems (Klassen and Locke, 2007; Garbary *et al.*, 2014). Therefore, it has been the focus of several studies, recently reviewed by Young and Elliott (2020). More recently, the seasonality in the body and energetic condition of shore crabs was investigated in the western Dutch Wadden Sea (Campos *et al.*, 2021) showing fluctuations related to environmental constraints such as thermal and prey availability patterns, and with the growth and reproduction of the crabs.

The rhizocephalan barnacle *Sacculina carcini* is a common endoparasite of the shore crab. Parasite prevalence can reach near 80% of the crab population with a great spatio-temporal variation (Lützen, 1984; Mathieson *et al.*, 1998; Torchin *et al.*, 2001; Werner, 2001; Zetlmeisl *et al.*, 2011). The life cycle of the parasite and its development on the shore crab host has been previously described (Høeg *et al.*, 2005). Shortly, infection occurs by female cypris larvae of *S. carcini* which settle on the carapace of recently moulted crabs and penetrate the exoskeleton into the haemolymph (Glenner and Werner, 1998; Glenner *et al.*, 2000). The parasite grows a root-like system (*interna*) throughout the host tissues, which serves as the nutrient-absorbing organ (O'Brien and Van Wyk, 1985). The infected host soon becomes a parasite genotype with a crab phenotype (O'Brien and Van Wyk, 1985). Later, an external reproductive sac-like structure (*externa*) produced by the parasite emerges out of the abdomen of the crab, in the brood chamber of the host (Glenner and Høeg, 1995; Høeg, 1995), and is fertilized by 1–2 cryptic dwarf males (Delage, 1884). After releasing the nauplii larva, the externa falls off leaving a scar on the abdomen of the host crab (Mouritsen *et al.*, 2018).

Sacculina infection severely impacts the general condition of the crab host (Mouritsen and Jensen, 2006; Larsen *et al.*, 2013), affecting growth, behaviour, morphology and physiology (Thresher *et al.*, 2000), feminizes males, and increases (doubles) the mortality rate of infected crabs (Goddard *et al.*, 2005). Externally infected crabs (often denominated as 'sacculinized' crabs) stop moulting (and hence cease growth) (Høeg, 1995), which favours the settlement and growth of fouling organisms, such as epizoic barnacles, on the carapace of crabs (Mouritsen and Jensen, 2006). Aside from morphological and behavioural feminization of male hosts (Rasmussen, 1959; Glenner and Høeg, 1995; Høeg, 1995; Werner, 2001), *S. carcini* also causes the degeneration of the gonads (Day, 1935; Zetlmeisl *et al.*, 2011), inducing 'parasitic sterilization' in both host genders (Høeg and Lützen, 1995; Werner, 2001; Zetlmeisl *et al.*, 2011).

Rhizocephalans can deplete energy stores of the host by utilizing the energy for their development or by causing the host to divert energy into a defence response against infection. Therefore, one would expect that infected crabs have lower energy content than uninfected crabs of similar size. However, it is not clear how *C. maenas* handles the extra energy requirements of infection. Although some morphological changes induced by *Sacculina*, such as the size reduction of walking legs (Kristensen *et al.*, 2012) and chelipeds of feminized males (Høeg, 1995; Mouritsen and Jensen, 2006; Lafferty and Kuris, 2009; Kristensen *et al.*, 2012) can impact the crabs' feeding behaviour, host food consumption appears to be unaffected by infection (Larsen *et al.*, 2013). The energy requirements of the parasite should then match the energy which the crab would allocate for its own growth and reproduction (Larsen *et al.*, 2013). If so, the nutritional status, including the energy content of the host (i.e. parasite included) would be identical to that of uninfected crabs of similar size.

This is the second of a series of 3 papers dedicated to a comprehensive investigation of the shore crab's condition in Europe's largest coastal wetland system, the western Dutch Wadden Sea. The aim of the present study was to evaluate the impact of *Sacculina* infection on the general condition of the shore crab *C. maenas*, more precisely on the condition of the pair 'crab-parasite' (sacculinized crab) as they cannot be physically separated. For that, we compared the nutritional status between sacculinized (i.e. parasite rootlets included) and non-sacculinized crabs, using both morphometric indices – the Fulton's K condition index and the percentage of dry weight – and biochemical data – the body energy content.

Material and methods

Sampling and laboratory procedures

A monthly sampling programme was carried out from August 2012 to March 2014 (except in September 2012 and 2013, October 2013, January and February 2014, due to adverse weather conditions) at 3 sites in the Marsdiep tidal basin in the western Dutch Wadden Sea (Fig. 1) as described in Campos *et al.* (2021). Shortly, a 2 m beam trawl with a single tickler chain and a mesh size of 1×1 cm was towed by a scientific vessel (*RV Stern*) at a speed of approximately 60 m min^{-1}. Each haul covered a surface area of at least 900 m^2. All sites were 5–12 m deep and had soft bottom sediments. In addition, water temperature and salinity were recorded at each site before the hauls. All catches were sorted immediately and the shore crabs were transported alive to the lab. In the 2012–2014 dataset, most parasite externa samples were below the minimum required weight for the calorimetry. Therefore, later, in May 2019, an additional sampling focused on obtaining the missing information on the energy content of the parasite externa was carried out to complement the available dataset.

All crabs were sexed according to the sexual dimorphism traits described in Squires (1990) (male with a triangular abdomen and 3rd–5th somites fused; female with a subtriangular rounded lateral abdomen and somites not fused), and measured to the nearest mm carapace width (CW) with a digital calliper. Each individual was classified into colour morphotype (green or red) based on the predominant colour of the thoracic sternum (McGaw and Naylor, 1992), and inspected for the presence of eggs in females, epibiont fouling, and parasites. Fouled crabs were excluded from the study to avoid extra confounding factors affecting energy determinations. Parasites were detected by lifting the abdomen of each crab from the thorax to check for the presence of *Sacculina carcini* externa (gonad mass sac).

Secondary features including scars of lost externa and broad abdomen in males were used to determine past infection and modified male (i.e. feminized) condition, respectively. Prevalence of infection was defined as the percentage of crabs that were externally parasitized (i.e. sacculinized crabs); exclusively internal (i.e. immature) phase of infection was not determined, nor were scarred nor modified male crabs without externa included in the calculation of prevalence, following previous studies (e.g. Zetlmeisl *et al.*, 2011).

Every month, for each sex, subsets of about 10 uninfected and 10 infected crabs (randomly chosen) per 10 mm size classes were selected for subsequent morphometric and calorimetric analyses. All animals were weighted (WW, wet weight), eviscerated (i.e. dissected to remove the stomach) and weighted again right after, to avoid mixing of stomach content in the calorimetric analyses. The externa of *S. carcini* was removed from infected crabs before weighing. Since *S. carcini* creates an internal root system in the whole body of the host, it was impossible to completely remove the parasite and exclusively analyse the host; thus the parasite roots had to be included. This means that any quantitative difference between non-sacculinized and sacculinized crabs is a conservative estimate. Therefore, comparative analyses were made between 'sacculinized' (i.e. crabs infected with *S. carcini* and presenting an externa sac) and 'non-sacculinized' (i.e. crabs presenting no externa, which can be uninfected or exclusively internally infected crabs) animals. For the 2019 samples, the externa of sacculinized crabs and the egg mass of ovigerous females were dried and analysed separately. Samples (crab whole body excluding stomach, eggs and *S. carcini* externa) were individually dried for 10 days at 60°C till constant dry weight (DW). The crabs Fulton's condition index (K) was determined by dividing the WW by the cubic CW.

Calorimetry

The energy content was determined using an IKA C2000 basic Calorimeter the same way as in Campos *et al.* (2021). After maceration of each dried crab, the powder sample was pressed in a mortar to build a pellet and taken into the calorimeter bomb, where it was combusted and analysed for caloric content (cal g^{-1} DW). Subsequently, values were corrected for minerals and water remains that did not account for the energy content of the animal. Sample ash contents higher than 30% make it difficult to obtain a complete combustion in the calorimeter (Cummins and Wuycheck, 1971). In *C. maenas*, the inorganic material consists largely of calcium (Adelung, 1971), and makes up a great part (about 40%) of the dry weight, affecting the calorimetric result since calcium reaction in the calorimeter is endothermic (Topley *et al.*, 1928). Therefore, the ash content was first determined by weighting the remaining ash after burning in the calorimeter to determine the calcium concentration in the samples. Then the ash was re-burned at 900°C in a muffle to remove the minerals that could not be removed during the calorimetric burning process and the remaining was weighed again to determine the ash-free dry weight (AFDW). The difference was used to calculate the percentage of calcium, which was then used to correct the energy content as follows:

Corrected caloric content (E)

$$= (\text{Caloric value} \times 100/\% \text{ AFDW}) - 1.4 \times \% \text{ CaCO}_3 (\text{cal g DW}^{-1})$$

where 1.4 cal g^{-1} is the caloric value of carbonate calcium in calorimetric reactions (Paine, 1964). Caloric content was also determined separately for the externa and the egg mass of crabs from the 2019 samples. Finally, caloric values were converted to kJoules per gram
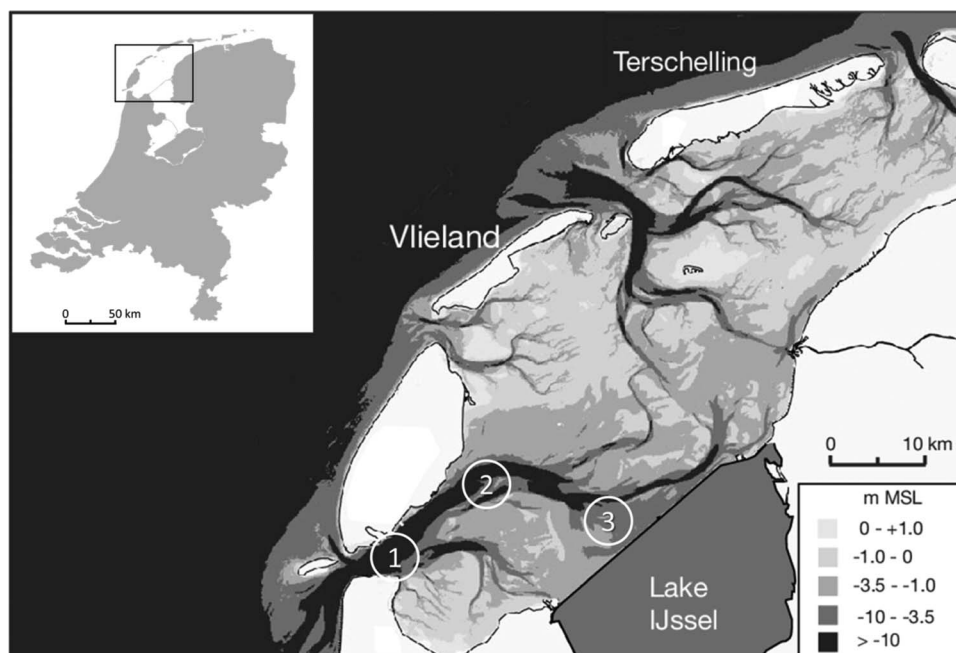
Fig. 1. Map of the sampling locations in the western Dutch Wadden Sea. (1) Gat van der Stier (N 52°57.27′ E 4°55.73′); (2) Texelstroom (N 53°02.03′ E 5°03.37′); and (3) Kornwerderzand (N 53°04.52′ E 5°16.55′); top left: sampling area in the Netherlands. (Adapted from Katwijk and Hermus, 2000).

of DW (kJ g DW^{-1}), hereafter E_{DW}, per gram of AFDW (kJ g AFDW^{-1}), hereafter E_{AF}, and total energy content (E_{tot}) of each crab was obtained multiplying E_{DW} by the respective DW.

A total of 617 crabs were analysed for energy, of which 114 crabs were externally infected with *S. carcini* (Table 1). No scarred crab was analysed for energy.

Data analysis

The crab condition was analysed for non-sacculinized and sacculinized crabs, considering crab sex and size, and comparing their mean values using Welch's *t*-test, a modification of the *t*-test. This test adjusts the number of degrees of freedom when the variances are thought not to be equal to each other and performs better than Student's *t*-test whenever sample sizes and variances are unequal between groups, as is the case here, providing the same result when sample sizes and variances are equal (Delacre *et al.*, 2017). The significance level was set at $\alpha = 0.05$.

The effect of *Sacculina* external infection was also assessed through regression modelling. A previous study, with *C. maenas* from the Dutch Wadden Sea, carried out on apparently uninfected crabs (not bearing the parasite externa), showed that crab sex and size, and sampling season were the most relevant predictors for crab condition, whereas crab colour was a negligible, as well confounding variable (being related to the animal's size), and sampling site was only relevant for %DW (Campos *et al.*, 2021). Therefore, these variables (crab colour and sampling site) were not considered in the models of the present study.

Here, the objective was to obtain insight into external infection effects on condition, independent of the sampling season or site, knowing that samples were not spatially and temporally independent. Furthermore, in the available data set, sampling season is a non-continuous variable (data collected in 2012–2014 and in 2019) with gaps, and sampling sites were sampled in an unbalanced way, containing data from different seasons, crab development stages and, thus, sizes. Therefore, linear mixed effect models were applied, with *Sacculina* external infection, crab size and sex as fixed factors, and sampling season and site as random factors. This way, the model accounted for temporal and spatial

Table 1. Number of sacculinized *Carcinus maenas* analysed for energy condition per sex (F: females, M: males, MM: modified males) and colour and percentage (%) of total sacculinized sample

| Sex	Colour	Total	%
F	Green	19	16.6
M	Green	15	13.6
MM	Green	27	26.9
	Green		57.1
F	Red	23	21.7
M	Red	18	11.7
MM	Red	12	9.5
	Red		42.9

variability, producing better estimates for the fixed effects parameters. Crossed LMER with random intercept and slope were applied (using the R lme4 package; Bates *et al.*, 2015), with CW standardized to mean zero (i.e. centred) and a standard deviation of 1 (i.e. scaled), to ensure that the estimated coefficients are all on the same scale, to allow comparison of effects. Model explained variance was obtained calculating the marginal R^2, which represents the variance explained by the fixed effects, and the conditional R^2, which is interpreted as the variance explained by the entire model, including both fixed and random effects (according to Nakagawa *et al.*, 2017), using the R package stargazer (Hlavac, 2018).

All data processing and statistical analyses were performed in R (R Core Team, 2020).

Results

General patterns of crab infection

A total of 11 068 shore crabs were collected and analysed for general biometry in the 2012/2014 sampling, from which 3.3% presented a visible externa and therefore were considered as
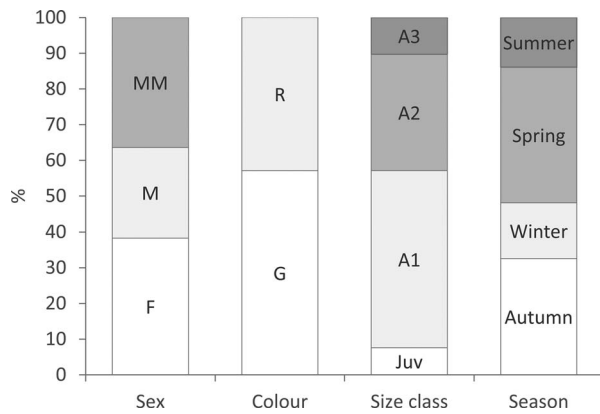
Fig. 2. Percentage distribution of the abundance of sacculinized crabs according to sex (F: females, M: males, MM: modified males), colour morphotype (G: green, R: red), size class (Juv, juveniles: ⩽25 mm CW; A1: 25–40 mm CW; A2: 40–55 mm CW; A3: >55 mm CW) and seasons.

sacculinized crabs (externally infected by *Sacculina carcini*). Additionally, 0.7% of the non-sacculinized crabs had a visible scar from lost externa on the abdomen. Distribution patterns of prevalence per sex, colour type, size class and season are presented in Fig. 2. Most sacculinized crabs were males (61.7%), of which more than half (60.0%) were modified males. No egg-bearing female was found externally infected or scarred, and non-sacculinized modified males represented only 0.24% of the total sample. In relation to the colour morphotype, despite the majority of the sacculinized crabs were green (57.1%), externally infected green morphs represented only 2.3% of all sampled green crabs, while for red morphs, sacculinized crabs represented 7.9% of all sampled red morphs. The size of externally infected crabs ranged from 14.8 to 88.6 mm, with an average of 40.9 ± 14.0 mm CW, but external infection was higher in adult crabs >25 mm CW, with almost half of the sacculinized crabs ranging from 25 to 40 mm CW (49.5% of the sacculinized crabs) and lowest in juveniles (<25 mm; 7.6%) and in crabs larger than 55 mm (10.3%) (Figs 2 and 3). Distribution of sacculinized crabs followed a seasonal pattern; abundance was highest in spring (38.0%) and autumn (32.6%), and lowest in summer (13.9%).

Model results

Table 2 presents the linear mixed effects model (GLMM) results. The models fitted particularly well for the E_{tot} condition ($R^2 > 80\%$), followed by Fulton's K (R^2 up to 69%), while for E_{DW}, E_{AF} and %DW the R^2 of the model was relatively low ($R^2 < 30\%$). The results suggested non-significant impacts of *Sacculina* external infection on the morphological condition (K and %DW) and on total energy (E_{tot}), and significant impact of external infection on the energy density (E_{DW} and E_{AF}). Other relevant factors affecting condition were sex and size. According to the GLMM models no predictor variable was significant for the variance found in %DW, only CW for K and E_{tot}, and only *Sacculina* infection for E_{DW} and E_{AF}.

Impact of Sacculina *external infection on crab's condition*

Although models did not reveal a significant impact of external infection on the morphological condition of crabs, sacculinized animals had slightly higher mean Fulton's K (1.42 ± 0.52 and 1.60 ± 0.64, respectively in non-sacculinized and sacculinized crabs; P value <0.01, Welsh's t-test), especially females (1.53 ± 0.56 and 1.82 ± 0.89, respectively in non-sacculinized and sacculinized crabs; P value = 0.051) and modified males
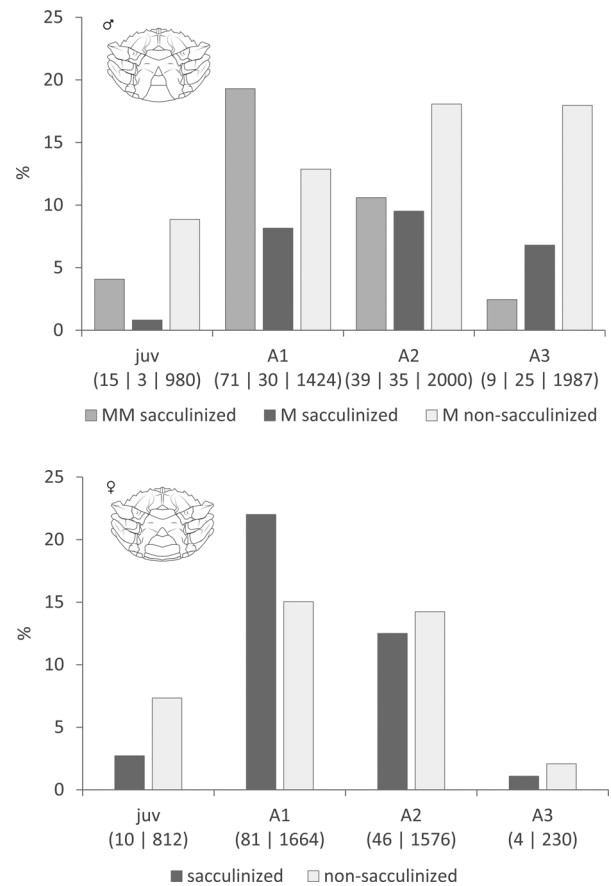
Fig. 3. Size distribution of sacculinized and non-sacculinized crabs per sex (F: females, upper panel; and M: males and MM: modified males, lower panel). Juv, juveniles: ⩽25 mm CW; A1: 25–40 mm CW; A2: 40–55 mm CW; A3: >55 mm CW. Note that MM non-sacculinized crabs represented only 0.24% of the sampled population.

(1.39 and 1.63 ± 0.41, respectively for the single non-sacculinized and sacculinized crabs) (Fig. 4). A general decreasing trend of K with size (CW) was also observed, as also identified as significant by the GLMM (Fig. 4, Table 2). In contrast, the %DW was unaffected by external infection (32.84 ± 4.58% and 32.09 ± 4.16%, respectively, in non-sacculinized and sacculinized crabs; P value = 0.099), though slightly lower in modified crabs (30.42 ± 5.44%), with no significant trend with size (Table 2).

Contrasting with the morphological condition, the energy density of crabs was significantly affected by external infection (model results, Table 2), and size was only significant for E_{tot}. The mean E_{DW} was 4.3% lower in sacculinized crabs (excluding the externa sac), overall, and up to 5.8% lower in crabs <40 mm CW (though size was not a significant factor affecting E_{DW}), with females more heavily affected (mean E_{DW} of F 12.14 ± 1.77 and 10.65 ± 1.28, P value <0.001; M 11.46 ± 1.54 and 11.20 ± 1.22, P value = 0.293, respectively, for non-sacculinized and sacculinized females) (Fig. 5). The 2019 dataset provided extra information on the externa sac energy content (Fig. 5) allowing determining the mean E_{DW} of the pair 'sacculinized crab-parasite including the externa sac', which was found to be very close to the mean E_{DW} of non-sacculinized crabs (only 1.2% lower), even though the sac itself had about the double of the energy density – mean E_{DW} of the sac 22.27 ± 5.52 *vs* 11.57 ± 1.49 kJ of non-sacculinized crabs (all 2019 crabs were <40 mm CW). The 2019 dataset further provided extra information on mean E_{DW} of the egg mass (Fig. 5). Mean E_{DW} of the egg mass (24.56 ± 5.21 kJ) was even higher than the E_{DW} of externa sac resulting in a mean E_{DW} of 'ovigerous females including the egg mass'

Table 2. Linear mixed effects model results, with predictor estimate and estimate error, *t*-value, significance (n.s. non significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) and the proportion of variance explained by the fixed effects (marginal R^2) and by the entire model (conditional R^2)

Condition variable	Predictor	Estimate	Error	*t*-value	R^2 fixed ef.	R^2 model
K	(Intercept)	1.4177	0.11174	12.687***	0.2146	0.6924
	Sacculina	−0.0690	0.16037	−0.430		
	Sex M	0.0932	0.07275	1.281		
	Sex MM	−0.1030	0.17728	−0.581		
	CW	−0.3719	0.13612	−2.732**		
	CW^2	0.0840	0.06245	1.346		
%DW	(Intercept)	32.6854	1.0459	31.252***	0.0184	0.2638
	Sacculina	−0.5112	0.7150	−0.715		
	Sex M	0.1214	1.1244	0.108		
	Sex MM	−1.4531	1.7195	−0.845		
	CW	−0.2596	0.2662	−0.975		
	CW^2	0.3424	0.2422	1.414		
E_{DW}	(Intercept)	11.6271	0.45350	25.639***	0.0598	0.3013
	Sacculina	−1.0421	0.39398	−2.645**		
	Sex M	0.3850	0.55513	0.694		
	Sex MM	−0.0621	0.64474	−0.096		
	CW	−0.1934	0.30768	−0.629		
	CW^2	−0.0763	0.12885	−0.592		
E_{AF}	(Intercept)	17.7666	0.35225	50.437***	0.0565	0.2859
	Sacculina	−0.7400	0.34678	−2.134*		
	Sex M	0.3866	0.44670	0.866		
	Sex MM	−0.2944	0.52080	−0.565		
	CW	−0.2099	0.22488	−0.933		
	CW^2	−0.0514	0.12855	−0.400		
E_{TOT}	(Intercept)	36.6110	3.7280	9.820***	0.8081	0.8480
	Sacculina	−5.7140	3.4700	−1.647		
	Sex M	5.0430	6.1320	0.822		
	Sex MM	−2.0400	6.1640	−0.331		
	CW	29.7650	2.0600	14.451***		
	CW^2	10.6190	1.5650	6.787***		

(12.79 ± 1.67 kJ), 10.8% higher than mean E_{DW} of (non-sacculinized) crabs of similar size.

The E_{AF} condition provided confounding results. Externally infected females reduced in 5.8% the E_{AF} in relation to non-sacculinized ones (mean E_{AF} 18.14 ± 1.66 and 17.08 ± 1.10, respectively, for in non-sacculinized and sacculinized females; P value <0.001), but infection did not affect males' E_{AF} (mean E_{AF} 17.55 ± 1.27 and 17.78 ± 0.91, respectively, for in non-sacculinized and sacculinized males; P value = 0.224) (Fig. 6).

In relation to E_{tot}, the energy of crabs increased with size (Fig. 6) and, considering similar sized crabs, E_{tot} was slightly lower for sacculinized crabs, externa sac excluded. The 2019 dataset, however, included data on E_{tot} of the parasites' externa sac (mean 2.93 ± 1.86 kJ, Fig. 7) and enabled to determine the total energy of the pair (sacculinized crab-parasite including externa). The same way, the E_{tot} of the egg mass measured with the 2019 dataset (mean 12.30 ± 7.39 kJ), enabled to determine the total energy of the pair (ovigerous female-egg mass) (Fig. 7). Though mean E_{tot} of the pair (sacculinized crab-parasite including externa) (19.49 ± 8.48 kJ) was much higher (about 30.8% higher) than the E_{tot} of non-sacculinized crabs of similar size (14.90 ± 6.87 kJ), mean E_{tot} of the pair [ovigerous females-egg mass] was even higher (48.5% higher; 41.93 ± 12.48 kJ and 28.23 ± 11.86 kJ, respectively for ovigerous and for non-sacculinized crabs of similar size).

Discussion

The present study described patterns in the external infection prevalence of the rhizocephalan parasite *Sacculina carcini* in a shore crab population from the western Dutch Wadden Sea. The results show a slight (up to 5.8%), but significant decrease in the energy density of sacculinized crabs (externa excluded), although the morphometric body condition was not compromised by external infection. The reduction in energy density was more evident in crabs up to 40 mm size, and in females. However, when the parasite sac was considered, the difference in energy condition between sacculinized and non-sacculinized
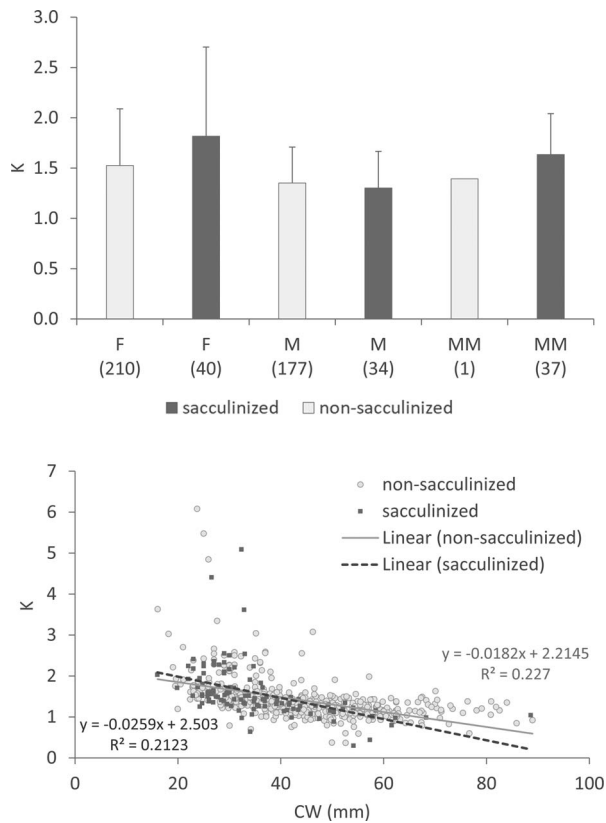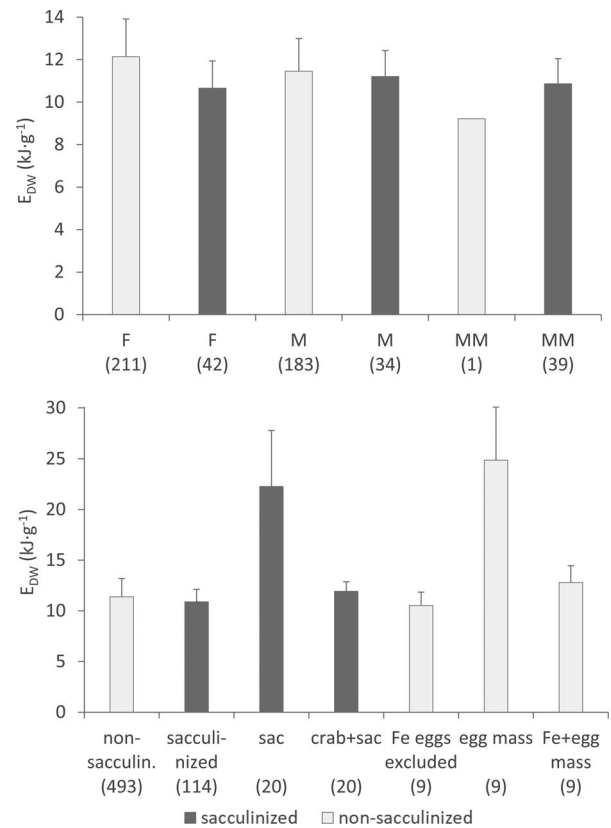
Fig. 4. Mean Fulton's K and error bars (as standard deviation) for sacculinized (dark grey) and non-sacculinized (light grey) crabs per sex (F: females; M: males) and modified males (MM) together with number of crabs between brackets (upper panel); and Fulton's K in relation to size (CW, mm) (lower panel). Sacculinized crabs in dark grey circles; non-sacculinized crabs in dark grey squares.

Fig. 5. Mean E_{DW} (kJ.g^{-1}) and error bars (as standard deviation) for sacculinized and non-sacculinized crabs per sex (upper panel); and mean E_{DW} (kJ.g^{-1}) and error bars (as standard deviation) for sacculinized, for non-sacculinized crabs, for the externa sac, the pair crab host-externa, the egg mass, and for the pair ovigerous females-egg mass (lower panel). Sacculinized crabs in dark grey; non-sacculinized crabs in light grey. Number of crabs between brackets.

crabs was attenuated because, despite small-sized (and hence with low amount of energy in total), the sac had almost the double of the energy density of the non-sacculinized crabs. In addition, the estimation of the energy content of the crab egg mass showed that both the energy density and total energy of the egg mass was even higher than that of the externa sac, and consequently, the pair ovigerous female-egg mass had the highest energy condition.

Patterns of Sacculina carcini *external infection prevalence*

A relatively small fraction of the crab population from the western Dutch Wadden Sea (only 3.3%) was externally infected by *S. carcini*, which is in accordance with a contemporaneous study in the area, reporting up to 3% prevalence of *Sacculina* infection (Waser et al., 2016). Prevalence of sacculinized crabs has a great spatial variation (Mouritsen et al., 2018). It is even lower (1.9–2.9%) in the Isfjord, Denmark (Lützen, 1984) and similarly low (2.9%) in the west coast of Sweden (Werner, 2001). Yet, while in the Wadden Sea, scarred crabs and modified males with no sac nor scar were nearly absent (respectively 0.7% and 0.2% of the total population), in Sweden scarred crabs accounted for an extra 2.6%, and uninfected modified males for an extra 2.7% of the population (Werner, 2001). Such a small fraction of scarred crabs did not allow evaluating the potential of recovery from infection. Elsewhere, the prevalence of *S. carcini* can range up to 20%, and can locally even reach 40–80% (Lützen, 1984; Mathieson et al., 1998; Torchin et al., 2001; Werner, 2001; Zetlmeisl et al., 2011). All these estimates (including present data) are conservative values, as the fraction of the immature phase of infection (interna, without externa) was not quantified. Quantification of internal infection requires the dissection of
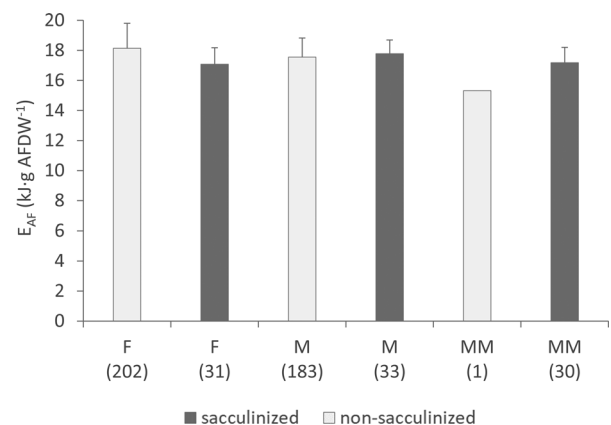

Fig. 6. Mean E_{AF} (kJ.g AFDW^{-1}) and error bars (as standard deviation) for sacculinized and non-sacculinized crabs per sex. Sacculinized crabs in dark grey; non-sacculinized crabs in light grey. Number of crabs between brackets.

the crabs (Belgrad and Griffen, 2015; Mouritsen et al., 2018), which is time consuming and out of the scope of the present study. Histological examination of shore crabs' hepatopancreas resulted in a mean of 24% prevalence of infected shore crabs in a population from South Wales (UK), though only 6.3% crabs were externally infected and 1.8% had scars (Rowley et al., 2020). Assuming a similar fraction, infected crabs in the Dutch Wadden Sea could be in the order of 12–13% of the population, internally infected crabs accounted.

A seasonal pattern in *Sacculina* prevalence has been described in previous studies. In general, prevalence peaks in
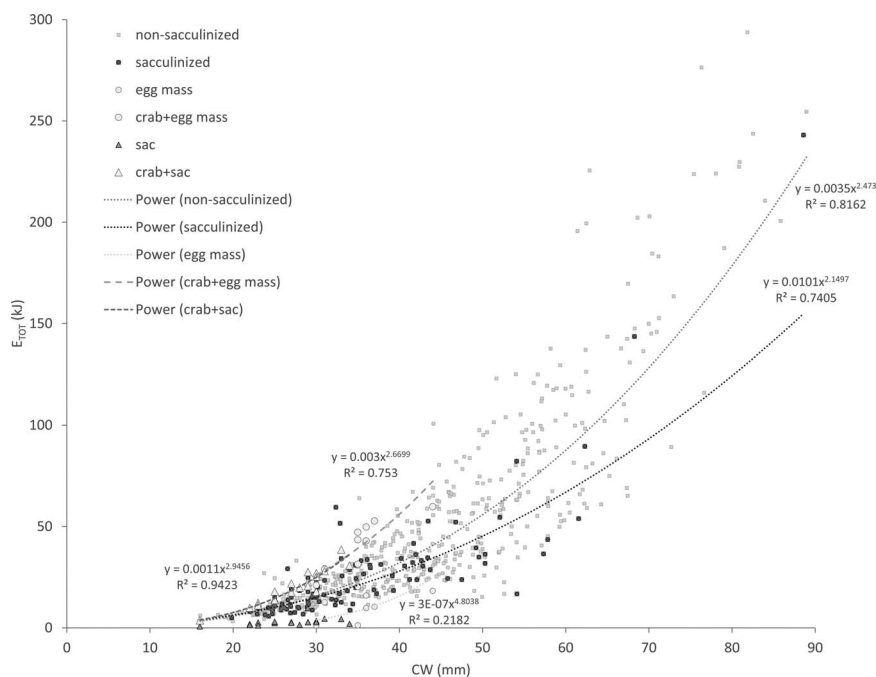
Fig. 7. Total energy (E_{tot}, kJ) in relation to size (CW, mm) for sacculinized and non-sacculinized crabs, for the externa sac, for the egg mass, and for the pairs [sacculinized crab-parasite including externa] and [ovigerous females-egg mass].

winter and minimums are observed in summer (Costa *et al.*, 2013; Jensen *et al.*, 2019), as described here for the Dutch Wadden Sea population. Therefore, increased mortality of externa has been related to the temperature rise during summer (Heath, 1971). Besides temperature, also salinity conditions influence the prevalence of infected crabs. The lower salinity limit for maintaining a permanent population of *Sacculina* seems to be around 20 (Kashenko and Korn, 2002; Mouritsen *et al.*, 2018), which fits the range (18–29) observed in the present (Campos *et al.*, 2021) and in the contemporaneous work in the area (Waser *et al.*, 2016). Yet, as in other sacculinid rhizocephalan species (Walker *et al.*, 1992), *S. carcini* eggs and larvae are more sensitive (Tolley *et al.*, 2006), requiring salinity >26 (Ramult, 1935), and condition the distribution of infected crabs in relation to salinity (Mouritsen *et al.*, 2018).

The prevalence of sacculinized crabs also varies with water depth (Rainbow *et al.*, 1979; Waser *et al.*, 2016). The deeper subtidal waters house older crabs with the highest levels of *S. carcini* (Waser *et al.*, 2016), while near none is found in the intertidal flats (Waser *et al.*, 2016). Water depth itself may not be a forcing reason but the depth distribution of crabs of different sizes, as older and larger crabs are typically more abundant in the subtidal (Atkinson and Parsons, 1973; Hunter and Naylor, 1993). Yet, preference for host size has not been documented (Werner, 2001; Waser *et al.*, 2016; Mouritsen *et al.*, 2018). In the present study, despite sampling took place always at the subtidal (5–12 m deep), *Sacculina* externally infected crabs mainly between 25 and 40 mm width, while only 10% of the largest crabs (>55 mm) were infected. This was also the size range with higher prevalence of infection previously reported for the area (Waser *et al.*, 2016) and for the Mondego estuary, in Portugal (Costa *et al.*, 2013). In the present work, the largest sacculinized crab was a male 88.6 mm CW and the smallest was a modified male 14.8 mm. This is smaller than previously reported (20 mm, Mouritsen *et al.*, 2018) and contradicts the assumption that crabs 3–16 mm do not become infected, as revealed by molecular analyses (Mouritsen *et al.*, 2018). When feminized males were accounted, prevalence of male external infection doubled the prevalence of sacculinized female crabs. Yet, preference for host gender has not been reported (Werner, 2001; Costa *et al.*, 2013; Waser *et al.*, 2016), with some populations revealing more

sacculinized males (e.g. Isefjord, Denmark, Lützen, 1984) and others more females (e.g. Mondego estuary, Portugal, Costa *et al.*, 2013; Danish coast, Mouritsen *et al.*, 2018; Scotland, Mathieson *et al.*, 1998).

The developmental stages of *S. carcini* externa seem correlated with the crab host colour cycle such that the youngest externa stages are predominant when the crabs are green, the intermediate stages when they are orange and the oldest when they are red (Lützen *et al.*, 2018). In the present study, the externa development stages were not evaluated, but external infection was more often found in green morphs, suggesting then that most parasites' externa would be on an early stage. Literature, however, refers that red crabs are more commonly infected than green ones (Zetlmeisl *et al.*, 2011; Costa *et al.*, 2013; Waser *et al.*, 2016), as infected animals remain longer in the intermoult stage which is prolonged in the red morphotype (Høeg, 1995).

Impact of external infection on crabs' condition

Infection by *S. carcini* causes castration, moulting arrest, behavioural and even body shape changes (Thresher *et al.*, 2000; Mouritsen and Jensen, 2006; Kristensen *et al.*, 2012; Belgrad and Griffen, 2015; Coates and Söderhäll, 2021), including the broadening of the abdomen in male crabs (Reinhard, 1956), a feminized attribute. All these effects stress the animals, suppress their defences, including a reduction in burrowing capacity, interrupt moulting and may favour increased colonization by epibionts (Mouritsen and Jensen, 2006). This would imply that infected crabs face extra energy costs due to the presence of the parasites, resulting in a lower general condition and a reduction in energy content. While the present findings revealed in fact a slight decrease in energy density in sacculinized crabs (externa excluded, and slightly less when sac was included), condition was evaluated for the pair host–parasite, because *Sacculina* internal roots cannot be dissected without losing relevant biological material. The main body of the parasite is a root-like system of branches that extends into most of the host's tissue, whereas the gonads of the parasites emerge externally under the abdomen of the crab (Høeg and Lützen, 1995). Therefore, the internal part of the parasite body was impossible to remove prior to calorimetric analysis resulting in a biased, i.e. apparently smaller, reduction in the host's

energetic condition: part of the measured organic weight and energetic content corresponded in fact to the parasite roots. This is probably the reason why the morphological condition of infected crabs was not affected by infection, as the extra weight of the parasite itself – corresponding to the internal roots – was included. It also means that the reduction in energy density of infected crabs accounts not only for the energy density of the host but also for the energy content of the parasite itself. And again this could explain why sacculinized crabs plus externa sac had higher total energy (about 30%) than non-sacculinized crabs of similar size, as an undetermined part of the energy belonged to the parasite. In addition, the extent of *S. carcini* rootlets within the crab internal tissues was not evaluated and might not be equivalent between sacculinized crabs (Powell and Rowley, 2008).

The arrest of moult and reproduction in sacculinized crabs, releases the energy pool which would be allocated for growth and gonad maturation of the host crab. In laboratory feeding trials with mussels, infection by *Sacculina* had no effect on food consumption by male crabs (Larsen *et al.*, 2013), which means that the energy released from crabs' growth is available for the parasite to spend on its own growth and reproductive requirements. Therefore, expectations of similar nutritional condition between non-sacculinized and sacculinized crabs including the parasite were partly confirmed in the present findings, as only a very slight decrease in energy density (1.2%) was found when the energy of the parasite sac was accounted for. However, the combined energy of host and parasite including externa in total was higher, suggesting high efficiency of the parasite allocating host energy. Yet, the energy content of the host, if isolated from the parasite, must be further reduced to an unknown extent in comparison to uninfected crabs.

Besides the impact on crabs' growth, *Sacculina* tend to feminize male hosts both morphological and behaviourally (Høeg, 1995; Mouritsen and Jensen, 2006; Lafferty and Kuris, 2009; Kristensen *et al.*, 2012). In shore crabs, sexual dimorphism is largely confined to a relatively broader abdomen and smaller master chelae in females (Shen, 1935; Elner, 1980). Several other female attributes can also be impacted by *S. carcini* infection. The externa sac is lodged in the same location of the crab's body where females carry the egg mass – no ovigerous female has ever been found sacculinized. Infected crabs (male or female) then develop a nurturing behaviour typical of an ovigerous female crab towards the parasite externa: the crab cares for the sac as if it was their own eggs (Høeg and Lützen, 1995; Thresher *et al.*, 2000). Further, the parasite takes advantage of the natural hatching process of the crab to release its larvae into the water when the externa is matured (Øksnebjerg, 2000). Infected crabs also tend to migrate to more saline and deeper areas (Rasmussen, 1959; Rainbow *et al.*, 1979; Lützen, 1984; Waser *et al.*, 2016), which favour *Sacculina* development (Kashenko and Korn, 2002), a migration pattern typical of ovigerous females (Baeta *et al.*, 2005).

As described above, *Sacculina* seems to use efficiently the energy left from the crabs' growth and maintenance. However, the comparison with the energy female crabs invest in reproduction is much more complicated. In fact, the energy density of the egg mass is much higher than the energy density of sacculinized crabs, even when the energy of the sac is accounted for, resulting in 4 times more energy in total of the egg mass in relation to the externa sac, though this may vary with externa stage of development and available data are restricted to a single month. Yet, while female crabs produce a single brood per year (Younge and Elliott, 2020) and hence the investment in reproduction is well represented in the energy density of the egg mass, *S. carcini* can produce several successive broods (Thresher *et al.*, 2000), and thus the snapshot information of the sac energy density is not enough to inform on the parasites' energy investment in reproduction, taken from the host.

Besides arresting the host growth and reproduction, and doubling the chances of mortality of crabs (Goddard *et al.*, 2005), the present findings describe the effects of *S. carcini* external infection on crabs' energetic condition, although conservatively, requiring that in future studies the extent of internal infection is disentangled. As for the implications of such energy reduction due to parasitism for the *C. maenas* population dynamics in the Dutch Wadden Sea further investigations are required.

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