Life history of *Sacculina carcini* Thompson, 1836 (Cirripedia: Rhizocephala: Sacculinidae) and the intermoult cycle of its host, the shore crab *Carcinus maenas* (Linnaeus, 1758) (Decapoda: Brachyura: Carcinidae)

Jørgen Lützen¹, Knut Helge Jensen² and Henrik Glenner²,³

¹Marine Biological Section, Biological Department, Københavns Universitet, Universitetsparken 4, 2100 Copenhagen Ø, Denmark; ²Marine Biodiversity, Biological Institute, Universitetet i Bergen, Thormøhlens Gate 53A, N-5020 Bergen, Norway; and ³Centre of Macroecology, Evolution and Climate, Museum of Natural History, Københavns Universitet, Universitetsparken 15, 2100 Copenhagen Ø, Denmark

Correspondence: H. Glenner: e-mail: henrik.glenner@uib.no

(Received 7 November 2017; accepted 3 May 2018)

**ABSTRACT**

The dominant colour through the intermoult cycle of *Carcinus maenas* (Linnaeus, 1758) changes from green to orange, then to red. The external developmental stages of the rhizocephalan parasite *Sacculina carcini* Thompson, 1836 are correlated with this cycle such that the youngest stages predominantly occur when the crabs are green, the intermediate stages when they are orange, and the oldest when they are red. Fouling by the barnacle *Balanus crenatus* Bruguière, 1789 increases through this cycle as well, with both sacculinised and unparasitised crabs of orange or red colour being significantly more fouled than green crabs. Sacculinised green crabs with younger external parasite stages are generally least fouled, whereas orange and red crabs with late parasite stages are most fouled, but only female crabs show a statistically significant positive association between *Sacculina* infection and fouling. For both sexes, time since moulting, indicated by crab colour, is the most important predictor for fouling by *B. crenatus*.

**Key Words:** *Balanus crenatus*, barnacles, colour morphs, Denmark, fouling, parasitism, susceptibility

**INTRODUCTION**

The life cycle of rhizocephalan barnacles can be divided into three phases: 1) the infective phase during which a free-swimming female larva detects, settles upon, and penetrates the integument of a crustacean host and injects a parasitic stage; 2) the endoparasitic phase during which the injected parasite develops an internal root system, the interna, within the hemolymph of the host; and 3) the reproductive phase during which the reproductive apparatus of the interna, the externa, emerges to the exterior by penetrating from inside through the host's integument and cuticle. The externa, still connected to the food-absorbing interna by a stalk, is exposed to seawater, enabling mating via the transformation of free-swimming male larvae into cryptogonochoristic dwarf males of various sorts (Lützen, 1984; Hoeg & Lützen, 1995). Species of *Sacculina* commonly parasitise brachyuran crabs, which are said to be “sacculinised.” Following its maturation, an externa of *Sacculina* will produce one or several batches of larvae, then senesce and fall off, leaving the parasitised crabs with a scar, the so-called “scarred” crabs.

It remains unknown at which intermoult period of a crab the youngest “virginal” external reproductive stage of *Sacculina carcini* (Thompson, 1836) emerges. Preliminary observations on the shore crab *Carcinus maenas* (Linnaeus, 1758) suggests that this may happen when the crab is in the green colour phase, a sign that it has quite recently moulted. Further comparisons between the older stages in the growth of the externa and the succeeding colour phases of the crab (orange and red) suggest that there might exist a correlation between these and the external developmental stages of *Sacculina*. An opportunity presented itself to explore such a relationship when we discovered that shore crabs were quite heavily sacculinised in the Limfjord, Denmark. Adult *C. maenas* are known to change colours throughout the intermoult period, the newly moulted exoskeleton, especially its underside, being initially green, then gradually turning orange and finally red (McGaw et al., 1992; Lewis, 2010; Audet et al., 2008). A conspicuous part of the adult male population is more or less permanently green, at least in the Limfjord. Studies have shown that the various coloured crabs have different tolerances to low salinity and oxygen while also showing...
differences in aggressiveness and other types of behaviour (Reid & Aldrich, 1989; McGaw & Naylor, 1992; Reid et al., 1997; Young et al., 2017).

Our investigation aimed to correlate the growth stages of the externae of \textit{Sacculina carcini} with the colour phases of the intermoult stages of the host crabs, \textit{Carcinus maenas}.

\section*{MATERIALS AND METHODS}

\subsection*{Collection and staging of specimens}

Sacculinised male and female \textit{C. maenas} were collected monthly in Sallingsund and Venoce Bay in the Limfjord, Denmark, from June to November in 2012, 2014, and 2016 by means of traps set at depths of 1–6 m. Water temperature varied between 7 °C (November) and 16 °C (July), and the average salinity varied from 28 to 30 psu. We recorded the developmental stage of \textit{Sacculina} and sex and colour of sacculinised crabs \((N = 732)\). To estimate how sacculinisation influences fouling of the host, we further recorded the presence or absence of epibionts, mainly the barnacle \textit{Balanus crenatus} Bruguière, 1789, on 297 crabs. These data were compared to field data for healthy crabs from regular samples taken through one year (May 2013 to April 2014) at Sallingsund. Most crabs were ≥ 30 mm in carapace width (CW), which is about the minimum size of the healthy, sexually mature male and female individuals we observed.

We distinguished four developmental stages of the externae of \textit{Sacculina carcini}, all located beneath the crab’s abdomen: colourless virgin externae 2–5 mm wide, with a duration of approximately two weeks; milky-white or yellowish immature externae 6–12 mm wide, with a duration of four to six weeks (Lützen, 1984); purple, brown, or dark brown sexually mature adults 11–22 mm wide; and senescent externae, the last stage, wrinkled and blackish and of the same size as the adults. Adult and senescent externae live much longer than the earlier stages and produce several batches of nauplius larvae during summer and autumn. Thereafter they perish and drop off, leaving a conspicuous black scar beneath the abdomen (Lützen, 1984).

A considerable part of the male crabs in the Limfjord are permanently green, while the other males and most females undergo a colour change. Crabs are green when newly moulted, then gradually turn orange and, if they live long enough, become red (McGaw et al., 1992). During each intermoult this colour sequence is repeated. In green crabs the sternum, third maxillipeds, legs, and claws are predominantly green or yellowish green. These parts are light orange or pink in orange crabs. Females tend to be pink rather than orange, especially on the anterior part of the body. Red crabs are tile-red on the undersurface of the body and on the legs and claws. These colour forms represent successive stages in the intermoult cycle in both sexes. Colour photographs of caged crabs were taken at intervals to document the changes. An overview of the collected material can be found in Table 1.

\subsection*{Laboratory experiments}

To investigate whether the presence of an external parasite affects moult ing, 20 unparasitised and 20 sacculinised crabs with senescent externae (older than six months) were caught in April 2016 and subsequently kept in individual cages until September of the same year. Seemingly healthy and scarred crabs were also individually kept under daily observation after they had first moulted. Several of these crabs developed externae after moult ing, and the growth of the externae as well as the colour changes of their hosts were recorded over the summer.

\section*{Statistics}

All statistics and plots were done by using R version 3.4.3 (R Development Core Team; http://www.r-project.org). For the first analysis, we compared the proportion of crabs in each of the three colour categories of green, orange, and red, infected with the five developmental stages of \textit{Sacculina carcini}: virgin, immature, adult, senescent, and scar. For these data we did a Chi-square test for a 3 × 5 contingency table, with each crab sex being tested separately.

The second analysis concerned data on the occurrence of \textit{Balanus crenatus} on individual crabs expressed as a binary response variable (0 and 1 for non-fouled and fouled crabs, respectively).

We did separate tests for each crab sex, where the fouling epifauna of \textit{B. crenatus} was analysed with the predictors \textit{S. carcini} infection status (infected versus uninfected), and crab colour. For the latter predictor, colour only contained the two levels, green and orange + red. This was done to reduce problems of low sample size for the orange group. We used a generalized linear model (GLM) with a binomial error term in these analyses. The R syntax for the models was: glm(epifauna ~ crab.colour*infection.status + family, family = binomial).

We also did more detailed analyses within each crab colour and sex where we used the same type of model as above but with a single predictor, infection stage, representing the four infection stages: virgin/immature, mature/senescent, scar, and infected. The combination of some stages was done to reduce problems of low sample size. If a model turned out to be significant, we performed a Tukey HSD multiple comparisons test to determine which of the four parasite infection stages differed with respect to the response variable.

\section*{RESULTS}

Observations on caged crabs demonstrated that male crabs remain green for one to three months after moult ing (Fig. 1). Caged crabs that possessed a senescent externa in April were almost exclusively

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Colour changes of the carapace of a moult ing, parasitised male \textit{Carcinus maenas} during four spring/summer months in 2016 (ventral view): the red, \textit{Sacculina}-scarred crab on 20 April, one month before molting (A); the old carapace (below) and the recently molted, now yellow/green crab on 20 June (B); the now reddish crab on 14 July, with a developing externa under the abdomen (C); the crab on 24 August, with a red carapace and a dark brown, fully developed externa of the rhizocephalan parasite (D).}
\end{figure}
red and fouled with epibionts by September. Of the 20 males among them, none was observed to moult between April and September in contrast to the control group of 20 infected males, which all moulted in May-July.

A clear relationship was evident between the sequence of developmental stages of *Sacculina carcini* externae and the dominant colours of the host crabs (Fig. 2). The green colour prevailed among both sexes of crabs that bore virgin and immature externae, whereas adult externae more equally occurred on the green, orange, and red crabs. Crabs with senescent externae or scars were predominantly red. The proportion of individuals in each of the three colour categories differed among the parasitic stages for both male and female hosts (Fig. 2: males: $\chi^2 = 102.24$, df = 8, $P < 0.001$; females: $\chi^2 = 215.48$, df = 8, $P < 0.001$).

In the first GLM testing, the occurrence of barnacle-fouled crabs showed no interaction between the two predictors, crab colour and infection status, for either female or male crabs (Fig. 3, Table 1). Thus, the effect of colour on the occurrence of barnacle-fouled individuals did not depend on the *S. carcini* infection status. There was a general increase in the occurrence of barnacle fouling for orange + red crabs compared to green crabs in both females and males (Fig. 3, Table 1). The occurrence of male crabs carrying barnacles did not depend on the *S. carcini* infection status (Fig. 3, Table 1). For females, in contrast, the occurrence of fouling was higher for individuals infected with *S. carcini* compared to uninfected crabs (Fig. 3, Table 1).

The more detailed GLM model based on rhizocephalan infection stages instead of infection status (Fig. 4) did not reveal any effect of the infection stages on the occurrence of barnacle fouling for green female crabs (residual deviance = 572.13, residual df = 636, deviance = 2.554, df = 2, $P = 0.279$). The occurrence of barnacle fouling for the orange + red female crabs, however, depended on infection stage (residual deviance = 1006.2, residual df = 829, deviance = 24.849, df = 3, $P < 0.001$), and the Tukey HSD multiple comparison test revealed that the parasite stages that differed in the occurrence of barnacle-bearing hosts were virgin/immature versus adult/senescent ($P = 0.045$) and adult/senescent versus uninfected ($P < 0.001$).

The same detailed GLM model applied to green males of *Carcinus maenas* showed the same trend (residual deviance = 1639.6, residual df = 2076, deviance = 7.768, df = 3, $P < 0.053$), with the Tukey HSD multiple comparison test revealing that this was due to differences in the occurrence of barnacle-bearing green male crabs with virgin/immature *Sacculina versus those with adult/senescent parasites ($P = 0.029$) and between crabs with adult/senescent *Sacculina versus uninfected crabs ($P = 0.054$). A similar situation was found for the orange + red males (residual deviance = 1234.8, residual df = 914, deviance = 13.824, df = 3, $P < 0.003$), but here the Tukey HSD revealed that the *S. carcini* stages differing in occurrence of barnacle-bearing crabs were virgin/immature versus adult/senescent ($P = 0.030$) and virgin/immature versus scar ($P = 0.008$), with a non-significant trend for virgin/senescent versus uninfected crabs ($P = 0.089$).

**DISCUSSION**

**Parasite life history and host colour phases**

The only studies that have so far linked the occurrence of *Sacculina carcini* to the colour of its host are those of Costa et al. (2013) from the Mondega Estuary, Portugal, and Waser et al. (2016) from the Wadden Sea, The Netherlands. Both studies found that red crabs were externally parasitised to a higher degree than green crabs, but the authors did not distinguish orange from green or red crabs, nor did they specify the growth stages of *Sacculina*. This makes it difficult to compare their results with ours.

Observations made by Lützen (1984) suggested that an overwhelming part of the sacculinised crabs with either a nucleus (the internal nascent stage of the virgin externa) or some virgin externa were green, and therefore had quite recently experienced a moult, but this was not followed up.

More than half of the adult male shore crabs in the Limfjord are permanently green all year round and go through two molts annually (summer and autumn), both resulting in a new green exoskeleton (our observations). The other part of the male population plus most of the females start out as green following a single annual moult (summer), but gradually turn orange within the subsequent one-year molting period, or, if they live long enough, they assume a red colour. The different colours of these male and female crabs, therefore, serve as an approximate marker of the time spent in the intermoult cycle. The youngest external stage of the developing *Sacculina* coincides with the beginning (green phase) of this cycle, since the great majority of the virgin and immature stages occur while the crabs are still green. Towards the end of the life of an externa (when it is senescent or represented by a scar), its host will mainly be red. Figure 2 shows the correlation between carapace colour and the developmental stage of the externa in a single specimen over five months.
It is difficult to tell why males with the prospect of being constantly green, when sacculinised, adopt the same colour change (to red) as male crabs with natural red colour morph. It may be a case of manipulation of the host as inasmuch as gravid rhizocephalans make host crabs behave as though they were berried (Devries et al., 1989). However that may be, it entails an arrest of the autumnal moult of the host that would otherwise be detrimental for the parasite.

Delage (1884) showed that the emergence of *S. carcini* occurred sometime after a moult, but he specified neither the time elapsed since the moult nor the colours of the crabs. Our laboratory observations on caged crabs (*N* = 5) indicated that the emergence of virgin externae takes place three to five days after the moult of the host crabs. At this time most crabs will still be green (Fig. 1), and the next growth stage of the rhizocephalan (immature) also occurs when most crabs have not yet turned orange. In another sacculinid crab, *Polyascus polygenea* (Lützen & Takahashi, 1997) (= *Sacculina senta* Boschma, 1933), Takahashi & Matsuura (1994) found that the new externa emerged within the first six days after the moult in 19 out of 20 cases.

During the process of emergence, the nucleus forces its way to the exterior by destroying the ventral abdominal integument of the crab, which is no longer capable of producing a cuticle, thus resulting in the formation of a hole through which the prospective externa protrudes (Delage, 1884). One may expect that this process is most easily accomplished during the early intermoult period when the cuticle is relatively thin rather than later, when, as documented by McGaw et al. (1992), the cuticle has become thicker in crabs that have turned orange or red. That this correlation sometimes fails in females with virgin externae in spite of their orange or red colour (Fig. 1) can perhaps be explained by the fact that the cuticle, due to the smaller size of the females, is probably thinner in the area of emergence. Another anomaly from the general pattern is that scars may occur in some of the green crabs (Fig. 2). This is probably because many virgin and immature *S. carcini* die young, as has been observed in caged crabs (Lützen 1984).

The period from emergence to the adult stage in *S. carcini* has been estimated as four to six weeks (Lützen 1984) on the basis of laboratory experiments. This is easily within the period of time crabs stay green before they change to orange.
Various authors have commented on the way sacculinisation influences the presence of epibionts on crabs. Delage (1884) reported that sacculinised shore crabs, especially those with senescent externae or scars, were often heavily fouled by epibionts. Linke (1939) and Crothers (1968) briefly remarked on the epifauna and/or the colour of sacculinised crabs. Epibionts (in this case, barnacles) become more abundant on host crabs as the growth stages become older and the crabs pass through different colour phases of the intermoult cycle.
Table 2. Left column: Fouling of healthy Carcinus maenas individuals in the western Limfjord (Sallingund), Denmark during May 2013 to April 2014, mostly by Balanus crenatus. Right column: Percentages of fouled sacculinised crabs from the same area.

<table>
<thead>
<tr>
<th>Colour</th>
<th>HEALTHY MALES</th>
<th>SACCU LINISE MALES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N fouled</td>
</tr>
<tr>
<td>Green</td>
<td>2003</td>
<td>271</td>
</tr>
<tr>
<td>Orange</td>
<td>451</td>
<td>176</td>
</tr>
<tr>
<td>Red</td>
<td>332</td>
<td>148</td>
</tr>
<tr>
<td>Total</td>
<td>2786</td>
<td>595</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Colour</th>
<th>HEALTHY FEMALES</th>
<th>SACCU LINIZED FEMALES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N fouled</td>
</tr>
<tr>
<td>Green</td>
<td>635</td>
<td>99</td>
</tr>
<tr>
<td>Orange</td>
<td>444</td>
<td>129</td>
</tr>
<tr>
<td>Red</td>
<td>328</td>
<td>97</td>
</tr>
<tr>
<td>Total</td>
<td>1407</td>
<td>325</td>
</tr>
</tbody>
</table>

We appreciate the thorough revisions by three anonymous reviewers, which greatly improved the manuscript. We are very grateful to the Carlsberg Foundation for covering all expenses connected with the study (grant 2008-01-0491). We also wish to thank the staff of the Danish Shellfish Center, Nykøbing Mors, Denmark, for collecting some of the material and providing laboratory facilities and lodging during our visits.

ACKNOWLEDGEMENTS

As a result, green crabs generally bear the youngest stages and are relatively clean, whereas orange + red (or scarred) crabs bear the oldest externae and are fouled with barnacles to a higher degree.

Mouritsen & Jensen (2006) reported that in the Limfjord, 75% of the sacculinised crabs (males and females together) in August were fouled by barnacles and calcareous tube worms, compared to only 29% of healthy crabs. Because they also showed that sacculinised crabs bury themselves less often than healthy ones, they reasoned that the former are more exposed to settling by larval epibionts. The average fouling percentages of healthy male and female crabs throughout one year (2013–2014) were a bit lower, at 21.4% and 23.1%, respectively, than for sacculinised males and females, at 34.5% and 43.5%, respectively (Table 2). The lower fouling frequency observed by us might be due to the fact that tube worms are rare in the western Limfjord, but very common in Livoe Broad where Mouritsen & Jensen (2006) collected their crabs. The figures thus support their data but extend them to cover both sexes separately. The males are only marginally more fouled (tube worms, barnacles, and other organisms) in sacculinised than healthy crabs, while sacculinised females are much more fouled than healthy ones are. This pattern might be correlated to the fact that female crabs in the Limfjord exhibit a higher prevalence of S. cancini (12.6%) than the male crabs there do (7.9%) (unpublished data). Rasmussen (1973) also found females to be more sacculinised than males (3.1% versus 1.8%) in shallow waters in the Isefjord. Similarly, Costa et al. (2013) found a 3.25:2.20 predominance ratio of parasitized females to parasitized males in the Mondega Estuary, Portugal and Waser et al. (2016) likewise found the same differences in the prevalence of the two genders in the Dutch Wadden Sea. There might exist a correlation between the higher parasite prevalence and fouling frequency in female crabs, but the causal link is probably complex and requires further investigation.

The annual moulting season for male crabs in Scandinavian waters is June and July (Rasmussen, 1973; Dries & Adelung 1982). This is the season when a maximum number of newly moulted and therefore green male crabs are present and the numbers of nuclei and virgins of Sacculina peak (Lützen, 1984; personal observation). Because a major fraction of the emerging virgin externae (on male crabs) start their life cycle in this limited season, it is possible to follow the more or less regular succession of successive growth stages. During winter and the following spring, many externae on male crabs die, the crabs in the meantime have mostly turned red, and only few survive until May (Lützen 1984). Evidently, therefore, the entire external life history of Sacculina can take place within one intermoult cycle of the male crabs if, as maintained by Brockhuysen (1936), such a cycle lasts about one year.


