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Body condition and energy content of the shore crab *Carcinus maenas* L. in a temperate coastal system: the cost of barnacle epibiosis

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Running title: Epibiosis cost on *Carcinus maenas* energy density

Abstract

The impact of barnacle epibionts on the condition of the shore crab *Carcinus maenas* was studied for the western Wadden Sea population. Approximately 39% of the crabs were fouled with the barnacle *Balanus crenatus*. Although the morphological Fulton's *K* condition decreased by 5.8% in fouled crabs, Linear Mixed-Effects Models (LMM) showed that only the energetic condition of the crabs was significantly affected by fouling. The energy density of fouled crabs was consistently poorer (4.1% in AFDW; 8.7% in dry weight) than that of non-fouled crabs, especially in females and green forms in dry weight (12.8 and 11.4% reduction, respectively). Cumulative infection with *Sacculina carcini*, detected in 4.5% of the fouled crabs, additionally reduced by 14.3% the energy density in dry weight and almost to half of the total energy of the fouled crabs. Impacts of energy density reduction on crabs' growth and reproduction are discussed.

Key words: *Carcinus maenas*, fouling, energy density, fitness, *Balanus crenatus*, Wadden Sea

Introduction

Epibiosis is a non-symbiotic, facultative association of organisms in which benthic invertebrates (epibionts) attach to the living substrate of hosts (basibionts) during a sessile life stage (Wahl 1989). This association can have advantages and disadvantages for both epibionts and basibionts, depending greatly on the context (for a review see Wahl 1989). Benefits for epibionts include increased dispersal, increased nutrient availability, and protection from predators, whereas epibiosis may be advantageous for basibionts, providing mimetic protection and cleansing (Fernandez-Leborans 2010). Nevertheless, epibiosis may imply more costs than benefits for basibionts, from increased weight, mobility limitations, increased competition for nutrients/prey, and increased predation risk to the impaired moulting, growth, mating, and functioning of several organs (Wahl 1989, 2008; Fernandez-Leborans 2010). Ultimately, all of these effects imply additional energy costs (Overstreet 1983; Dick et al. 1998) and reduce host fitness (Wahl et al. 1997; Wahl 2008; Fernandez-Leborans 2010). However, there is limited information on the effects of epibiosis on the condition of the basiobiont, particularly on its energy density.

The calcified carapace of crustaceans is a suitable substrate for colonization by a wide variety of epibiotic organisms (Gili et al. 1993; Savoie et al. 2007; Fernandez-Leborans 2010), although it is only available for a relatively short time due to moulting of the basibiont host (Abelló & Corbera 1996). The shore crab *Carcinus maenas* is often colonized by a variety of epibionts, including barnacles, hydrozoans, bryozoans, bivalves, algae, and tube-forming polychaetes (Abelló et al. 1997). Although native to the northeastern Atlantic (Crothers 1968), this epi-benthic decapod has a high invasive character worldwide (Cohen et al. 1995; Darling et al. 2008). European populations of the shore crab are consistently abundant, reflecting high reproductive success and physiological plasticity: Adult crabs can withstand salinities from 4 to 52‰ (Cohen & Carlton 1995), have a high thermal tolerance (Cohen & Carlton 1995; Freitas et al. 2007), high behavioural plasticity (Souza et al. 2019), and are voracious opportunistic omnivores with an extensive list of prey species (eg, Cohen et al. 1995; Baeta et al. 2006).

Barnacles are the most conspicuous epibiont taxa in *C. maenas* from European populations, with a prevalence of up to 40% in certain North Sea areas (Zetlmeisl 2001). The additional weight of heavy epibionts can limit crab mobility, which reduces foraging efficiency, and is associated with high metabolic costs (Dick et al. 1998). As a result, deterioration of the general condition of the crab host can be expected. The barnacle-colonized shore crab is therefore an excellent candidate for studying the effects of epibiosis on the energy density of the epibenthic host.

This study is the second in a series of 3 papers investigating the factors causing variation in the body condition of the shore crab in Europe's largest coastal wetland system, the western Dutch Wadden Sea. Seasonal variation in body condition of crabs was described and linked to variation in environmental conditions, such as thermal and prey availability patterns, and consistent with crab growth and reproductive patterns (Campos et al. 2021). The effects of *Sacculina carcini* infection on crab body condition were investigated in a separate study (Campos et al. 2022). The present paper aims to evaluate the effects of epibiosis by barnacles on the general condition of the shore crab *C. maenas*, using both morphometric, physiological and biochemical information.

Materials and methods

Sampling and laboratory procedures

From August 2012 to March 2014 (with the exception of September 2012 and 2013, October 2013, January and February 2014), a monthly sampling programme was conducted at three sites in the western Dutch Wadden Sea (Figure 1). Crabs were collected with a 2 m beam trawl with a single tickler chain (1 cm mesh size) towed by boat (sampling details in Campos et al. (2021)). Water temperature and salinity were recorded at each site.

Carapace width (CW) of sampled crabs was measured to the nearest mm with a digital calliper. Sex determination was based on the sexual dimorphism characteristics described in Squires (1990) (males: triangular abdomen, 3rd to 5th somites fused; females: sub-triangular, laterally rounded abdomen, somites not fused). Each individual was assigned into two colour morphotypes, green or red, based on the predominant colour of the thoracic sternum (McKnight et al. 2000), and examined for reproductive condition (berried females) and epibiont growth. The presence of external parasites such as *Sacculina carcini*; Sacculinized crabs (i.e., crabs with a *S. carcini* externa) without epibiont barnacles were excluded from further analysis. For subsequent morphometric and calorimetric analyses, approximately 10 crabs of each sex per size class (10 mm) were randomly selected. The wet weight (WW) of each crab was recorded to the nearest 0.0001 g, and for fouled crabs (crabs were considered fouled when barnacles comprised more than 5% of the total weight or if more than 10 individual barnacles colonized the carapace), weight was recorded after removal of all epibionts by scraping the carapace surface. All crabs were eviscerated and weighed immediately thereafter, to avoid mixing of stomach contents during the calorimetric analysis. Samples (crab whole body excluding stomach) were dried to a constant dry weight (DW, g) (10 days, at 60°C) to determine the dry weight condition (percentage of dry weight, %DW). The Fulton's condition index (*K*) of each crab was determined by dividing the WW by the cube of CW.

Calorimetry

Energy density was determined using a IKA C2000 Calorimeter. After maceration of each dried crab, the powder sample was pressed into a pellet in a mortar, and transferred to the calorimeter, where it was burned and analysed for caloric content (cal.g^{-1} DW). The resulting ash was weighed. Complete combustion of samples with ash content greater than 30% is not guaranteed (Cummins & Wuycheck 1971). In *Carcinus maenas,* the inorganic material is largely calcium (Adelung 1971), and constitutes about 40% of the dry weight, which affects the calorimetric result because the reaction of calcuim in the calorimeter is endothermic (Topley 1928). Therefore, the ash was reburned at 900°C in a muffle furnace to remove the minerals that could not be removed during calorimetric burning, and the remaining ash was reweighed to determine the ash-free dry weight (AFDW). The difference was used to calculate the percent of calcium, which was then used to correct the energy content, using 1.4 cal.g^{-1} as the caloric value of calcium carbonate in calorimetric reactions (Paine 1964). Finally, the caloric values were converted to kJoules per gram of DW (hereafter E_{DW}, kJ.g^{-1} DW) and per gram of AFDW (hereafter E_{AF}, kJ.g^{-1} AFDW), and the total energy content (E_{tot}, kJ) of each crab was determined by multiplying E_{DW} by the respective DW.

A total of 629 crabs were analysed for energy content, of which 124 crabs were colonized by the barnacle *Balanus crenatus* and 36 of them were infected with *Sacculina carcini* (Table 1).

Data analysis

Fulton's condition (*K*), energy density (E_{DW}, E_{AF}) and total energy (E_{tot}) of shore crabs were analysed using three data sets: (1) non-fouled, healthy crabs, i.e., crabs with no or few barnacles and no *Sacculina* infection; (2) fouled crabs, with no *Sacculina* infection; and (3) fouled and *Sacculina* infected crabs.

Comparisons of crab condition and energy between groups defined by their sex/reproductive status and fouling/infection levels were based on Welch's t-test, which adjusts the number of degrees of freedom when variances are not expected to be equal and performs better than Student's *t*-test when sample sizes and variances are unequal between groups, as is the case here, and yields the same result when sample sizes and variances are equal (Delacre et al. 2017). The significance level was set at alpha = 0.05.

Linear mixed effects models (LMM) were applied to evaluate the effects of fouling and fouling+infection on crab condition using crab status (healthy, fouled, fouled and infected), sex, and size as fixed effects. To control for temporal and spatial variability in the data, which were considered noise in our data, sampling season and location were included in the model as random effects, which provided better estimates for the fixed effects. Given the observed temperature patterns, April to June were defined as spring, July and August as summer, October and November as autumn and December to March as winter. Crossed LMM 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 standard deviation of one (i.e., scaled) to ensure that the estimated coefficients were all on the same scale to allow comparison of effects. The explained variance of the model was obtained by calculating the marginal R^2, which is the variance explained by the fixed effects, and the conditional R^2, which is interpreted as the variance explained by the entire model, including fixed and random effects (Nakagava et al. 2017), using the R package stargazer (Hlavac 2022).

Crab colour was not included in the model because this variable was found to be of little importance for crab condition (Campos et al. 2021), and was also a confounding variable that is related to the animal's size (i.e., larger crabs tend to be predominantly from the red colour morphotype).

All data processing and statistical analyses were performed in R (R Core Team 2019).

Results

Patterns of barnacle epibiosis

A total of 11068 shore crabs were collected and analysed for general biometrics, of which 40.8% were fouled. The barnacle *B. crenatus* was by far the most prevalent epibiont (95.1%), with the remaining 4.9% of epibionts corresponding to *Elminius modestus* (2.2%), seaweeds (1.7%), sea squirts (0.5%), the american slipper limpet *Crepidula fornicata* and sea anemones (0.25% each). The intensity of epibiosis ranged from a single to 159 epibiont barnacle per crab; about 90% of the fouled crabs had up to 42 individual barnacles, with a mean of 16.6 ± 19.4 barnacles per crab. The present results refer to epibiosis by *B. crenatus* on shore crabs, crabs covered with other epibiont species were not analysed.

Only 31.5% of all green morphs were fouled, whereas barnacle epibiosis was observed in 72.1% of all red morphs (Figure 2). The red morphs also had significantly more individual barnacles per crab, with an average of 23.3 ± 23.4 barnacles (maximum of 159) compared to 13.3 ± 16.1 barnacles (maximum of 136) for the green morphs (Chi-square: $p<0.0001$).

Most fouled crabs were males (Figure 2). About 8.9% of the fouled crabs were ovigerous females (69.4% of all ovigerous females were fouled). The size of fouled crabs ranged from 10.9 to 97.1 mm, with an average CW of 48.2 ± 14.3 mm, but the barnacle prevalence differed with size (Figure 2). Larger crabs tended to have a greater maximum number of epibiotic barnacles than smaller crabs, corresponding to approximately 2.3 barnacles per mm of CW. Most fouled crabs were collected in Texelstroom, followed by Kornwerderzand and Gat van de Stier. However, considering the local abundance of crabs at each site, fouled crabs accounted for 45.1% of the crabs in Gat van de Stier, 43.0% of crabs in Texelstroom and 31.7% of crabs in Kornwerderzand (Chi-square: $p<0.0001$). The abundance of fouled crabs was highest in spring, followed by summer, autumn and lowest winter (Figure 2).

About 4.5% of the crabs fouled with barnacles were also infected with *Sacculina carcini* (1.75% of the total crab population); fouled crabs accounted for 57.4% of the sacculinized crabs. The frequency of fouled and sacculinized crabs was higher in Gat van de Stier (2.6% of the local population) and Texelstroom (2.3% of the local population) and negligible in Kornwerderzand (0.8%) (Chi-square: $p<0.0001$).

Model results

The results of the linear mixed-effects models (LMM) are shown in Table 2. The models fit the Fulton's K and E_{tot} condition indices well ($R^2>70\%$), and the E_{DW}, E_{AF} and %DW poorly ($R^2<35\%$). The effect of barnacle fouling and the cumulative effect of barnacle fouling with *Sacculina* infection were both found to be significant only for E_{DW}. While size had a significant effect on Fulton's K and E_{tot}, sex was not a significant predictor of variance for the five condition indices tested.

Effect of Balanus crenatus *epibiosis on crab condition*

Figure 3 shows the results of condition indices for non-fouled and fouled crabs, and for fouled crabs that were also infected with *Sacculina*.

Although epibiosis was not significant in the LMM of morphometric indices, Fulton's K was reduced by approximately 5.8% in fouled crabs ($K = 1.42 \pm 0.52$ and 1.33 ± 0.29, respectively in non-fouled and fouled crabs; $p < 0.05$). The reduction in Fulton's K was even greater in ovigerous females (11.9%: $K = 1.30 \pm 0.61$ and 1.15 ± 0.16, respectively in non-fouled and fouled crabs; $p < 0.05$) and in green morphs (15.5%: $K = 1.41 \pm 0.48$ and 1.20 ± 0.21, respectively in non-fouled and fouled crabs; $p < 0.001$). However, the cumulative effect of *Sacculina* infection resulted in a non-significant change in Fulton's *K*. The %DW was not significantly affected by epibiosis ($p = 0.37$) but increased significantly by 4.9% with cumulative infection ($p < 0.01$): %DW = 32.8 ± 4.58, 33.35 ± 4.78 and 34.5 ± 3.06, respectively for non-fouled, fouled, and fouled and infected crabs.

Both energy density indices significantly decreased by 4.1% in E_{AF} ($E_{AF} = 17.56 \pm 1.53$ and 16.84 ± 1.34 KJ.g^{-1}AFDW, respectively in non-fouled and in fouled crabs; $p < 0.001$) and by 8.7% in E_{DW} ($E_{DW} = 11.39 \pm 1.81$ and 10.40 ± 1.60 KJ.g^{-1}DW, respectively in non-fouled and in fouled crabs; $p < 0.001$). The reduction in energy density was greater in females (6.2% reduction in E_{AF}: $E_{AF} =18.14 \pm 1.66$ and 17.01 ± 1.62, respectively in non-fouled and in fouled crabs; $p < 0.001$; and 12.8% reduction in E_{DW}: $E_{DW} =12.14 \pm 1.77$ and 10.59 ± 1.80, respectively in non-fouled and fouled crabs; $p < 0.001$) and in green morphs (7.6% reduction in E_{AF}: $E_{AF} =17.91 \pm 1.41$ and 16.54 ± 1.95, respectively in non-fouled and in fouled crabs; $p < 0.01$; and 11.4% reduction in E_{DW}: $E_{DW} =11.92 \pm 1.62$ and 10.56 ± 1.61, respectively in non-fouled and in fouled crabs; $p < 0.01$). Cumulative infection with *Sacculina* further decreased energy condition by 14.3% in E_{DW} ($E_{DW} =9.76 \pm 1.48$; $p < 0.001$), although E_{AF} was not further affected.

According to LMM models, trends in E_{tot} responded positively to CW, i.e., larger crabs (larger CW) had higher E_{tot}, and similarly, larger fouled crabs (and larger fouled and infected crabs) had higher E_{tot} (Figure 4). However, only the effect of epibiosis combined with *Sacculina* infection was significant ($p < 0.001$), resulting in a decrease to almost half of the E_{tot} (47.3% less E_{tot}: $E_{tot} = 52.36 \pm 49.63$, and 27.59 ± 26.67 for non-fouled and fouled crabs with *Sacculina* infection, respectively).

Discussion

Patterns of Balanus crenatus *epibiosis*

Although epibiosis is widespread among *C. maenas* populations throughout Europe (Crothers 1967; Zetlmeisl 2001; Zetlmeisl et al. 2011) and in invaded areas (Young et al. 2017), few studies have examined the incidence patterns in natural populations (Heath 1976; Wolf 1998; Zetlmeisl 2001). Epibiosis can be absent (Zetlmeisl 2001) or affect 40% (Zetlmeisl 2001; Zetlmeisl et al. 2011; present study) to nearly half of the crab population, as found in North Wales (Heath 1976) and in SW Great Britain (Crothers 1967). In these cases, barnacles make up the majority of the epibiont species.

Similarly to the North Wales (Heat 1976) and Danish populations (Lutzen et al. 2008), in the Dutch Wadden Sea, the native barnacle *B. crenatus* was by far the most abundant epibiont, colonizing 39% of the crabs. Nevertheless, the intensity of epibiosis (i.e., the average number of epibionts per basibiont) was three times higher in the Wadden Sea (16.6 ± 19.4 barnacles per crab) than in North Wales (5.17 barnacles per crab). The potential impact must therefore be greater for crabs from the Wadden Sea population. Nevertheless, some of the available studies reporting epibiosis on shore crabs were conducted decades ago, requiring a re-evaluation of present situation as epibiosis intensity may have changed.

Most epibiont species do not settle exclusively on one host species or exhibit obligate epibiosis (Wahl & Mark 1999; Leonard et al. 2007; Fernandez-Leborans 2010). In the Wadden Sea, epibiosis by *B. crenatus* also occurs on the blue mussel *Mytilus edulis*, albeit to a lesser extent (10% of subtidal mussels, Buschbaum & Saier 2001), and on the periwinkle *Littorina littorea*, where it can affect 86% of the population (Buschbaum & Reise 1999). Barnacles *B. crenatus* are also the most common epibionts found on the cephalothorax of the spider crab *Maja squinado* in Spanish waters (Parapar et al. 1997), in *Cancer* spp. from British Columbia (*C. gracilis*, 42%; *C. magister*, 64%; and *C. productus*, 79%; McGaw 2006), and in the red king crab *Paralithodes camtschaticus* (43%, Dvoretsky & Dvoretsky 2009). Elsewhere, other hard-shelled organisms may harbour *B. crenatus* epibionts (eg, Barnes & Bagenal 1951; Dick et al. 1998; Giri & Wicksten 2001; Savoie et al. 2007; Fernandez-Leborans & Gabilondo 2008; Dvoretsky & Dvoretsky 2022) to an unknown extent.

The extent of epibiosis in crustaceans is influenced by several factors (Wahl & Lafargue 1990), including biotic conditions associated with the basibiont: its size, frequency and stage of moutling, duration of intermoult (period between two successive moults or ecdysis), reproductive stage and parasitism, and efficiency of antifouling defences (Barnes & Bagenail 1951; Davis & White 1994). Larger basibiont crabs tend to be more fouled (Heath 1976; Key et al. 1999) because they are a larger target for settling larvae (McGaw 2006; Dvoretsky & Dvoretsky 2009) and also because they moult less frequently (Scrocco & Fabianek 1969). In the Wadden Sea population, prevalence and intensity of epibiosis was higher in adults greater than 40 mm in width. This may simply be due to the fact that large (and older) crabs have a greater surface area available for colonization, and may explain why fouled males, which are larger on average, were more abundant than fouled females – as also observed in *Cancer* spp. (McGaw 2006). While young crabs may moult multiple times a year, moults become less frequent with age (McGaw 2006), to about once a year when approaching sexual maturity (Crothers 1967). Because ecdysis is an efficient way to shed the epibiont load (Wahl

1989), epibiont colonization may not be as successful in juveniles (Abelló et al. 1994; Stanski et al. 2018), justifying the relatively low frequency of epibiosis observed in juvenile crabs in the present study – as well as found in *Cancer* spp. (McGaw 2006). However, it remains controversial whether recently moulted crabs can be immediately colonized by very small barnacles (McGaw 2006), because larvae are attracted to arthropodin, which has the highest concentrations in newly moulted crabs (Crisp 1974), or whether they are not colonized because recently moulted crabs still lack the bacterial film necessary for larval settlement (Gili et al. 1993).

The longer the period between two successive moults of the basibiont, the longer the skeleton surface is available for colonization, and the higher is the likelihood of successful epibiotic settlement on the carapace (McGaw et al. 1992). Prolonged intermoult results then in a higher incidence of epibiosis (Wahl 1989; McGaw et al. 1992). While the likelihood of epibiont settlement should be similar across colour forms (Reid et al. 1997), prolonged intermoult may account for the greater epibiont burden commonly reported in red morphs (eg, Crothers 1968; McGaw et al. 1992; Wolf 1998; Zetlmeisl et al. 2011; Young et al. 2017), and confirmed in the present study (72% of all red crabs versus 32% of all green morphs had barnacles): red crabs are associated to a reproductive stage (Abelló et al. 1997; Reid et al. 1997; Styrishave et al. 2004), and therefore are under a prolonged intermoult (McGaw et al. 1992; Wolf 1998; Young et al. 2017). Similarly, ovigerous females are in a prolonged intermoult until spawning (Abelló et al. 1994; Abelló & Corbera 1996), and had a high incidence of barnacle epibiosis in the present study (69% of the ovigerous females had barnacles), similar to other crustaceans (Abelló et al. 1990; Firstater et al. 2009). Finally, the higher incidence of barnacles in sacculinized crabs (57% of infected crabs had barnacles) than in uninfected ones, which has also been reported in other crustaceans (Abelló & Corbera 1996), may be due to prolonged intermoult enforced by the rhyzocephalan parasite, as *Sacculina carcini* inhibits moulting of its host (Abelló & Corbera 1996). In Danish waters, epibiosis incidence was even higher in sacculinized crabs (75% of sacculinized crabs had multiple epibionts versus 29% of the uninfected crabs), although the intensity of barnacle epibiosis (7.7 barnacles per crab) (Mouritsen & Jensen 2006) was almost half that of the present study. Moreover, the burying response of sacculinized crabs is reduced by more than half, making the crabs more exposed and susceptible to epibiosis. Such an effect on behaviour has also been demonstrated in another portunid crab, *Charybdis longicollis*, infected with the rhizocephalan *Heterosaccus dollfusi* (Innocenti et al. 1998).

Most red morphs are larger than 35mm, and tend to dominate in the subtidal (McGaw et al. 1992), where exposure to barnacle colonization is probably higher than in the intertidal, where green morphs dominate. Red morphs are also less tolerant to environmental stress and less efficient osmorregulators, avoiding low salinity waters (McGaw & Naylor 1992; Abelló et al. 1997; Lee et al. 2003; Baeta et al. 2005) which favour an increase in grooming behaviour. The thicker carapace of red crabs (McGaw et al. 1992; Reid et al. 1997; McKnight et al. 2000; Souza et al. 2011) may be a better attachment surface for barnacle larvae than the thinner integument of green morphs. In contrast, green morphs are in an active growth phase, with short moulting cycles to maximize growth (Wolf 1998). Carapace thickness vary with other factors such as the population density, which in turn can affect the frequency of dyadic disputes among crabs and affect the duration of intermoult stages (Souza et al. 2011).

Barnacle cyprid larvae are gregarious and tend to settle on surfaces where other barnacles or their remains are already present (Miron et al. 1996; Anil et al. 2012). Therefore, crabs under a prolonged intermoult or in terminal ecdysis are more likely to maintain older barnacle generations and attract new ones. The frequency of epibiosis is probably directly proportional to the time elapsed since the last moult (Abelló et al. 1994), because barnacles prefer to settle on crabs during the intermoult stage (Kaiser et al. 1990, 1993). The abundance of epibionts may then be useful in determining the presence of a terminal moult of the host (Fernandez-Leborans 2010), i.e., a state in which animals no longer moult (Carlisle 1957), with near complete coverage by sessile epibionts in a Brachyuran basibiont suggesting a terminal anecdysis (Abelló et al. 1990). In the present study, the percentage of carapace cover by barnacles was not determined. However, large shore crabs, especially the red forms, are known to enter a state of terminal anecdysis (Carlisle 1957; Crothers 1967; McGaw et al. 1992; Styrishave et al. 2004), so such a high degree of cover can be expected. In the German Wadden Sea, epibionts cover did not approach the total cover, although higher in red morphs (23% versus 13% in green crabs, Wolf 1998). In other crustaceans, females reach terminal anecdysis earlier (after pubertal moulting), while males continue moulting (Overstreet 1983; Crisp 1983), but in *C. maenas* this distinction is not clear. In blue crabs *Callinectes sapidus*, only females enter terminal ecdysis, while males continue moulting, and thus the prevalence of the barnacle epibiont *Chelonibia patula* is higher in female (70%) than in male (54%) crabs (Key et al. 1997).

Abundance of fouled crabs was lowest in winter, both in the Dutch (this work) and German Wadden Sea (Wolf 1998), but the period of highest abundance differed: spring and summer/autumn, respectively in the Dutch and German areas, respectively, likely due to settlement patterns of *B. crenatus* in the area. In the Clyde Sea (United Kingdom), *B. crenatus* has a long settlement period, ranging from spring to autumn (Pyefinch 1948; Blom & Nyholm 1961), with the main release of nauplii and subsequent settlement occurring in spring, followed by moderate release and settlement in August (Pyefinch 1948). Knowledge of barnacle settlement time can be useful to estimate the time that has elapsed since the last moult of the basibiont crab (Gili et al. 1993; Dick et al. 1998). For example, if two generations of barnacles are present on the crab's carapace, this indicates that the basibiont crab has stopped moulting for about a year (Crothers 1967). Similar to other epibiotic associations (Costa et al. 2010), it is possible that epibiont barnacles have a synchronous life cycle with their basibiont crabs.

Environmental factors such as temperature, salinity, and water currents also influence the degree of epibiosis in crustaceans (Wahl & Lafargue 1990). Barnacle larvae respond to environmental cues such as heterogeneity, hardness and texture, and local hydrodynamics in their search for a suitable attachment surface (Hudon et al. 1983; Miron et al. 1996). Epizoic colonization is favoured when hard substrates are limited (Pineda & Caswell 1997), as in the Wadden Sea. The heterogeneity of crab carapace topography, with its grooves, lobes, depressions, and especially its texture (roughness), creates a wide range of microenvironments that may promote *B. crenatus* attachment to some extent (Crisp & Barnes 1954; Hills et al. 1998; Bers & Wahl 2004). In complex substrates, *Balanus* sp. preferentially settle in grooves of 1 and 10 mm (Lemire & Bourget 1996), at the base of roughness elements of 0.6 – 5.7 mm height (Walters & Wethey 1996).

Barnacle larvae detect conspecifics by chemical signal, and reject substrates in which they are not present (Miron et al. 1996). Because larvae have a gregarious behaviour, barnacles form dense colonies with a high number of individuals. Most gregarious invertebrates are highly selective about where they settle, because once settled their ability to move is lost or restricted. In this study, epibionts occurred from a single individual to 159 individuals, with a mean of 16.6±19.4 barnacle epibionts per crab, three times more than the average reported for the North Wales population (Heath 1976).

Salinity influences successful settlement and limits the distribution of *B. crenatus*, as it does for other barnacle species (Dineen & Hines 1994). It is an osmoconforming species, capable of evading excessive changes in salinity as an adult by retreating into the protection of the shell. *B. crenatus* can acclimate to salinities of 14 to 17‰ (Foster 1970) and therefore occurs in estuarine waters. However, salinity also affects the behavioural response of crabs (McGaw & Naylor 1992). They occur at salinities between 10 and 33‰, but can also be found in freshwater flooded intertidal zones with salinities as low as 1.4‰ (Crothers 1968; McGaw & Naylor 1992; Cohen & Carlton 1995). Shore crabs are efficient osmoregulators and respond to low salinity by increasing the frequency of antennal, antennular, and mouthpart cleaning (McGaw et al. 1999). A decrease in grooming, caused by a change in salinity may allow the settlement of greater numbers of cyprids (Giri & Wicksten 2001). Although not statistically significant, fouled crab abundance was slightly lower at the site where salinity was also lower (Kornwerderzand), possibly due to lower barnacle abundance. Although *B. crenatus* usually occurs in sheltered areas, water movement increases settlement and attachment (Miron et al. 1996), and differences in fouling prevalence between sites may be due to differences in local hydrodynamics (Wolff 1959).

Impacts of Balanus crenatus *epibiosis on crabs' condition*

The effects of barnacle epibiosis on the basibiont may be context-specific and vary in strength. In this study, the shore crab was able to tolerate epibiosis, but the presence of barnacles affected its body condition by significantly degrading both morphological and energetic condition (although the SD of the results indicate marked individual variability). A negative effect on the morphological condition of the basibiont was also reported for the association between the barnacle *Chelonibia patula* and the blue swimmer crab *Portunus pelagicus* in the Persian Gulf (Bastami et al. 2012). However, a positive effect of epibiosis with the algae *Enteromorpha* sp in the mole crab *Emerita analoga* was associated with a reduction in energy expended for in locomotion, as fouled crabs tended to remain less active in the subtidal (Firstater et al. 2009).

The barnacle and crab association appears to be detrimental to the host crab and should not be considered a commensal ecological but a parasitic interaction, whereas it can be beneficial for the barnacles, as has been reported for other epibiotic associations (eg, Wahl 1989, 1997; Abelló et al. 1990; Key et al. 1997; Fernandez-Leborans 2010). Benefits to the epibiont barnacle range from physical advantages (e.g., attachment surface) to enhancement of feeding (Harder 2008). Because most epibiont species, including *B. crenatus,* are suspension feeders, growing on the carapace of crabs is advantageous because the host's movement and feeding activities ensure access to food (Jorgensen 1966). Host movement can optimize epibiont dispersal and gene flow, and host movement or respiration generates water currents that enhance removal of epibiont metabolic residues (Wahl 1989; Key et al. 1997; Fernandez-Leborans 2010). Yet, the temporary surface is only suitable for organisms with a short life cycle and/or a rapid growth phase (Seed 1985), such as barnacles (Gili et al. 1993), and hence some epibionts can coordinate their life cycle with the moulting events of their hosts (Jeffries et al. 1992).

Dealing with epibiosis generally involves trade-offs between tolerance and investment into defence, which utilises resources of the host (Aucker et al. 2004; Leonard et al. 2007). Often costs of epibionts outweigh their benefits for the basibiont (eg, Wahl 1989, 1997; Becker et al. 2000; Buschbaum & Saier 2001). Therefore, many organisms have behavioural and/or physiological antifouling mechanisms (Becker & Wahl 1996; Fernandez-Leborans 2010) to shed epibionts by grooming, or preventing them from initially attaching, by hiding and burrowing, or using bioactive compounds like surface waxes, and cuticular structures (eg, Gili et al. 1993; Becker & Wahl 1996; Wahl et al. 1998). In crustaceans, ecdysis is an effective way of removing any existing epibionts (Dyrynda 1986; Thomas et al. 1999). Crustaceans also secrete waxes onto their cuticles, reducing cuticular wettability and possibly making it harder for epibionts to adhere (Becker et al. 2000; Callow & Callow 2002). Also, the microtopography of the cuticle can prevent colonization or growth (Callow & Callow 2002; Bers & Wahl 2004). Nevertheless, in energy-limited conditions, producing such defences can be costly (Fagerstrom 1989) and hence tolerating epibionts releases energy reserves otherwise invested in growth or reproduction (Bazzaz et al. 1987; Van Alstyne 1988).

The decrease in energy density of *C. maenas* fouled with barnacles reflects an epibiont burden that may result from increased energetic costs to drag the barnacle load (Overstreet 1983, Dick et al. 1998), or extra energy expenditure to counteract harmful exudates of the epibionts or mechanical damage (Becker et al. 2000). Barnacles mainly attach to the dorsal face of the carapace of the crabs and do not appear to mechanically interfere with locomotion and feeding appendages. Yet, epibiont barnacles may compete for food or interfere with foraging and prey handling of crabs, resulting in additional difficulties in successfully prey and feed, altering nutrient acquisition and reducing the energy content of the crabs.

Furthermore, epibiosis may negatively affect basibiont crabs by decreasing flexibility (Wahl & Mark 1999) and impairing the locomotion ability, affecting the escape response to predators and hence increasing their vulnerability to predation (Key et al. 1997; Harder 2008). The damage of the body surface made by epibionts may increase the risk of infections (Becker et al. 2000), of parasitism (Thieltges & Buschbaum 2007) and secondary epibiosis by other species (fouling cascade, Gutiérrez & Palomo 2016). Additional stress caused by epibiosis can also make the basibiont host more susceptible to natural and anthropogenic stressors (Pucket & Carman 2002).

The reduced energy content of fouled crabs may disrupt the moulting cycle and affect their growth. While still debated if the presence of barnacles affects mussel growth (Buschbaum & Saier 2001; Sievers et al. 2013) or not (Garner & Litvaitis 2013), periwinkle basibionts *L. littorea* with *B. crenatus* epibionts grow slower (Wahl 1997; Buschbaum & Reise 1999). Reduced growth keeps the basibiont snails in the window of higher vulnerability to predation for a longer period of time (Buschbaum & Reise 1999). Yet, in the case of crabs, epibiosis was more common in adults, which have already outgrow their most vulnerable sizes. Nevertheless, faster growth and better physiological condition will increase survival potential within the population (Suthers 1998). Also, if a certain amount of tissue growth is not achieved, moulting is delayed (Adelung 1971), which in turn will favour epibiosis prevalence. Besides growth, the reproduction of basibiont snails is also impaired by barnacle epibiosis, through reduced egg production due to reduced fitness (Buschbaum & Reise 1999), while for crabs no information exists. In addition, mortality of periwinkles overgrown by *B. crenatus* is three times higher. Altogether, epibiosis can result in significant impacts on the dynamics of the perinwinkle population (Buschbaum & Reise 1999).

There are other effects of epibiosis that may counterbalance the decrease in energy density. For crustaceans, potential benefits of epibiosis include a decrease in predation risk by protection or camouflage (eg, Wahl & Hay 1995; Parapar et al. 1997) or palatability changes due to the presence of epibionts, by which epibiosis may improve the survival of fouled crabs. This is a well known case in Majid crabs which combine epibiosis with a marked masking behaviour, and create a complex camouflage to ward off predators (Parapar et al. 1997). When epibionts are less attractive than their host or even repellent, consumer (predation) pressure can decrease and the benefit for the crab host survival may overcome the decrease in condition (Wahl et al. 1997). As an example, the presence of *Balanus improvisus* on the blue mussel *Mytilus edulis* facilitates handling and, consequently consumption, by *C. maenas* (Enderlein et al. 2003) but reduces predation by the starfish *Asterias rubens* (Laudien & Wahl 1999). Epibionts ranking lower than their host generally reduce predation pressure on the latter (eg, Wahl & Hay 1995; Karez et al. 2000; Dougherty & Russell 2005), while epibionts ranking higher in attractiveness to the consumer than the basibiont may have two opposing effects regarding the trophic interaction commonly named attractant/ decoy or shared doom (Wahl & Hay 1995).

However, further research is required to understand the extent of barnacle epibiosis impact, namely if epibiosis impairs the crab fitness, by affecting its growth, reproduction (gonad maturity, fecundity) and survival, and ultimately the dynamics of the population, acting as an ecological lever, modulating effects of biotic and abiotic stress, either greatly amplifying or buffering, as suggested in other populations (Wahl 2008).

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The authors report there are no competing interests to declare.

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Caption of figures and tables

Figure 1. Map of the sampling locations in the western Dutch Wadden Sea. 1, Gat van de Stier (N 52°57.270´ E 4°55.730´); 2, Texelstroom (N 53°02.030´ E 5°03.370´); and 3, Kornwerderzand (N 53°04.520´ E 5°16.550´); top left: sampling area in the Netherlands (Adapted from Katwijk & Hermus 2000).

Figure 2. Percentage distribution of the abundance of fouled crabs according to sex (F: females, M: males, MM: modified males), colour morphotype (Red; Green), size class (Juv, juveniles: ≤25 mm CW; A1: 25-40mm CW; A2: 40-55mm CW; A3: >55mm CW), seasons (Sum., summer; Spr., spring; Win., winter; Aut., autumn) and site (Korn., Kornwerderzand; Gat v.S., Gat van de Stier; Texel., Texelstroom).

Figure 3. Fulton's condition index (*K*), percentage of dry weight (DW, %), and energy density (E_{DW}, $kJ.g^{-1}$ DW; and E_{AF}, $kJ.g^{-1}$ AFDW) overall and per sex for non-fouled (white bars), fouled crabs (pink bars), and fouled crabs infected with *Sacculina* (purple bars), with the respective number of crabs (F: females; Fe: females with eggs; M: males; MF: modified males) (mean values and one SD error bars are presented).

Figure 4. Fulton's condition index (*K*) and total energy (E_{tot}, kJ) in relation to size of the crab (CW, mm).

Table 1. Number of shore crabs *Carcinus maenas* fouled with barnacles and analysed for energy condition per sex and colour, separately for uninfected crabs and for crabs infected with *Sacculina carcini*, and percentage (%) of total sample.

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).

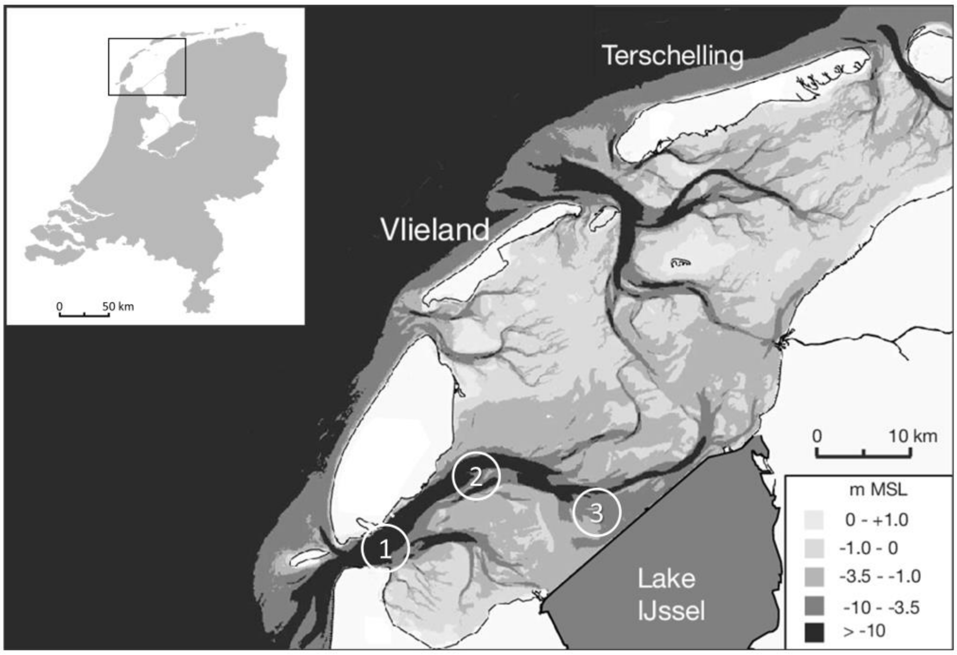
Table 1. Number of shore crabs *Carcinus maenas* fouled with barnacles and analysed for energy condition per sex and colour, separately for uninfected crabs and for crabs infected with *Sacculina carcini*, and percentage (%) of total fouled sample.

Uninfected colour	crabs sex	total	%	Infected colour	crabs sex	total	%
Green	F	159	28	Green	F	19	3
Green	M	123	21	Green	M	15	3
Green	MF	0	0	Green	MF	27	5
Green	All	282	49	Green	All	61	11
Red	F	149	26	Red	F	23	4
Red	M	32	6	Red	M	18	3
Red	MF	1	0	Red	MF	12	2
Red	All	182	31	Red	All	53	9

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)	1.3195	0.0978	13.492	***	0.2614	0.7148
	fouled	0.0588	0.0539	1.091			
	Infected&fouled	0.0592	0.1260	0.470			
	Sex M	0.1929	0.0891	2.165			
	Sex MF	-0.0921	0.1133	-0.813			
	CW	-0.3525	0.1192	-2.957	**		
	CW^2	0.1062	0.0476	2.233	*		
%DW	(Intercept)	32.9086	0.9682	33.988	***	0.0233	0.3529
	fouled	-0.2523	1.4549	-0.173			
	Infected&fouled	1.2331	1.5409	0.800			
	Sex M	0.9621	1.4712	0.654			
	Sex MF	0.3731	1.9198	0.194			
	CW	0.1940	0.3833	0.506			
	CW^2	0.1837	0.2642	0.695			
E_{DW}	(Intercept)	11.7096	0.5240	22.346	***	0.0806	0.3355
	fouled	-1.1895	0.3999	-2.975	**		
	Infected&fouled	-1.2329	0.5017	-2.458	*		
	Sex M	0.2665	0.5278	0.505			
	Sex MF	-0.1322	0.9654	-0.137			
	CW	-0.1753	0.2430	-0.721			
	CW^2	-0.0781	0.1371	-0.569			
E_{AF}	(Intercept)	17.8500	0.3852	46.335	***	0.0645	0.3253
	fouled	-0.9191	0.4928	-1.865			
	Infected&fouled	-0.3898	0.5283	-0.738			
	Sex M	0.1428	0.3833	0.373			
	Sex MF	1.1136	1.2626	0.882			
	CW	-0.1803	0.1841	-0.979			
	CW^2	-0.0571	0.1064	-0.536			
E_{tot}	(Intercept)	41.2185	3.9391	10.464	***	0.7858	0.8477
	fouled	-2.5568	4.2452	-0.602			
	Infected&fouled	1.9349	6.0477	0.320			
	Sex M	10.8866	7.9539	1.369			
	Sex MF	0.5147	7.7529	0.066			
	CW	34.2994	2.5002	13.719	***		
	CW^2	10.3286	2.1091	4.897	***		

Fig.1


Terschelling
Vlieland
1
2
3
Lake IJssel
0
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0
50 km
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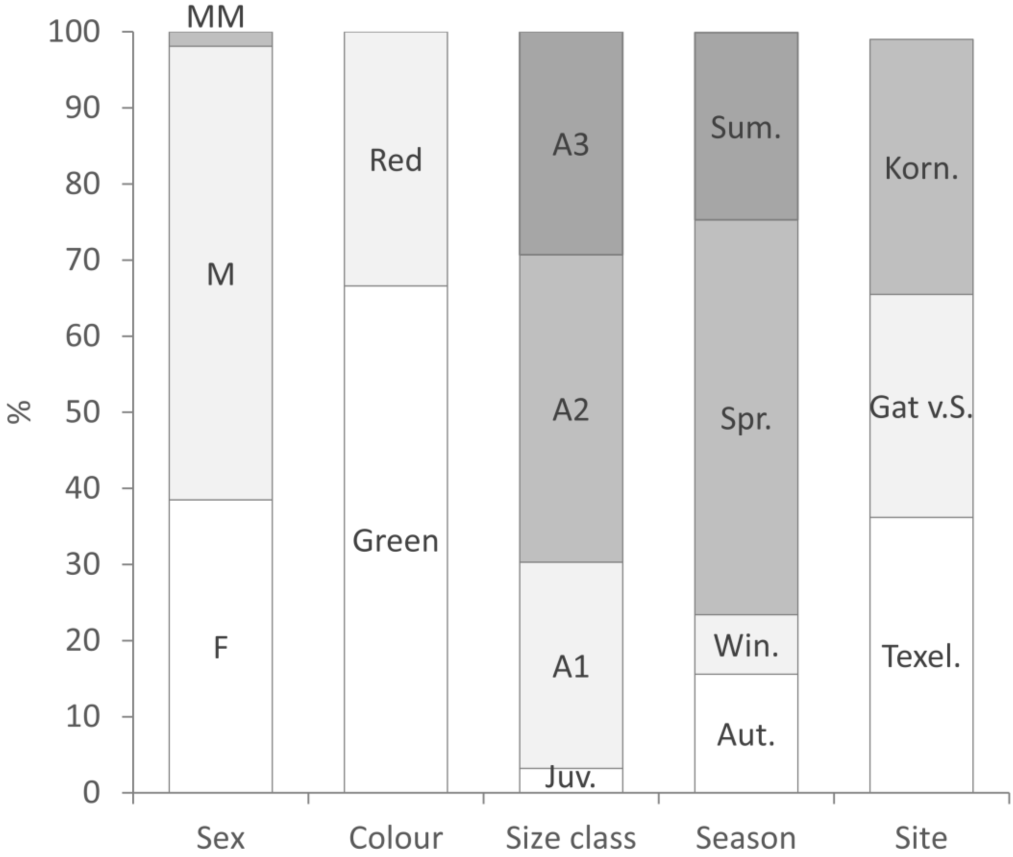
-1.0 - 0
-3.5 - -1.0
-10 - -3.5
> -10

Fig.2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90
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MM
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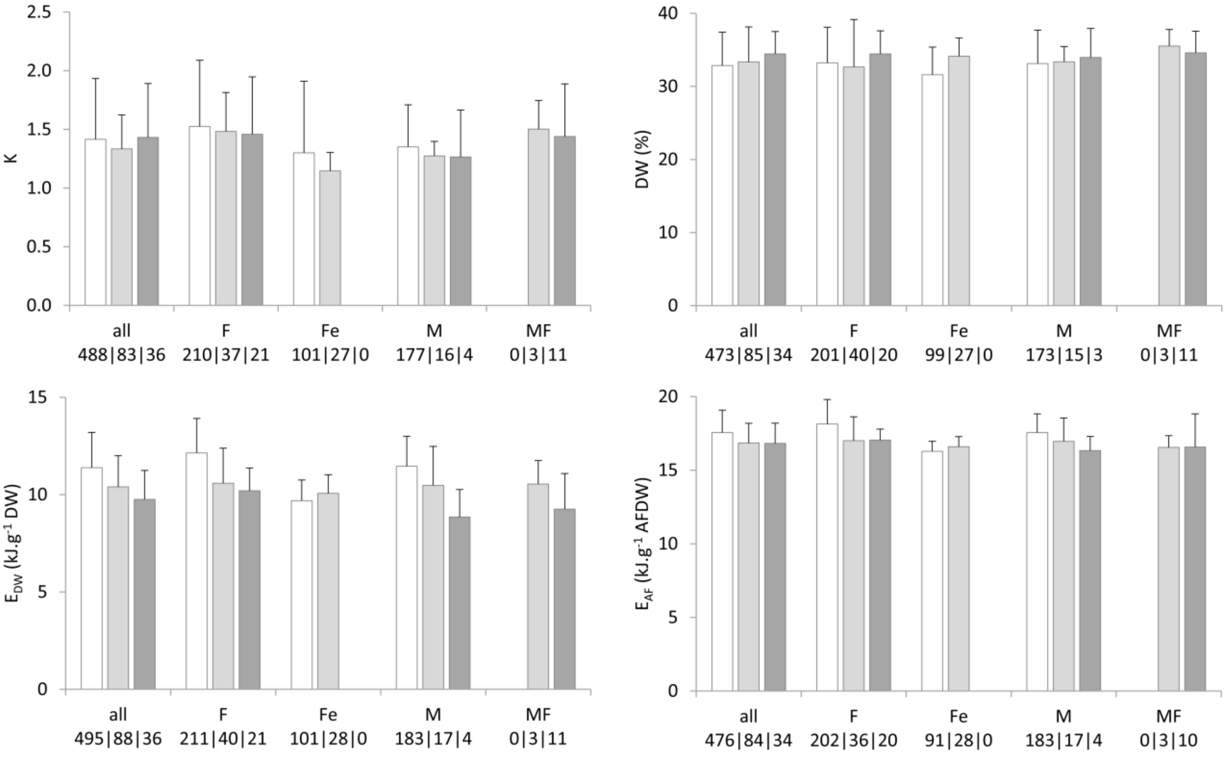
F
Red
Green
A3
A2
A1
Juv.
Sum.
Spr.
Win.
Aut.
Korn.
Gat v.S.
Texel.
Sex
Colour
Size class
Season
Site

Fig.3


K
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2.0
1.5
1.0
0.5
0.0
all
488|83|36
F
210|37|21
Fe
101|27|0
M
177|16|4
MF
0|3|11
DW (%)
40
30
20
10
0
all
473|85|34
F
201|40|20
Fe
99|27|0
M
173|15|3
MF
0|3|11
E_{DW} (kJ.g^{-1} DW)
15
10
5
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all
495|88|36
F
211|40|21
Fe
101|28|0
M
183|17|4
MF
0|3|11
E_{AF} (kJ.g^{-1} AFDW)
20
15
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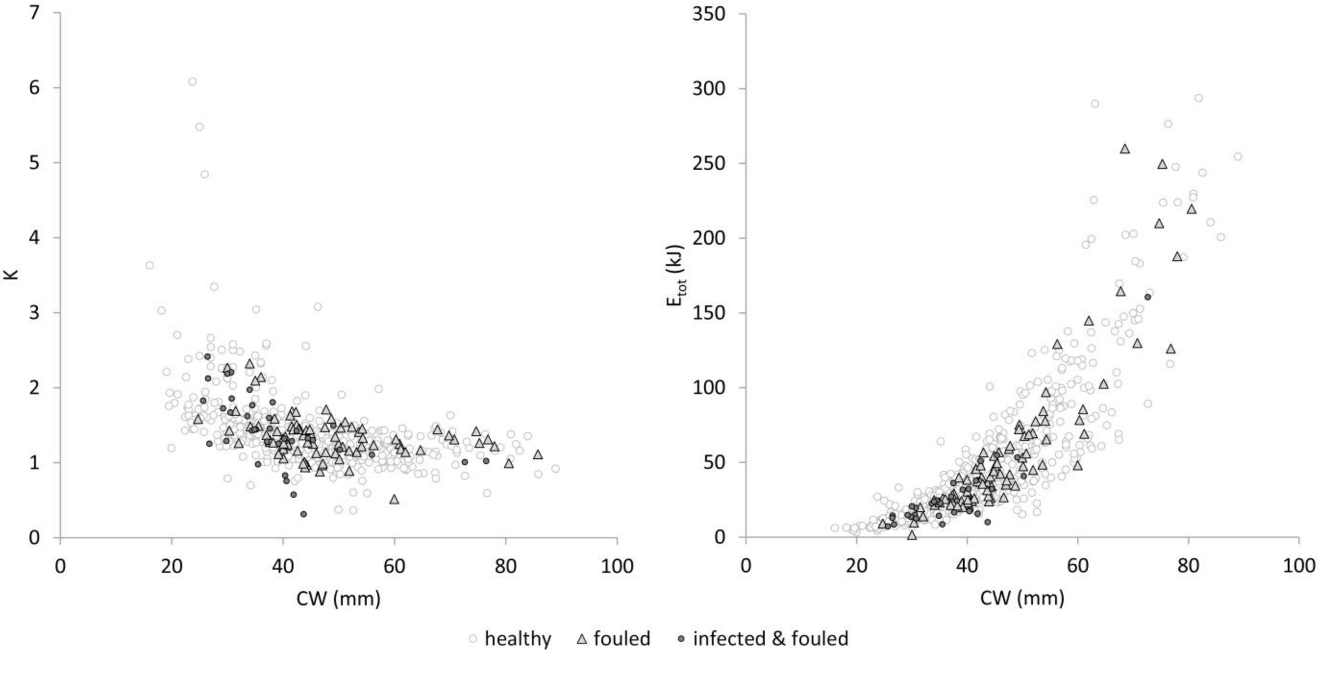
91|28|0
M
183|17|4
MF
0|3|10

Fig.4


K
7
6
5
4
3
2
1
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40
60
80
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CW (mm)
E_{tot} (kJ)
350
300
250
200
150
100
50
0
0
20
40
60
80
100
CW (mm)
○ healthy
△ fouled
● infected & fouled