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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. 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 40mm 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. 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 four times lower than total energy of egg mass. 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.

Key words: Decapoda, energy density, rhizocephalan, parasitism, Wadden Sea

Key findings

- 3.3% of the population was externally infected with *S. carcini* and 0.7% had scars of previous infection
- energy density was significantly reduced by up to 5.8% in sacculinized crabs under 40mm carapace width
- total energy content of *Sacculina* externa was about four times lower than total energy of egg mass
- *S. carcini* seems efficient in consuming energy that would be used for host growth and maintenance

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 with 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 Elliot (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 one to two 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 sterilisation" 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 2m beam trawl with a single tickler chain and a mesh size of 1 x 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 to 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 to 5th somites fused; female with a subtriangular rounded laterally 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. feminised) 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 10mm 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^{o}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

158 combusted and analysed for caloric content (cal g^{-1} DW). Subsequently, values were
159 corrected for minerals and water remains that did not account for the energy content of the
160 animal. Sample ash contents higher than 30% make it difficult to obtain a complete
161 combustion in the calorimeter (Cummins and Wuycheck, 1971). In *C. maenas*, the
162 inorganic material consists largely of calcium (Adelung, 1971), and makes up a great part
163 (about 40%) of the dry weight, affecting the calorimetric result since calcium reaction in
164 the calorimeter is endothermic (Topley, 1928). Therefore, the ash content was first
165 determined by weighting the remaining ash after burning in the calorimeter to determine
166 the calcium concentration in the samples. Then the ash was re-burned at 900ºC in a muffle
167 to remove the minerals that could not be removed during the calorimetric burning process
168 and the remaining was weighed again to determine the ash-free dry weight (AFDW). The
169 difference was used to calculate the percentage of calcium, which was then used to correct
170 the energy content as follows:

171

172 Corrected caloric content (E) = (Caloric value x 100/ % AFDW) – 1.4 x %$CaCO_3$ (cal g
173 DW^{-1})

174

175 where 1.4 cal g^{-1} is the caloric value of carbonate calcium in calorimetric reactions (Paine
176 1964). Caloric content was also determined separately for the externa and the egg mass of
177 crabs from the 2019 samples. Finally, caloric values were converted to kJoules per gram of
178 DW (kJ g DW^{-1}), hereafter E_{DW}, per gram of AFDW (kJ g $AFDW^{-1}$), hereafter E_{AF}, and
179 total energy content (E_{tot}) of each crab was obtained multiplying E_{DW} by the respective
180 DW.

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

183

184 *Data analysis*

185 Crab condition was analysed for non-sacculinized and sacculinized crabs, considering crab
186 sex and size, and comparing their mean values using Welch's t-test, a modification of the t-
187 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 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 standardised to mean zero (i.e. centred) and a standard deviation of one (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 Nakagava *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 11068 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 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 Figure 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 88.6mm, with an average of 40.9±14.0mm CW, but external infection was higher in adult crabs >25mm CW, with almost half of the sacculinized crabs ranging from 25-40mm CW (49.5% of the sacculinized crabs) and lowest in juveniles (<25mm; 7.6%) and in crabs larger than 55mm (10.3%) (Fig. 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 models (GLMM) results. The models fitted particularly well 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%). 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 (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 <40mm 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 versus 11.57±1.49kJ of non-sacculinized crabs (all 2019 crabs were <40mm 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±5.21kJ) was even higher than the E_{DW} of externa sac resulting in a mean E_{DW} of 'ovigerous females including the egg mass' (12.79±1.67kJ), 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±1.86kJ, 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±7.39kJ), 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±8.48kJ) was much higher (about 30.8% higher) than the E_{tot} of non-sacculinized crabs of similar size (14.90±6.87kJ), mean E_{tot} of the pair [ovigerous females-egg mass] was even higher (48.5% higher; 41.93±12.48kJ and 28.23±11.86kJ, 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. 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 40mm size, and in females. However, when the parasite sac was considered, the difference in energy condition between sacculinized and non-sacculinized 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-saculinized 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.

311 *Patterns of* Sacculina carcini *external infection prevalence*

312 A relatively small fraction of the crab population from the western Dutch Wadden Sea
313 (only 3.3%) was externally infected by *S. carcini*, which is in accordance with a
314 contemporaneous study in the area, reporting up to 3% prevalence of *Sacculina* infection
315 (Waser *et al.,* 2016). Prevalence of sacculinized crabs has a great spatial variation
316 (Mouritsen *et al.,* 2018). It is even lower (1.9-2.9%) in the Isfjord, Denmark (Lützen, 1984)
317 and similarly low (2.9%) in the west coast of Sweden (Werner, 2001). Yet, while in the
318 Wadden Sea, scarred crabs and modified males with no sac nor scar were nearly absent
319 (respectively 0.7% and 0.2% of the total population), in Sweden scarred crabs accounted
320 for an extra 2.6%, and uninfected modified males for an extra 2.7% of the population
321 (Werner, 2001). Such a small fraction of scarred crabs did not allow evaluating the
322 potential of recovery from infection. Elsewhere, the prevalence of *S. carcini* can range up
323 to 20%, and can locally even reach 40–80% (Lützen, 1984; Mathieson *et al.,* 1998; Torchin
324 *et al.,* 2001; Werner, 2001; Zetlmeisl *et al.,* 2011). All these estimates (including present
325 data) are conservative values, as the fraction of the immature phase of infection (interna,
326 without externa) was not quantified. Quantification of internal infection requires the
327 dissection of the crabs (Belgrad & Griffen 2015; Mouritsen et al., 2018), which is time
328 consuming and out of the scope of the present study~~i~~. Histological examination of shore
329 crabs' hepatopancreas resulted in a mean of 24% prevalence of infected shore crabs in a
330 population from South Wales (UK), though only 6.3% crabs were externally infected and
331 1.8% had scars (Rowley *et al.,* 2020). Assuming a similar fraction, infected crabs in the
332 Dutch Wadden Sea could be in the order of 12-13% of the population, internally infected
333 crabs accounted.

334 A seasonal pattern in *Sacculina* prevalence has been described in previous studies.
335 In general, prevalence peaks in winter and minimums are observed in summer (Costa *et al.,*
336 2013; Jensen *et al.,* 2019), as described here for the Dutch Wadden Sea population.
337 Therefore, increased mortality of externa has been related to the temperature rise during
338 summer (Heath 1971). Besides temperature, also salinity conditions influence the
339 prevalence of infected crabs. The lower salinity limit for maintaining a permanent
340 population of *Sacculina* seems to be around 20 (Kashenko and Korn, 2002; Mouritsen *et*
341 *al.,* 2018), which fits the range (18-29) observed in the present (Campos *et al.,* 2021) and in

342 the contemporaneous work in the area (Waser *et al.*, 2016). Yet, as in other sacculinid
343 rhizocephalan species (Walker *et al.*, 1992), *S. carcini* eggs and larvae are more sensitive
344 (Tolley *et al.*, 2006), requiring salinity >26 (Ramult, 1935), and condition the distribution
345 of infected crabs in relation to salinity (Mouritsen *et al.*, 2018).

346 The prevalence of sacculinized crabs also varies with water depth (Rainbow *et al.*,
347 1979; Waser *et al.*, 2016). The deeper subtidal waters house older crabs with the highest
348 levels of *S. carcini* (Waser *et al.*, 2016), while near none is found in the intertidal flats
349 (Waser *et al.*, 2016). Water depth itself may not be a forcing reason but the depth
350 distribution of crabs of different sizes, as older and larger crabs are typically more abundant
351 in the subtidal (Atkinson and Parsons, 1973; Hunter and Naylor, 1993). Yet, preference for
352 host size has not been documented (Werner, 2001; Waser *et al.*, 2016; Mouritsen *et al.*,
353 2018). In the present study, despite sampling took place always at the subtidal (5-12m
354 deep), *Sacculina* externally infected crabs mainly between 25 and 40mm width, while only
355 10% of the largest crabs (>55mm) were infected. This was also the size range with higher
356 prevalence of infection previously reported for the area (Waser *et al.*, 2016) and for the
357 Mondego estuary, in Portugal (Costa *et al.*, 2013). In the present work, the largest
358 sacculinized crab was a male 88.6mm CW and the smallest was a modified male 14.8mm.
359 This is smaller than previously reported (20mm, Mouritsen *et al.*, 2018) and contradicts the
360 assumption that crabs 3-16mm do not become infected, as revealed by molecular analyses
361 (Mouritsen *et al.*, 2018). When feminized males were accounted, prevalence of male
362 external infection doubled the prevalence of sacculinized female crabs. Yet, preference for
363 host gender has not been reported (Werner, 2001; Costa *et al.*, 2013; Waser *et al.*, 2016),
364 with some populations revealing more sacculinized males (e.g. Isefjord, Denmark, Lützen,
365 1984) and others more females (e.g. Mondego estuary, Portugal, Costa *et al.*, 2013; Danish
366 coast, Mouritsen *et al.*, 2018; Scotland, Mathieson *et al.*, 1998).

367 The developmental stages of *S. carcini* externa seem correlated with the crab host
368 colour cycle such that the youngest externa stages are predominant when the crabs are
369 green, the intermediate stages when they are orange, and the oldest when they are red
370 (Lützen *et al.*, 2018). In the present study, the externa development stages were not
371 evaluated, but external infection was more often found in green morphs, suggesting then
372 that most parasites' externa would be on an early stage. Literature, however, refers that red

crabs are more commonly infected than green ones (Zetlmeisl, 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 Soderhall, 2021), including the broadening of the abdomen in male crabs (Reinhard, 1956), a feminised 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, 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 four 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 is restricted to a single month. Yet, while female crabs produce a single brood per year (Younge and Elliot, 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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Author contribution

HWV, JC and ATS conceived and designed the study; FR conducted the data gathering; AB performed statistical analyses and graphical work; JC and VF wrote the article; HWV, ATS and FR reviewed and edited the manuscript.

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Conflicts of Interest

The authors declare there are no conflicts of interest.

Ethical Standards

Not applicable.

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651 **Caption of figures and tables**

652 Figure 1. Map of the sampling locations in the western Dutch Wadden Sea. 1, Gat van der
653 Stier (N 52º57.27´ E 4º55.73´); 2, Texelstroom (N 53º02.03´ E 5º03.37´); and 3,
654 Kornwerderzand (N 53º04.52´ E 5º16.55´); top left: sampling area in the Netherlands
655 (Adapted from Katwijk and Hermus, 2000).

656 Figure 2. Percentual distribution of the abundance of sacculinized crabs according to sex
657 (F: females, M: males, MM: modified males), colour morphotype (G: green, R: red), size
658 class (Juv, juveniles: ≤25 mm CW; A1: 25-40mm CW; A2: 40-55mm CW; A3: >55mm
659 CW) and seasons.

660 Figure 3. Size distribution of sacculinized and non-sacculinized crabs per sex (F: females,
661 upper panel; and M: males and MM: modified males, lower panel). Juv, juveniles: ≤25 mm
662 CW; A1: 25-40mm CW; A2: 40-55mm CW; A3: >55mm CW. Note that MM non-
663 sacculinized crabs represented only 0.24% of the sampled population.

664 Figure 4. Mean Fulton's *K* and error bars (as standard deviation) for sacculinized (dark
665 grey) and non-sacculinized (light grey) crabs per sex (F: females; M: males) and modified
666 males (MM) together with number of crabs between brackets (upper panel); and Fulton's *K*
667 in relation to size (CW, mm) (lower panel). Sacculinized crabs in dark grey circles; non-
668 sacculinized crabs in dark grey squares.

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

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

678 Figure 7. Total energy (E_{tot}, kJ) in relation to size (CW, mm) for sacculinized and non-
679 sacculinized crabs, for the externa sac, for the egg mass, and for the pairs [sacculinized
680 crab-parasite including externa] and [ovigerous females-egg mass].

681 Table 1. Number of sacculinized *Carcinus maenas* analysed for energy condition per sex
682 and colour and percentage (%) of total sacculinized sample.

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

687

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

691

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

692

693

694 Table 2. Linear mixed effects model results, with predictor estimate and estimate error, t-
695 value, significance (n.s. non significant, *p<0.05, **p<0.01, ***p<0.001) and the
696 proportion of variance explained by the fixed effects (marginal R^2) and by the entire model
697 (conditional R^2).
698

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	***		

Graphical Abstract

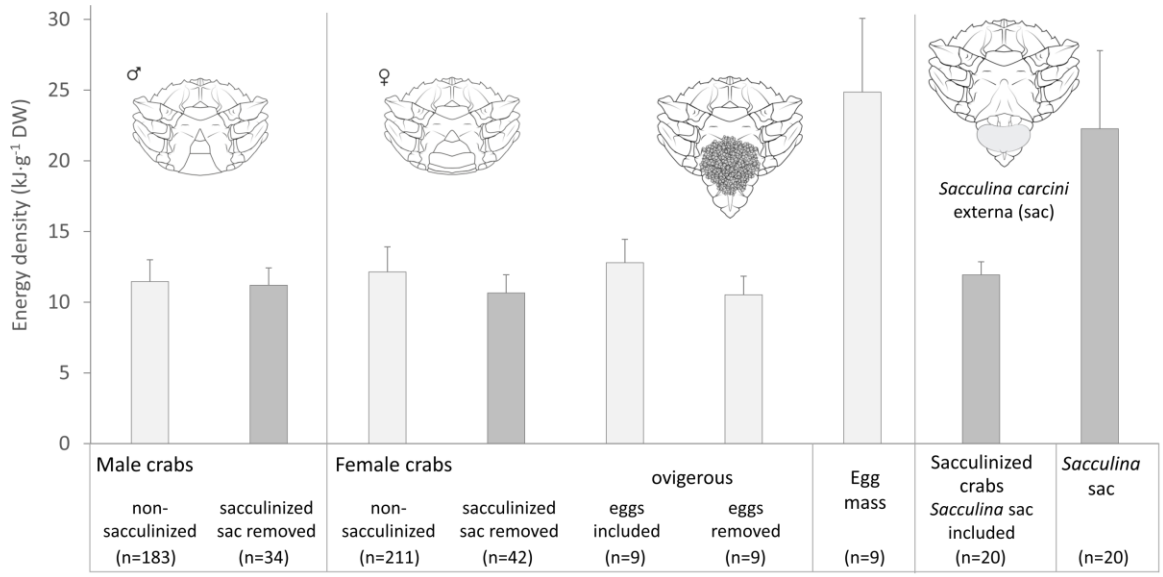


Fig. 1

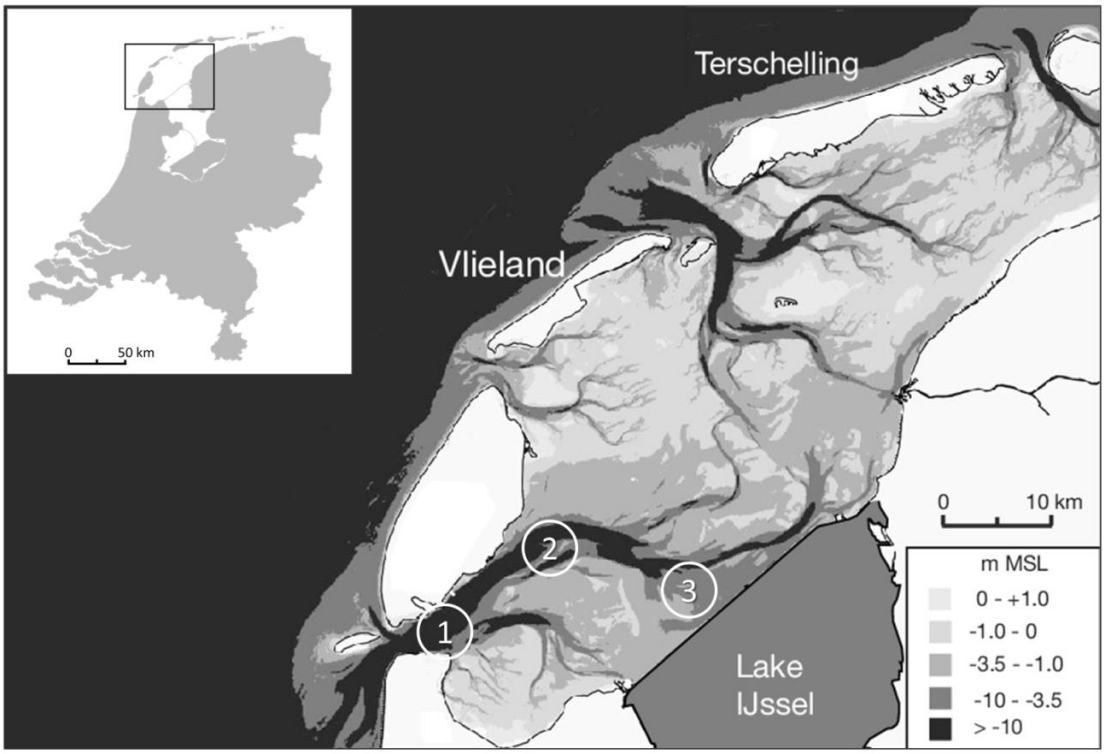


Fig. 2

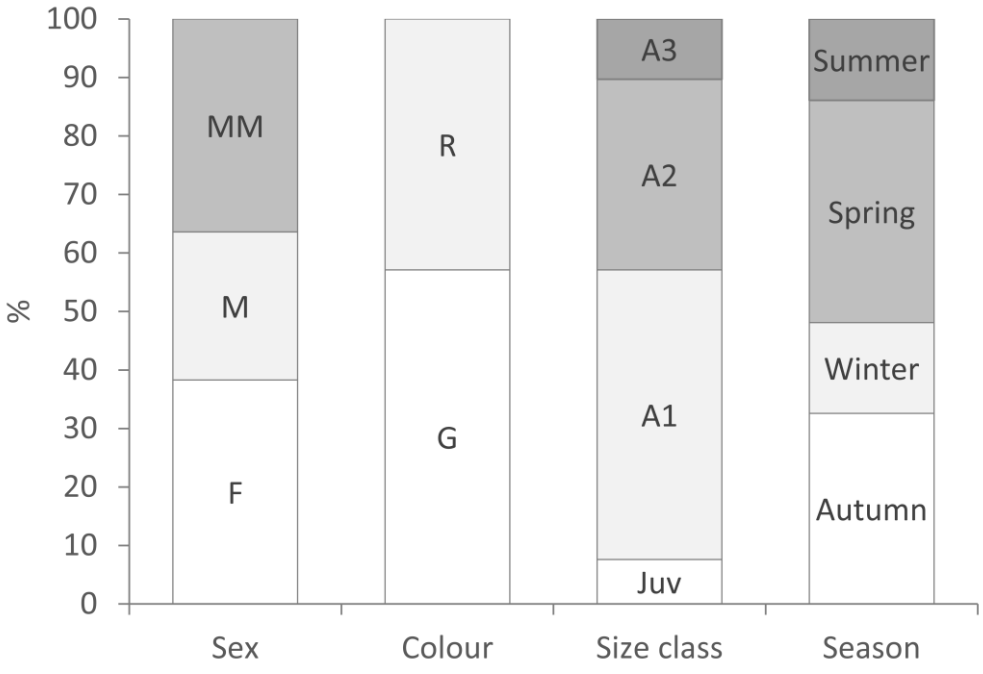


Fig. 2

Fig. 3

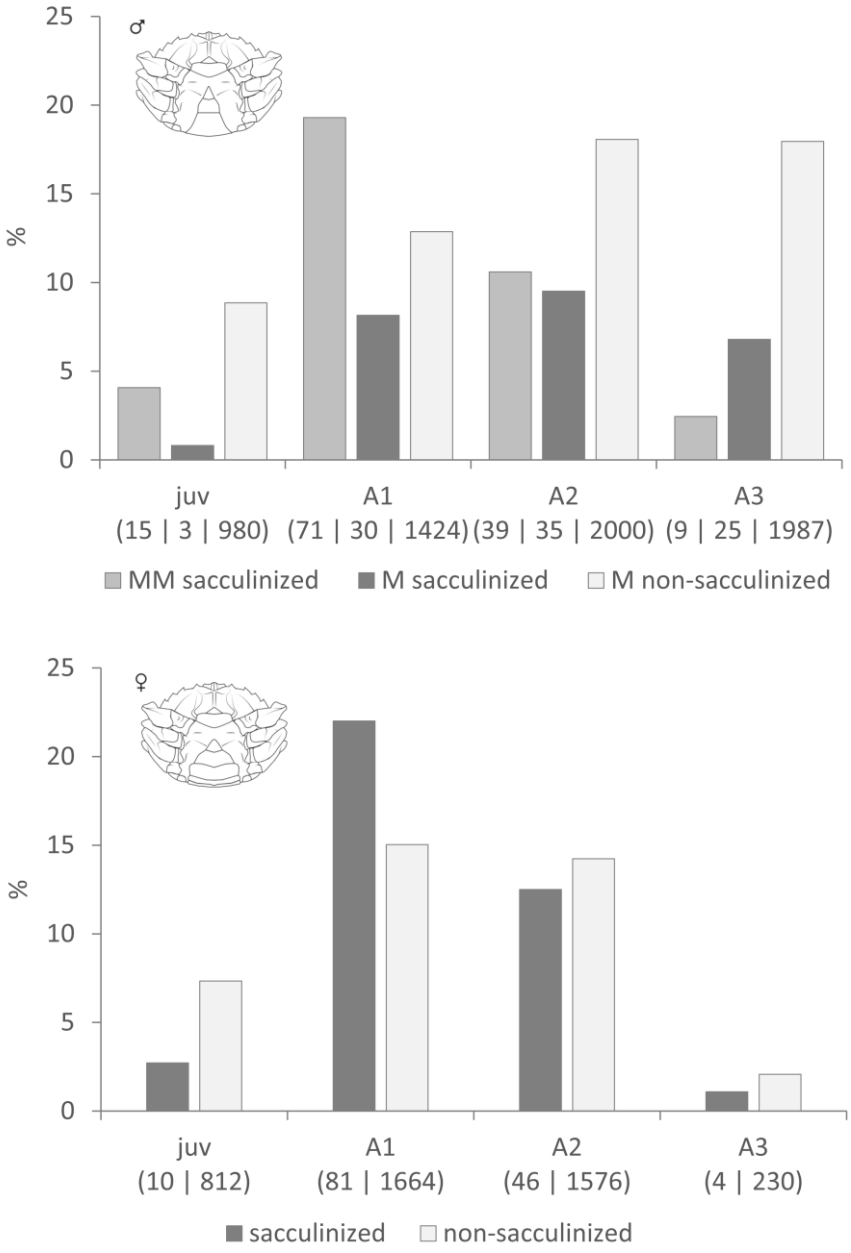

♂
25
20
15
10
5
0
%
juv
(15 | 3 | 980)
A1
(71 | 30 | 1424)
A2
(39 | 35 | 2000)
A3
(9 | 25 | 1987)
MM sacculinized
M sacculinized
M non-sacculinized
♀
25
20
15
10
5
0
%
juv
(10 | 812)
A1
(81 | 1664)
A2
(46 | 1576)
A3
(4 | 230)
sacculinized
non-sacculinized

Fig. 4

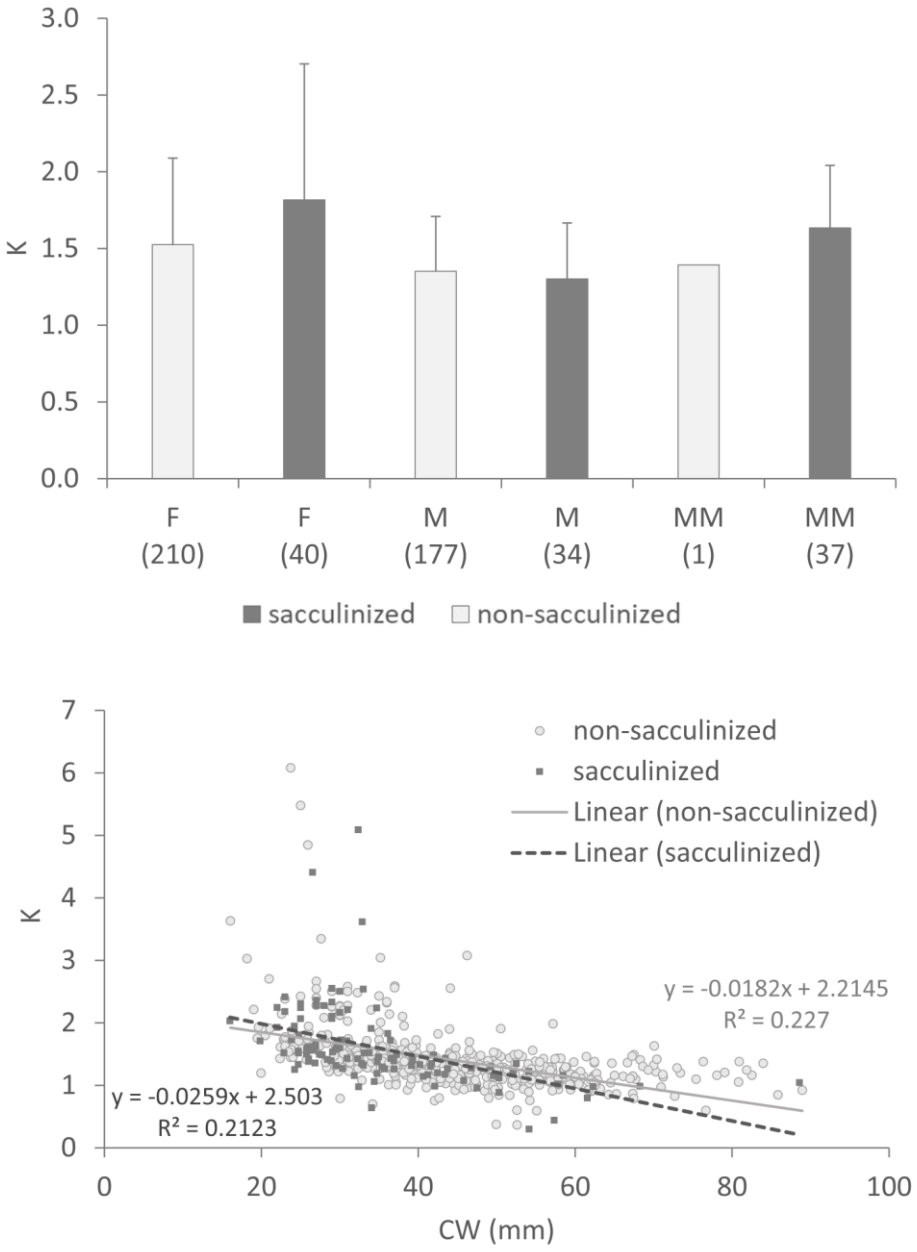

3.0
2.5
2.0
1.5
1.0
0.5
0.0
K
F (210)
F (40)
M (177)
M (34)
MM (1)
MM (37)
sacculinized
non-sacculinized
7
6
5
4
3
2
1
0
K
0
20
40
60
80
100
CW (mm)
non-sacculinized
sacculinized
Linear (non-sacculinized)
Linear (sacculinized)
y = -0.0182x + 2.2145
R² = 0.227
y = -0.0259x + 2.503
R² = 0.2123

Fig. 5

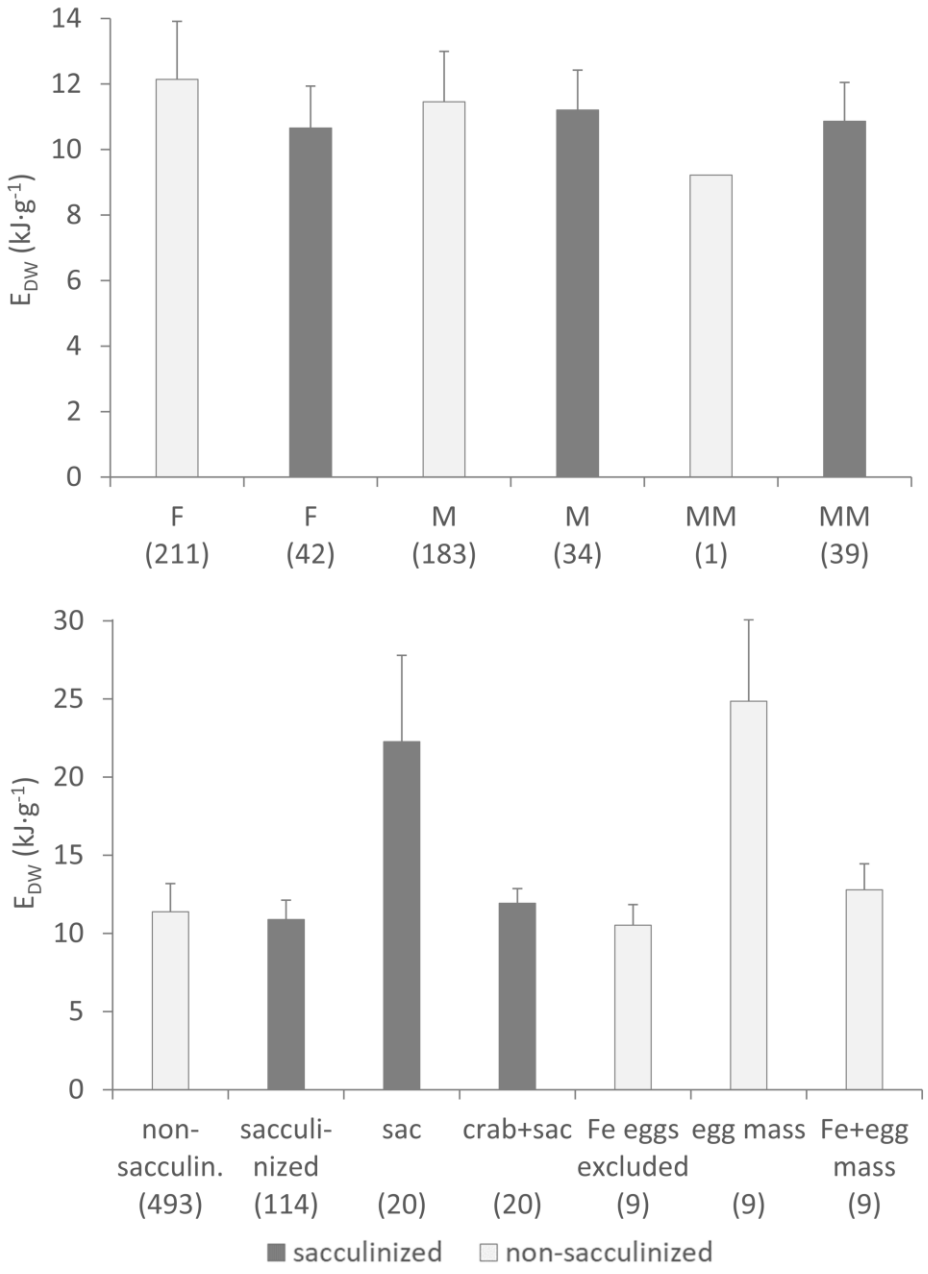

14
12
10
8
6
4
2
0
E_{DW} (kJ·g^{-1})
F (211)
F (42)
M (183)
M (34)
MM (1)
MM (39)
30
25
20
15
10
5
0
E_{DW} (kJ·g^{-1})
non-sacculin. (493)
sacculi-nized (114)
sac (20)
crab+sac (20)
Fe eggs excluded (9)
egg mass (9)
Fe+egg mass (9)
sacculinized
non-sacculinized

Fig. 6

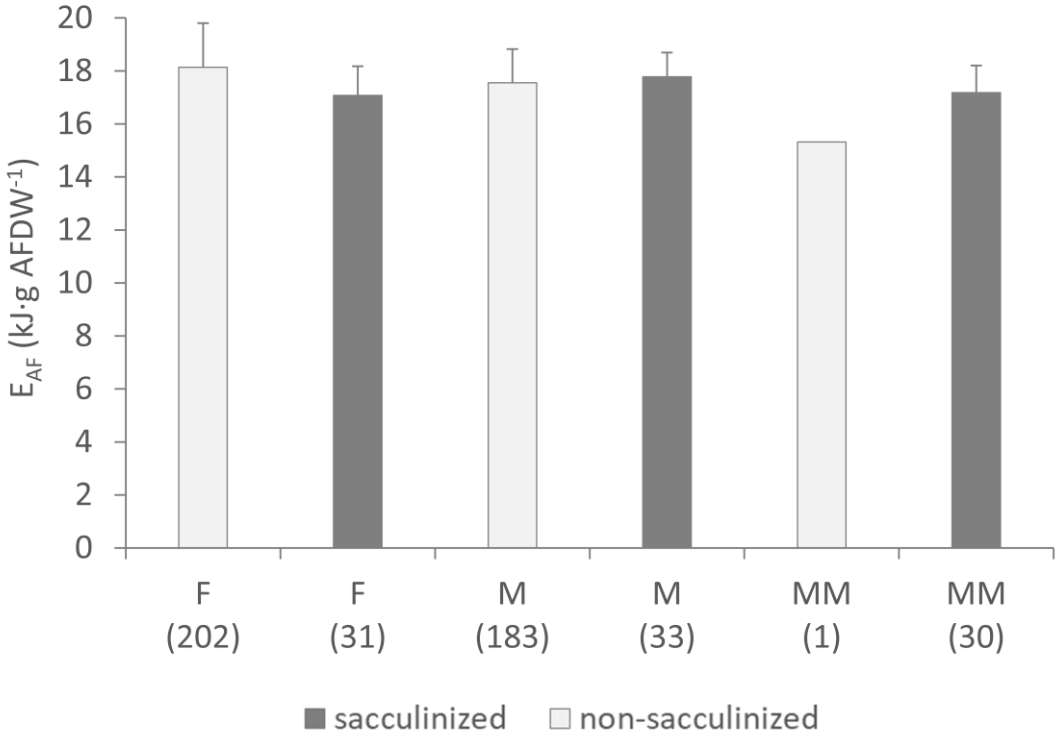
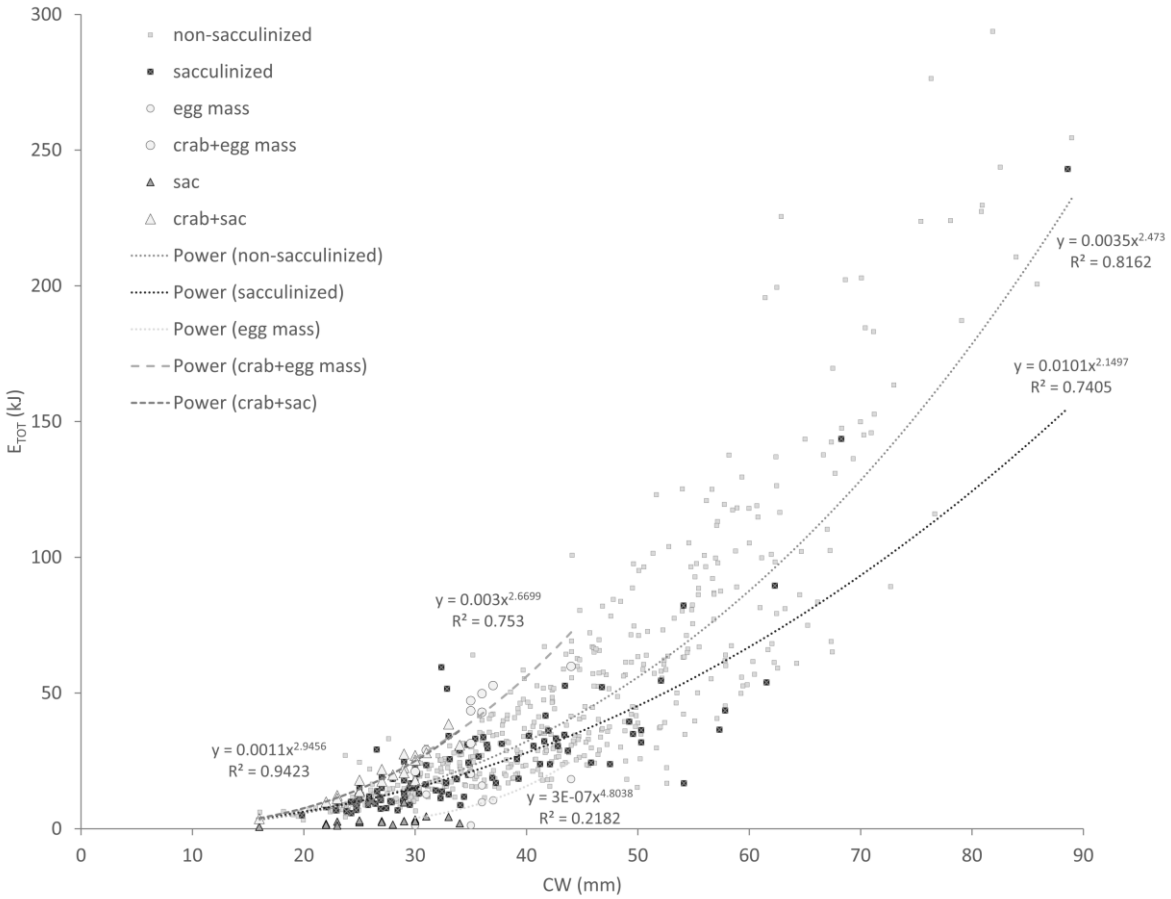

20
18
16
14
12
10
8
6
4
2
0
E_{AF} (kJ·g AFDW^{-1})
F (202)
F (31)
M (183)
M (33)
MM (1)
MM (30)
sacculinized
non-sacculinized

Fig. 7


non-sacculinized
sacculinized
egg mass
crab+egg mass
sac
crab+sac
Power (non-sacculinized)
Power (sacculinized)
Power (egg mass)
Power (crab+egg mass)
Power (crab+sac)
y = 0.0035x^2.473
R² = 0.8162
y = 0.0101x^2.1497
R² = 0.7405
y = 0.003x^2.6699
R² = 0.753
y = 0.0011x^2.9456
R² = 0.9423
y = 3E-07x^4.8038
R² = 0.2182
E_TOT (kJ)
CW (mm)
0
50
100
150
200
250
300
0
10
20
30
40
50
60
70
80
90