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ORIGINAL ARTICLE



Variation in juvenile stages and success of male acquisition in Danish and French populations of the parasitic barnacle *Sacculina carcini* (Cirripedia: Rhizocephala) parasitizing the shore crab *Carcinus maenas*

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ABSTRACT

Population and developmental characteristics of the rhizocephalan parasite *Sacculina carcini* were studied in the Limfjord, Denmark and at Roscoff, France. The frequency of juvenile externae in the Limfjord was higher during summer (69.8% of all externae) than during winter (43%). At Roscoff, 62.6% of externae were juvenile in May–June, but only 8.4% in September. The percentage of juveniles with settled males in the Limfjord was higher in summer (20.7% of all juveniles) compared to winter (6.7%). Juveniles at Roscoff in summer had less success in male acquisition (18.0%). The mean number of settled male cyprids per juvenile externa was 0.56 in summer and 0.12 during winter in the Limfjord, but only 0.27 in the summer at Roscoff. Laboratory kept virgin externae invaded by male cyprids ceased to attract additional males within 5–6 days after the first male settlement and then grew into adults within 3 weeks. Externae without males, kept in isolation, did not grow or show any other signs of maturation. Our results confirm and extend previous data on the role of males and their effect on externa development in *S. carcini*, and show that the success of male acquisition is susceptible to both seasonal and regional variation.

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Introduction

Parasitic barnacles (Rhizocephala) are an interesting and important group due to their complicated life cycle, and the fact that they are believed to exert comprehensive control over their crustacean hosts, including partial or complete sterilization of both sexes, and in many species also arrestment of the moult cycle (Høeg and Lützen 1995). Furthermore, since rhizocephalans can occur at high prevalence, they influence the dynamics of the host population by lowering the carrying capacity of the host population (Thresher et al. 2000; Lafferty and Kuris 2009). All rhizocephalans have separate sexes (dioecy, see Høeg 1995). Infection of the host by a female cypris larva is followed by an internal growth stage, where the parasite develops a system of rootlets and establishes its control of the host. Eventually, the parasite emerges on the outside of the host as a small virgin externa, which soon becomes attractive to male cyprids that settle and become implanted as dwarf males (Høeg 1991). Before males are obtained, the virgin parasite will

neither grow nor mature sexually, but following a successful acquisition, the female parasite resumes growth and will rapidly reach sexual maturity (Yanagimachi 1961; Høeg 1982; Høeg and Ritchie 1985). The adult externa will, in most species (the kentrogonid rhizocephalans), release a long succession of broods of larvae, all the result of fertilization by dwarf males (Ritchie and Høeg 1981; Walker 1987; Høeg and Lützen 1995).

The successful acquisition of dwarf males and ensuing growth to sexual maturity are both pivotal for parasite success. Nevertheless, while the complicated metamorphosis of male cyprids into dwarf males is very well described morphologically (Høeg 1987), few studies have focused on the early development of the female externa in terms of the success of recruiting males, the duration of the juvenile growth stages leading to sexual maturity and the frequency of these stages at various seasons in the parasite population. Such lack of information on the juvenile parasite is the case for the 'classical' text-book rhizocephalan *Sacculina carcini* Thompson, 1836, which parasitizes

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the European shore crab *Carcinus maenas* (Linnaeus, 1758), a very prominent member in many near shore habitats along the coast of northern Europe (Høeg and Lützen 1985; Werner 2001; Kristensen et al. 2012; Waser et al. 2016). The most extensive population level data on juvenile development of *S. carcini* was given by Lützen (1984), who together with Høeg (1984, 1987) and Walker (1985) first established that *S. carcini* have a sexual system relying on dwarf males.

Here we extend Lützen's (1984) study by sampling *S. carcini* infected *C. maenas* populations from two localities; Roscoff, France and the Limfjord, Denmark. The aim is to investigate whether there is a general seasonality in the frequency of the external stages of development as found for the Roscoff population by Høeg and Lützen (1995). Furthermore, we wanted to examine the success of virgin parasites in acquiring dwarf males at various seasons, and to follow growth into maturity of virgin parasites after accurately timed implantation of males in the laboratory. The answers to these questions are crucial for understanding the population dynamics and reproductive success of this 'model' rhizocephalan parasite and, therefore, also for its potential regulatory effect on *C. maenas* populations.

Materials and methods

Study of frequency of external developmental stages and success of male acquisition

From 2009–2012 samples of *C. maenas* were regularly taken (ca. monthly except during winter months) in the western part of the Danish Limfjord near the island of Mors (Figure 1(C)). To test if sample depth and sample location could have an effect on male acquisition, three different sites and three different depths were chosen. Another sample was taken in 2016 to study the growth rate of the externa in the laboratory. The Limfjord is a strait, but with very narrow connections at both the western and eastern ends. The three sampling localities were all located around the island of Mors, and we treat all samples together in this paper since we did not detect any significant differences between sites or depths (data not given).

At Roscoff, France, the crabs were sampled in 1983, 1986 and 2011 by Jens T. Høeg on the sandy flat called l'Aber, located at the exit of a small freshwater stream (Figure 1(B)). The crabs were hand collected at low tide from underneath flat stones on the far side of l'Aber with respect to the station (Supplementary video 1). This is the same locality and method used by Høeg (1984, 1987) and Høeg and Lützen (1995).

At Roscoff the samples were taken in May and June in 1983. May, June, August and September in 1986 and June, July and September in 2011. Monthly samples were not taken every month at Roscoff, so we pooled all data from both localities into the summer period (April–September) and winter period (December–March). We recorded the prevalence of externally sacculinized crabs and scored the size (diameter) and developmental stage of the parasite externae (see below). Large subsets of parasite externae in the juvenile (pre-adult) stages were preserved in formalin for a closer examination under a dissection microscope. This included details on general development (moulting stage, presence of a mantle aperture and maturation of the ovary) and the presence and number of male cypris larvae attached on the juvenile parasite.

Study of externa development

From the 2016 Limfjord sample, we isolated hosts carrying small sized externae of stages 1–3 for long time maintenance and observation of parasite growth in the laboratory. In one series of experiments, the virgin externae that emerged in the laboratory (stage 2; 2–3 mm wide) were experimentally exposed to male cyprids for two days (Figure 8). In the other series of experiments, crabs with externas in stage 2 or 3 were sampled in the field by traps (Figure 9). These externas were about 4 mm in diameter (stage 3). The development of all externae from both experiments was individually followed and photo documented (Supplementary Figure 1) until the first brood of nauplius larvae was released. Both specimen lots were kept under the same temperature regime as in the field (Figure 2).

At Roscoff in 1986, we isolated stage 3 virgin externae until they were exposed to male cyprids reared in the laboratory (see definition of stages below). Exposure to cyprids was overnight and thereafter the externae were examined for male settlement. Those that had received male cyprids were then kept in aquaria and observed and measured regularly until they reached the adult stage. In another experiment at Roscoff in 1983, a number of stage 2 virgins (attractive but without males) were kept in isolation under similar conditions as controls. These experiments followed the procedures described in Høeg (1984, 1987). All experiments were done in seawater with the same temperature and salinity as in the ambient water (Figure 2).

Stages of externa development in *S. carcini*

Based on morphology, growth and presence of males we divide the external development of the female *S.*

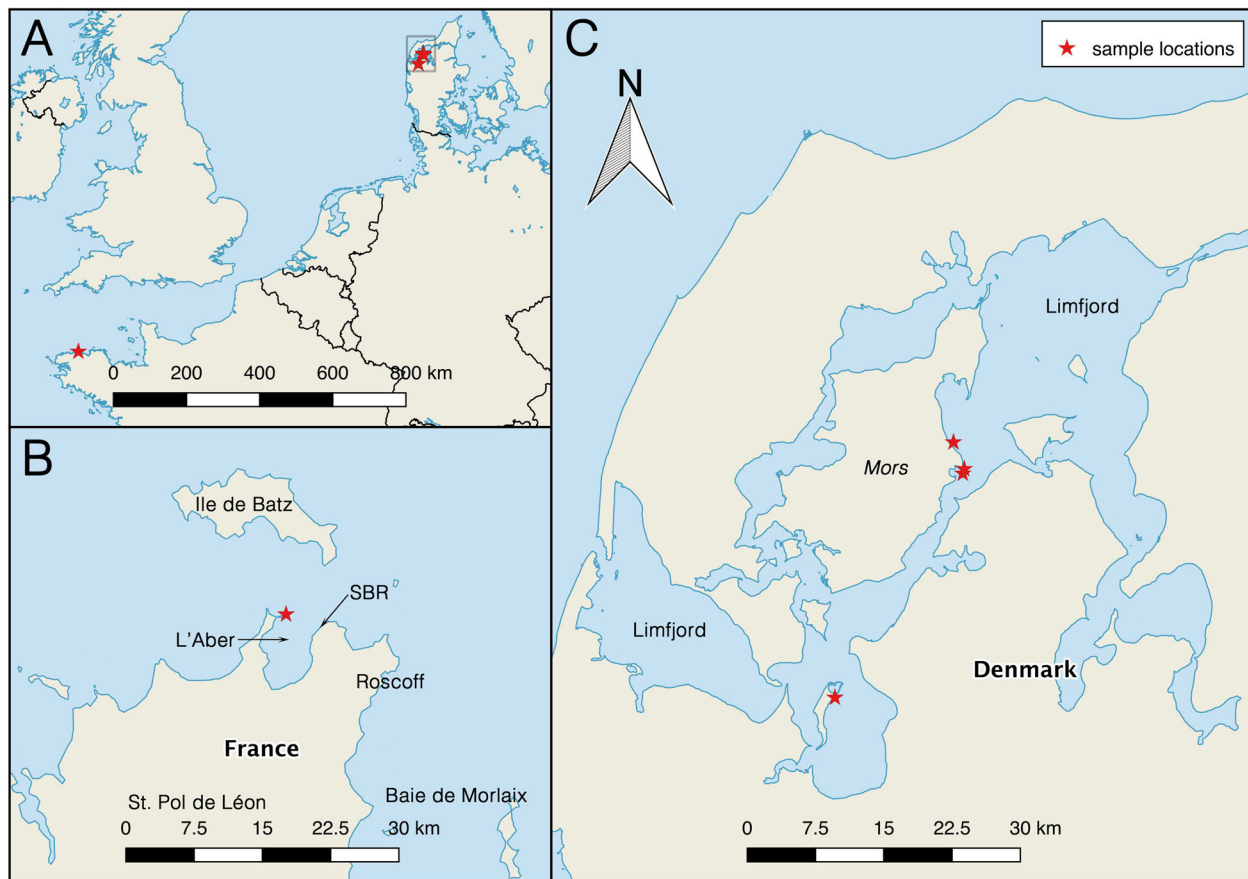


Figure 1. Sample locations. A. North Western Europe. B. France, Brittany, Roscoff; the sample area is a sandy inlet, l'Aber, covered in the western part with stones. C. Denmark, western part of the Limfjord and the narrow connection with the North Sea; the three sample sites were all located near the island of Mors.

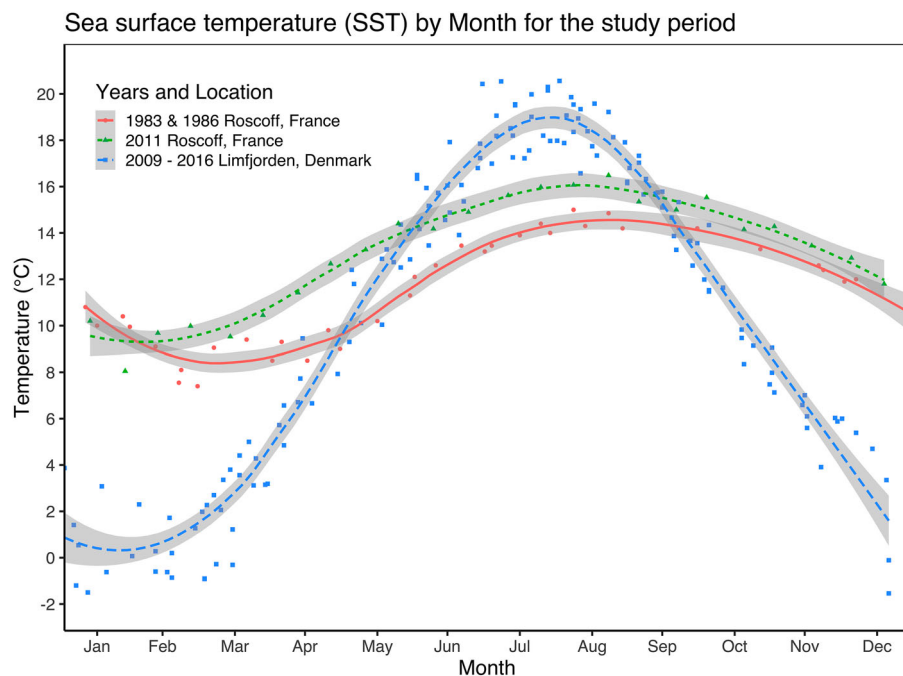


Figure 2. Monthly variation in Sea surface temperature (SST). The variation in SST for the two sample sites during the study period. Points represent the actual measurements, while the lines represent the smoothed conditional means and the gray area is the Standard Error. Measured near Roscoff in 1983, 1986 and 2011 (Dauvin et al. 1989; SOMLIT), and near The Danish Shellfish Centre, Limfjord in 2009–2016 (recorded by The Danish Shellfish Centre).

carcini into seven clearly separated stages (Lützen 1984; Høeg 1987; Høeg and Lützen 1995). They can easily be separated either by eye or using only low power magnification aids and are therefore useful for population studies of *S. carcini* (Figures 3 and 4). Stages 1–3 we collectively call ‘virgin’, even if a stage 3 may no longer be a true ‘virgin’. The reason is that

only inspection for settled males at the mantle aperture or sections of the receptacles can accurately decide if a stage 3 parasite has successfully received males (Supplementary video 2). Stage 3 externa are morphologically identical to stage 2 (Figure 3(c,d)), but biologically different in as much as the presence of males enables them to grow into sexual maturity.

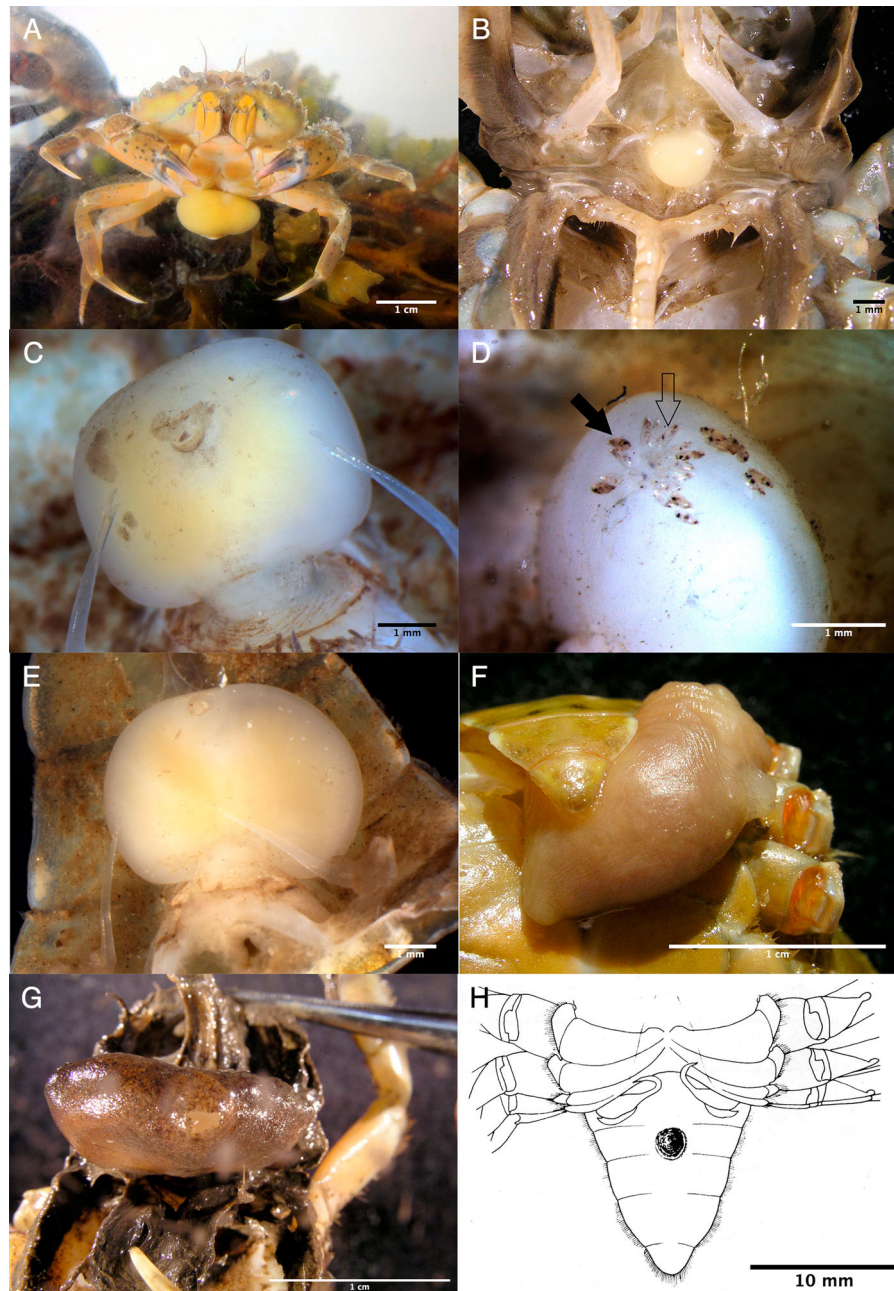


Figure 3. Developmental stages of *S. carcini* female externae. A. Adult externa (stage 5) under the abdomen of the host *C. maenas*. B. Stage 1 (pre-attractive virgin externa) no mantle aperture. C. Stage 2 (attractive virgin externa without males); D. Stage 3 (attractive virgin externa with males); identical to stage 2 but male cyprids now settled around the mantle aperture; virtually transparent cyprids (open arrow) have already metamorphosed into trichogon larvae; tissue filled cyprids (black arrow) still un-metamorphosed. E. Stage 4 (immature externa) with oocytes maturing in ovary. F. Stage 5 (adult externa); light brown colouration caused by developing nauplius eye in the brooded embryos; compare to yellow coloured externa in A, an adult soon after oviposition. See also text and Figure 4. G. Stage 6 (Old externa); darker colour and wrinkled surface that becomes progressively fouled. H. Stage 7 (Scar); Circular, blackish rings or areas marking the base of the peduncle of lost externa on the crab abdomen. Modified from Lützen (1984).

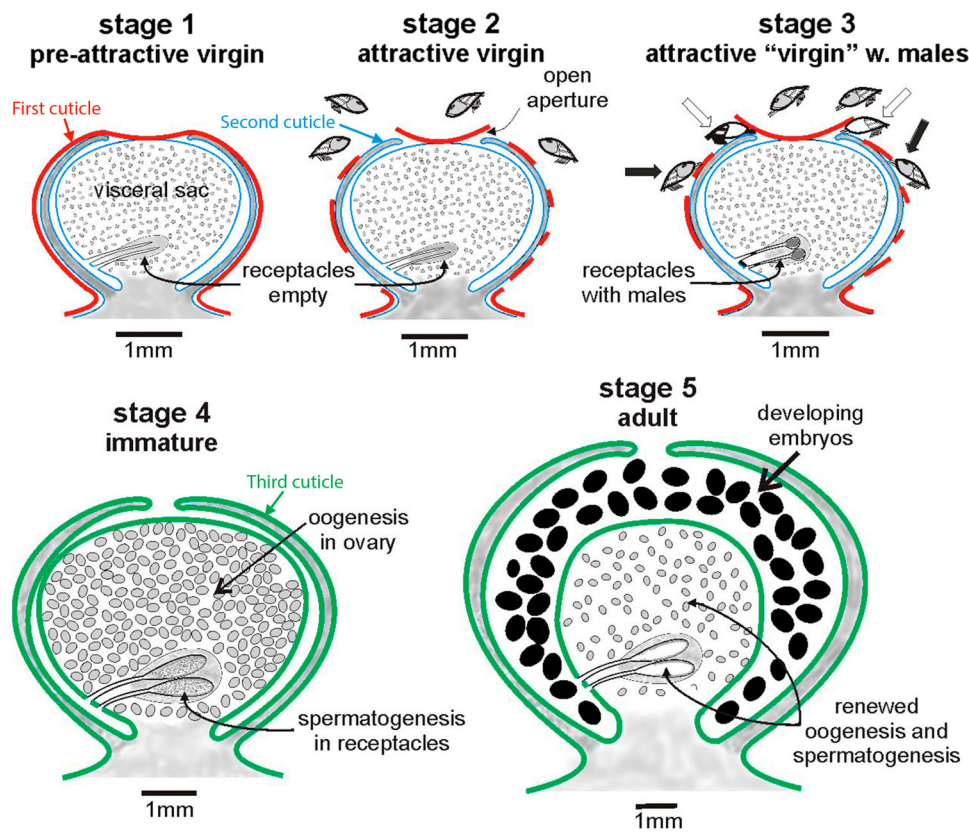


Figure 4. Schematic drawings of the developmental stages of the *S. carcini* externae. Stage 1 (pre-attractive virgin externa): First externa cuticle (red) continuous above the developing mantle, now forming as a part of the second externa cuticle (blue). Stage 2 (attractive virgin externa without males): First externa cuticle (red) now being shed as variably sized pieces, one piece always covering the mantle aperture; externa now attractive to male cyprids, but none have yet settled. Stage 3 (attractive virgin externa with males): Morphologically as stage 2, except for male cyprids now settled around 'hood' covering mantle aperture; two cyprids (white arrow) already metamorphosed and implanted into the two receptacles as dwarf males; any males from later metamorphosing cyprids (black arrow) will be denied access to the two already filled receptacles. Stage 4: (immature externa): Second externa cuticle (blue) now shed together with remains of settled cyprids and replaced with third externa cuticle (green); spermatogenesis and oogenesis in progress. Stage 5 (adult externa): Eggs spawned into the mantle cavity and fertilized by sperm from the receptacles; another reproductive cycle is about to begin in ovary and receptacles.

For ecological purposes, it is therefore crucial to separate stages 2 and 3, as this confers essential information on the success rate of recruiting males. Stage 3 externae are already on the path to sexual maturity and biologically not different from stage 4. Lumping stages 1–3 together due to their outward similarity confers little ecological information. Stages 1–4 externae are called juveniles to separate them from the reproductively mature stage 5 and 6. The complete life cycle of *S. carcini* with development times for the different stages, both for externa, male and female larvae, is illustrated in Supplementary Figure 2. Recently it has also been shown that the development of the parasites is correlated with the dominant colour through the intermoult stages of the host *C. maenas* (Lützen et al. 2018). If the crab has a single annual moult, the colour changes from green to orange to red and can serve as an approximate

marker of the time spent in the intermoult stage. This information is included in our description of the *S. carcini* externa stages, as it could be helpful in future ecological studies.

Stage 1 - Pre-attractive virgin externa (duration < 6 days). This stage extends from the emergence of the externa on the host crab and until it acquires an open mantle aperture at its first moult. The stage 1 externa is 2–3 mm wide (Figure 5), whitish and almost transparent, because oogenesis is arrested at this very early stage. The externa cuticle is continuous around the apex with no mantle aperture, although late in the stage this prospective opening is becoming visible beneath the 1st externa cuticle. Male cyprids are not attracted to and never found settled on this stage. The emergence of the externa takes place 3–4 days after the host has moulted (Lützen et al. 2018). Predominantly on green crabs (Lützen et al. 2018).

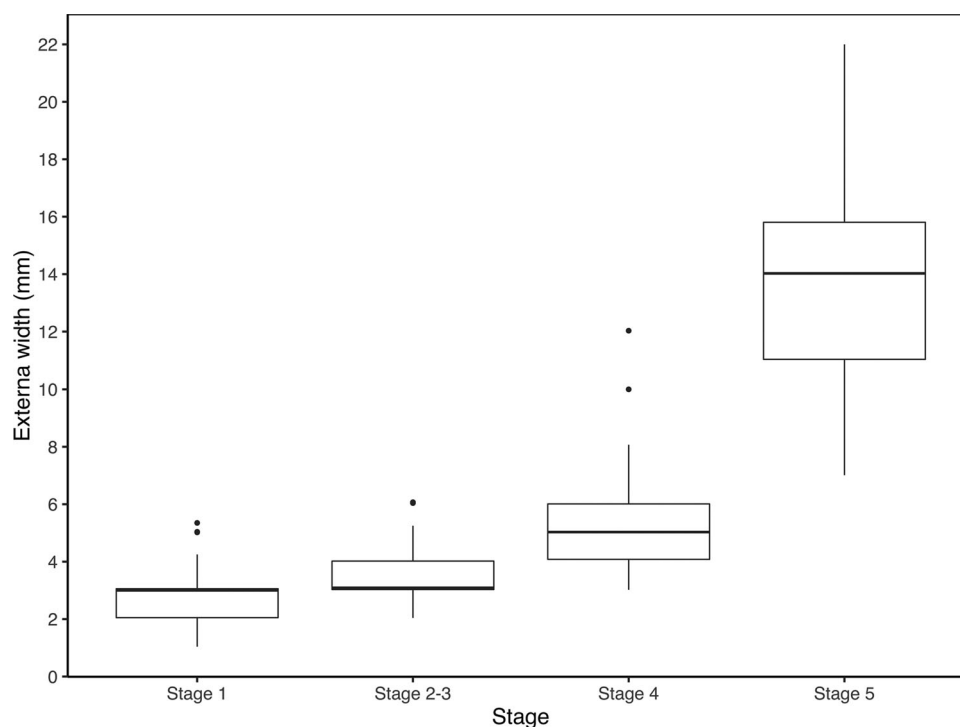


Figure 5. Size variation in external growth stages of *S. carcini*. From the Limfjord and Roscoff field samples ($n = 637$), there was no significant difference between the externa sizes at the two localities. The black dots represent outliers. Stage 1, $n = 258$. Stages 2–3, $n = 204$. Stage 4, $n = 145$. Stage 5, $n = 30$.

Stage 2- Attractive virgin externa without males (duration variable). This stage is only slightly larger, 3–5 mm width (Figure 5), than stage 1 and extends from the first appearance of an open mantle aperture and until settlement of a male cypris larva. The moult from stage 1–2 is gradual, since the 1st externa cuticle peels off as variably sized pieces. One of these cuticle pieces always remains as a hood that partially covers the aperture, thus restricting access into the mantle cavity to narrow crevices between hood and 2nd externa cuticle (see Høeg 1987). Male cyprids settle and metamorphose around the edge of this hood, and their absence serves to separate this stage from the following, which otherwise have exactly the same size and morphology. Failing to receive any male, the externa remains at this stage and becomes progressively overgrown with epibionts, so it acquires a brownish, ‘dirty’ look. Such aging, uninvaded virgin stage 2 externae were also collected from the field. The epibionts growth around the mantle aperture can become so extensive that it may prevent settled males from entering into the externa altogether. Such old virgin externae also seem to gradually ‘swell up’ and they eventually perish, probably within a few weeks after the moult to stage 2. Predominantly on green crabs (Lützen et al. 2018).

Stage 3- Attractive virgin externa with males (duration ca. 6 days). This extends from the settlement of the first male and until the externa resumes growth and ceases to be attractive to other male cyprids. Following settlement, the male cyprid metamorphoses into a very slender trichogon larva that penetrates through the narrow crevices beneath the hood covering the mantle aperture and thence through the mantle cavity and into one of the two receptacles located in the visceral sac. Only a single male can enter each receptacle, whence an externa can maximally host two males (Høeg 1987). The arrival of the first male into a receptacle stimulates the externa to resume development, and it only remains attractive for another 6 days, so any additional male cyprids must settle within this time window, after which the parasite enters stage 4. Cyprids metamorphosed into trichogons, remain as empty almost transparent shells at their original settlement site around the mantle aperture (Figure 3(D), and Supplementary video 2). A successfully implanted male can often be spotted as a pigmented body in the visceral sac of the still almost transparent externa. Predominantly on green crabs (Lützen et al. 2018).

Stage 4 – Immature growth phase (duration ca. 2–4 weeks). This stage begins when the stage 3 externa ceases to attract males and starts to increase in size (Figures 3(e) and 4), externa width 4–12 mm (Figure

5). The shift from stage 3–4 is also marked by a moult that involves shedding the 2nd externa cuticle that lines both the external surface and the mantle cavity. As a result, any cyprid remains around the mantle aperture are shed with the cuticle. The same is true of any supernumerary trichogons ‘stranded’ in the mantle cavity because access was blocked by earlier arriving ones (Høeg 1987). During stage 4 the externa increases almost linearly in width and the visceral sac becomes increasingly opaque due to progressing oogenesis in its ovary (Supplementary video 1). Predominantly on green crabs (Lützen et al. 2018).

Stage 5 – Adult externa (duration ca. 1 year; width > 10 mm). The adult stage begins with the release of the first batch of eggs into the mantle cavity concomitant with the release of sperm from the receptacles, and it is reached ca. 30 days after the virgin received its first male. Due to oviposition, the visceral sac has shrunk in size, rendering space for the now embryo filled mantle cavity that is ventilated by vigorous peristalsis of the mantle musculature. Following the first oviposition, a second reproductive cycle starts in ovary and receptacles. The newly released embryos provide the externa with a yellowish colour, but it eventually becomes very dark brown due to the developing nauplius eyes in the prospective nauplius larvae (Figure 3 (f) and Supplementary video 1). When collected and placed in clean, aerated seawater, such very dark coloured externa will almost invariably release nauplii within 1–2 days. The younger stage 5 externae are bright yellow or orange coloured at oviposition and have a smooth and clean outer surface. The total time from the emergence of the externae (stage 1) to the adult externa (stage 5) is estimated to be 4–6 weeks (Lützen 1984). The adult externae is equally frequent on the green, orange and red crabs (Lützen et al. 2018).

Stage 6 – Old externa (ca. 3–4 months). These aged externae can be distinguished from younger ones by having a darker colour and wrinkled surface that becomes progressively fouled. Often, the broods contained in such externae consist to a large part of unfertilized eggs. Very old externae can be very extensively overgrown with various organisms, almost black in colour and in obvious ill health. These changes in appearance can give an estimate of the age of the externa (Lützen 1984). In our study, old externae, whatever their appearance, were for simplicity always included in our stage 5 category. Predominantly on red crabs (Lützen et al. 2018).

Stage 7– Scar (duration variable). Circular, blackish rings or areas marking the base of the peduncle of lost externa on the crab abdomen. Predominantly on red crabs (Lützen et al. 2018).

Results

Limfjord samples

In this locality, 49,682 *C. maenas* were caught during the period 2009–2012. Of this 9.42% were infected visibly with externae of *S. carcini*, with some variation between years and seasons (Table I). This study was part of a larger study exploring factors governing infection patterns, see Mouritsen et al. (2018) for a detailed exploration of these factors. The seasonal variation of external developmental stages is shown in Table II. A contingency table test shows that the numbers of juvenile and adult externae deviate significantly between the summer and winter seasons (chi-square = 231.92, $P < .001$). A subsample of 627 juvenile externae (= stages 1–4), covering both seasons, were examined in detail, including an allocation to stage and recording the number of settled male cyprids (Table III). This externae comprised 26.3% of all juveniles (= 2383 stage 1–4 externae) sampled during 2009–2012 in the Limfjord. Both the ratio of virgins invaded by males (stage 3) and the mean number of settled cyprids on these externae varied between seasons. In the Limfjord, more males settled on the females during the summer months ($p < .001$), both when calculated as a mean on all juvenile externae (summer = 0.56, winter = 0.12) or as a mean of the attractive stages 2 + 3 only (summer = 1.17, winter = 0.62). Likewise, more externae were found to carry male cyprids

Table I. Prevalence (%) of *S. carcini* on 49,682 *C. maenas* sampled in the Limfjord, Denmark, 2009–2012.

Year	Summer (Apr.–Sep.)	Winter (Dec.–Mar.)	Total
2009	4.31	8.55	6.43
2010	9.55	9.86	9.59
2011	10.25	11.52	10.69
2012	7.17	20.19	11.38
Total	8.64	10.97	9.42

Notes: All external developmental stages of *S. carcini* are included, but not crabs with scars from lost externae. Samples pooled into summer period (April–September) and winter period (December–March).

Table II. Seasonal variation of external developmental stages of *S. carcini* in the Danish Limfjord.

	Virgin (stages 1–3)	Immature (stage 4)	Total juvenile (stages 1–4)	Adult (stage 5)	All stages
Limfjord					
Summer	1031	655	1686 (69.8%)	728 (30.2%)	2414 (100%)
Winter	256	441	697 (43.0%)	924 (57.0%)	1621 (100%)
Total all year	1287	1096	2383 (59.0%)	1652 (41%)	4035 (100%)

Notes: A contingency table test shows that in the Limfjord the numbers of juvenile and adult externae deviate significantly between the Summer and Winter seasons (chi-square = 231.92, $P < .001$).

Table III. Frequency of juvenile externa stages (1–4) and male settlement success in the Danish Limfjord and at Roscoff, France.

	External stage of development						Cypris males		
	1 Unattractive	2 Attractive no males	3 Attractive with males	4 Immature	1 – 4 All juveniles	2 + 3 All attractive	3 Attractive with males		Mean # of males per stage
							Of all juveniles (1 – 4)	Of attractive (2 + 3)	
<i>Limfjord</i>									
Summer	91	90	68	80	329	158	20.7%*	43.0%*	0.56* 1.17*
Winter	159	32	20	87	298	52	6.7%*	38.5%*	0.12* 0.62*
Total	250	122	88	167	627	210	14.0%	41.9%	0.35 1.03
<i>Roscoff</i>									
Summer	9	242	80	113	444	32	18.0%	24.8%*	0.27* 0.37*

Note: The values marked with * were tested for differences between summer and winter for the Limfjord and for differences between the Limfjord and Roscoff (see details in text).

during the summer, whether calculated as a ratio of all juveniles (summer = 20.7%, winter = 6.7% $P = .005$) or as ratio of the attractive stages 1 + 2, only (summer = 43.0%, winter = 38.5%, $P = .034$).

Roscoff samples

In this locality, we sampled 444 externae during the summer seasons of 1983 and 1986, of which 80 (18.0%) were at stage 3 and thus carrying male cyprids. Here, the mean number of attached cyprids was less than in the Limfjord summer population, with only 0.27 cyprids per externae on all juveniles (stages 1–4) and 0.37 per the attractive stages 2 + 3. Winter samples were not available from this locality, but there was a distinct variation during the summer season in the ratio of virgin stages (stages 1–3), with a high 46.6% among a total of 179 externae in late May and early June to a very low 1.1% among 285 externae in September. A sample taken in late June 2011 fell between these two extremes with 6.1% stages 1–3 among a total of 66 externae. The latter sample was not closely inspected for separation into stages 1–3. The prevalence of *S. carcini* at the l'Aber was not recorded for all samples and is hard to estimate accurately, since during September 1986 it varied on a daily level from 10.4% to 43.0%, apparently owing to weather (rain versus sun), precise time during the low tide and the level of the tide at sampling (personal observations). In the 2011 sample, special care was taken to catch all crabs under the stones and the prevalence was 14%, which matched a prevalence of 12% obtained from baited traps put out in the same area.

Externae growth

The time from emergence to the attractive stage 2 was ca. 3 days. There was a very clear difference in growth

between virgin externae without males (stage 2) and those that had received male cyprids (stage 3) by controlled settlement in the laboratory (Figures 6, 8, 9). The virgins without males were kept in isolation and showed no signs of oogenesis in the ovary and they remained at an externa width less than 5 mm and never moulted. Externae with males (stage 3) started to increase in size ca. 6 days after male settlement, thus entering stage 4, and thereafter they grew almost linearly until the adult stage 5 was attained at an externa width of ca. 12 mm. The juveniles sampled in the Limfjord and observed over time gave comparable results, even though for these we did not know the exact time of male settlement (Figures 7, 8, 9). The time from stages 2–3 obviously depends upon the availability of cyprids in the plankton. The total time from male settlement to sexual maturity (stages 3–5) was approximately one month at both localities (Figures 6 and 7).

Discussion

The rhizocephalan *S. carcini* is the most common parasite on the European shore crab *C. maenas*, a key member of shallow water and intertidal communities. *S. carcini* exerts multiple and far reaching effects on this host crab, including partial or complete parasitic sterilization and arrestment of the moult cycle. The parasite is, therefore, a potentially important regulator of *C. maenas* populations (Thresher et al. 2000). For understanding the distribution and prevalence of *S. carcini*, and hence the effect on the host crab populations, it is essential to take into account the role played by the males of this parasite. We have studied *S. carcini* in Danish and French populations and found distinct variations in the relative frequency of external developmental stages and in the success rate with which virgin parasites acquire the male larvae essential for developing into adulthood. Furthermore, we

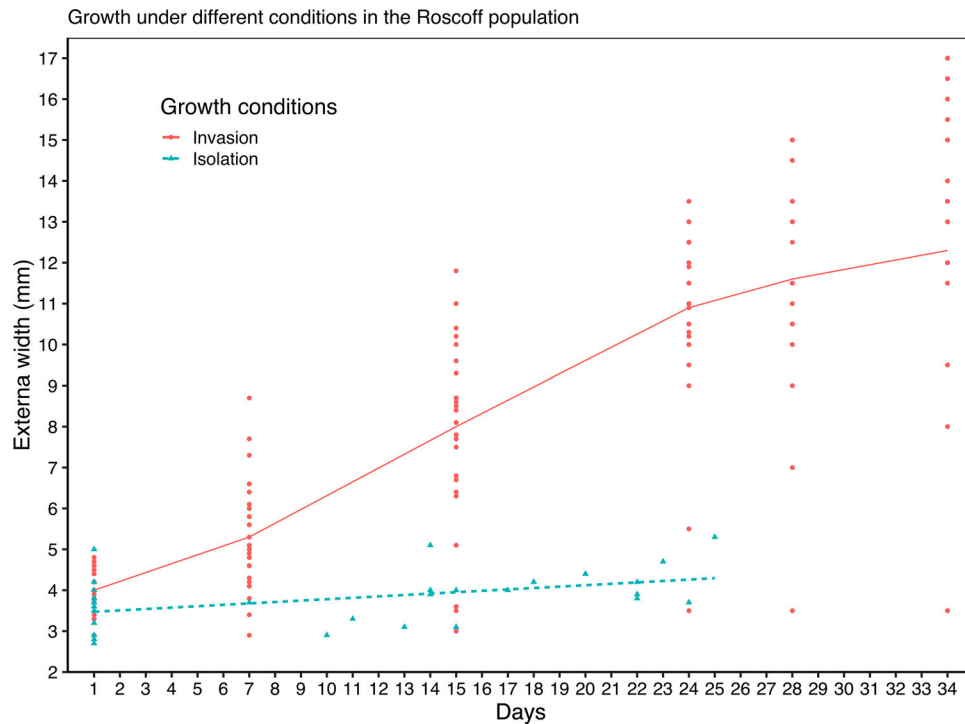


Figure 6. Growth of *S. carcini* externae under different conditions in the Roscoff population. Externa width (EW) in mm plotted against days since experiment start (male invasion), the lines represent the average. Points represent measurements of the externa and assessment of the externa developmental stage. Invasion (solid line): Virgin externae (stage 2) experimentally invaded by male cyprids in the laboratory ($n = 37$). Isolation (dashed line): Externae kept in isolation ($n = 10$). Virgins that received males (stage 3) started immature growth within ca. 5 days and reach adulthood (stage 5) in ca. 30 days. Attractive virgin externae (stage 2) that were experimentally prevented from receiving males grew little if at all within the same time frame.

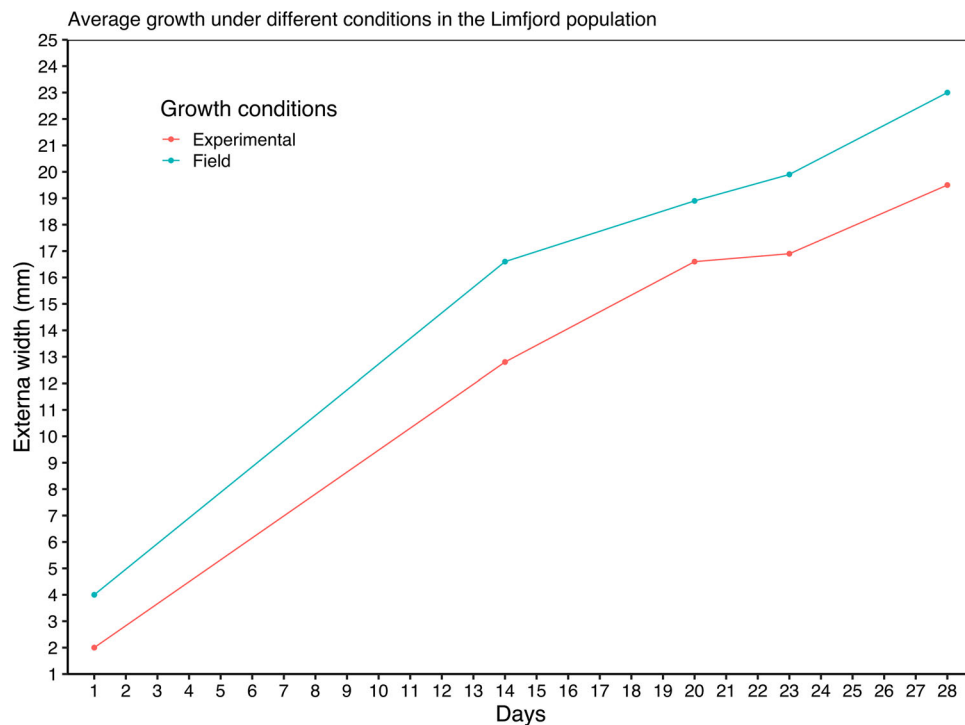


Figure 7. Average growth of *S. carcini* externae under different conditions in the Limfjord population. Experimental: Virgin externae (stage 2) reared and experimentally exposed to male cyprids in the laboratory ($n = 8$). Field: 'Virgin' externa (stage 3 – invaded by males in the field) collected in the wild and reared in the laboratory ($n = 4$). The externa collected in the field that did not grow (externa 9, 13 and 15, see Figure 9) were excluded when calculating the average.

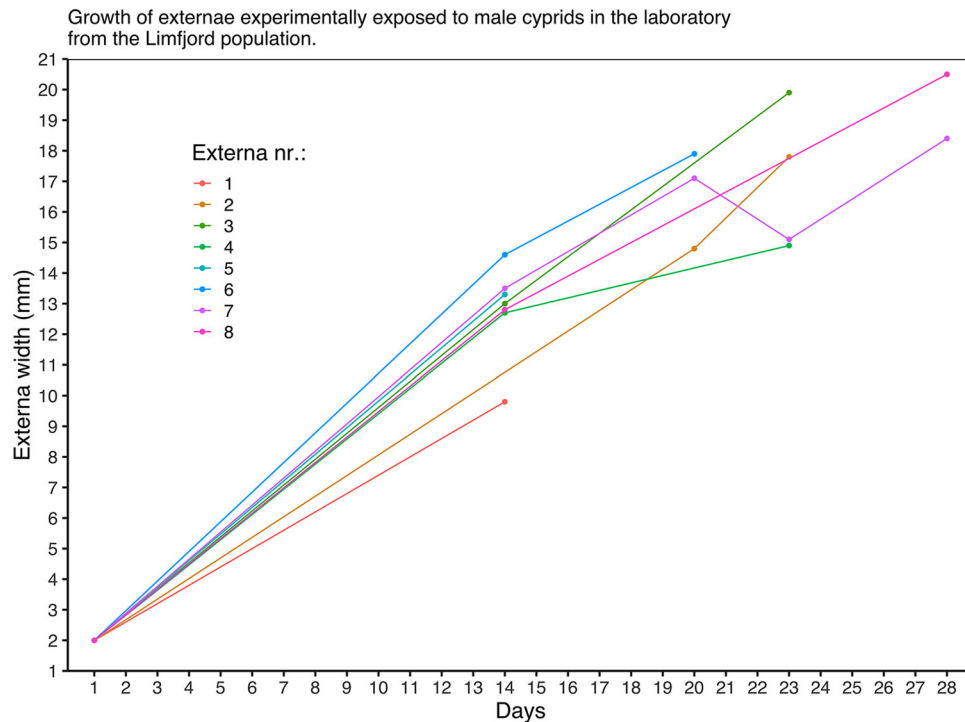


Figure 8. Growth of *S. carcini* externae reared and experimentally exposed to male cyprids in the laboratory from the Limfjord population. Virgin externae (stage 2) reared and experimentally exposed to male cyprids in the laboratory ($n = 8$). Host crabs where kept separately so the individual growth of each externa could be followed. The end of the line symbolized the death of the host crab, i.e. externa 8 and host died at day 14. The measurement for externa 7 at day 23 is a measurement error or the externa measured when contracting, not negative growth.

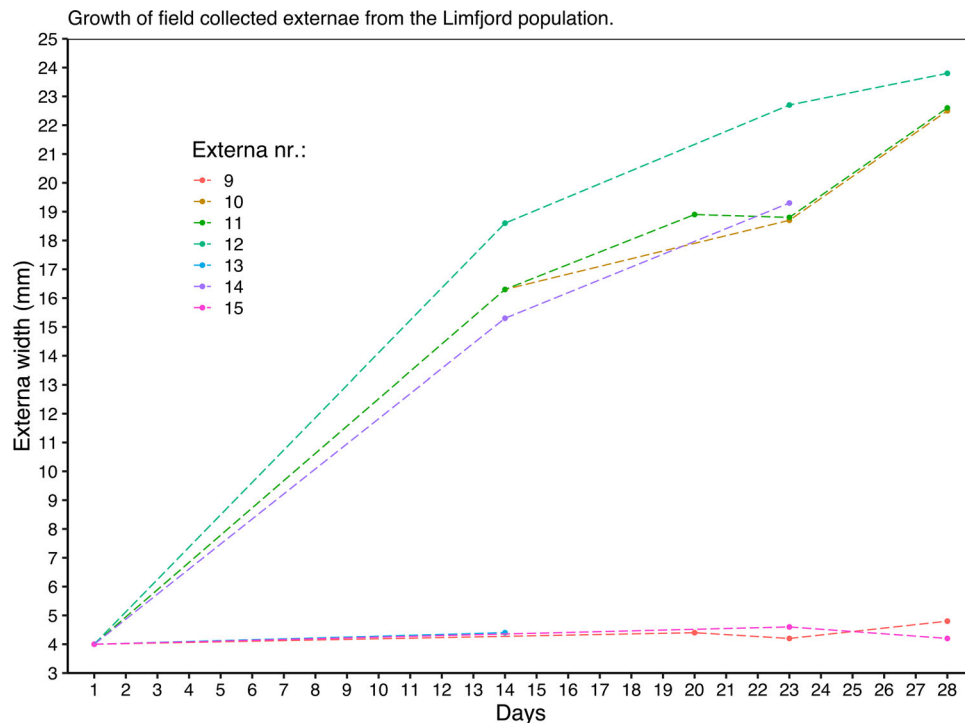


Figure 9. Growth of field collected *S. carcini* externae from the Limfjord population. 'Virgin' externa (stage 3 – invaded by males in the field) collected in the wild and reared in the laboratory ($n = 7$). Externa 9, 13 and 15 did not grow. Host crabs where kept separately so the individual growth of each externa could be followed.

followed for the first time the growth of juvenile parasites into adulthood following controlled implantation of males by laboratory reared larvae.

Frequency of external developmental stages

At Roscoff, France there was a distinct seasonality between early and late summer in the frequency of juvenile externae (stages 1–4) of *S. carcini*. Juveniles predominated in late May and early June but decreased over the summer until constituting less than 10% of the population in September. In the Danish Limfjord we also found seasonal variation in the frequency of juveniles, but here between summer and winter samples, the latter not being covered at Roscoff (Tables II and III). Also in contrast to Roscoff, juvenile externae never amounted to less than 30% in the Limfjord population. For a more in depth exploration of the frequency of external developmental stages in the Limfjord population see Mouritsen et al. (2018). The relative frequency of growth stages has never been analyzed in this detail in any other rhizocephalan, but a seasonality seems also to exist in *Lernaeodiscus porcellanae* Müller, 1862, parasitizing porcelain crabs in southern California and in the circumarctic *Clistosaccus paguri* Lilljeborg, 1861, parasitizing hermit crabs (Ritchie and Høeg 1981; Høeg 1982).

Male acquisition by virgin parasites

Both the frequency of virgins that had acquired males and the mean number of male cyprids actually settled on male attracting parasites varied with both season and locality (Table III). In the Limfjord, the success rate of virgin externae was high during the summer and low during the winter, while at Roscoff the acquisition of males during the summer was much lower than during the same season in the Limfjord, even though the parasite prevalence at the two localities was comparable. Some individual stages 2 and 3 externa would, of course, have accumulated additional males if they had spent more time in the field. Nevertheless, the differences observed, including the failure of some virgins to obtain any males at all, most likely reflects the situation in the field, because, as discussed below, attractive virgin externae of *S. carcini* have only a relatively short time window in which to acquire their males before they perish.

In the Limfjord, the relatively low success of virgins during winter is probably explained by a low density of male cyprids in the plankton, but the presence of recently settled males in juvenile externae sampled in November and December shows that reproduction of *S. carcini* also extends into this cold part of the year.

This fits with observations (data not given) that adult externae with nauplii ready for release can also be found this late in the season.

At Roscoff, the low success in acquiring males during the summer compared to the Limfjord may be due to differences in hydrodynamics affecting larval dispersal. The Limfjord is a semi-enclosed, comparatively stable water body not affected by tides (Figure 1(C)). We, therefore, suggest that larvae are contained rather locally. Also contributing to the density of parasite larvae would be the high prevalence levels we found for *S. carcini* in all our three sample locations separated by some kilometres (Figure 1(C), data not given). At Roscoff, our sample area directly faces the narrow channel between the mainland and Ile de Batz, a location famed for an extreme tidal range and resulting violent currents. Therefore, *S. carcini* larvae are likely to be dispersed far away, whence the local high prevalence at our sampling site (12–14% for the 2011 sample) will not necessarily be accompanied by a high local density of larvae. In fact, our multiple surveys of other sampling sites near Roscoff often yielded no parasites at all, so the high parasite prevalence at our sample site does not reflect the general situation along this part of the Brittany coast.

The acquisition of male larvae by virgin externae is pivotal to rhizocephalan populations (Yanagimachi 1961; Lützen 1984; Høeg and Ritchie 1985; Høeg and Lützen 1995), and variations in virgin success may well contribute both to differences in parasite prevalence between localities and the existence of host populations completely free of parasites. In the Limfjord the *S. carcini* virgins found in the early winter, even with their low success rate of recruiting males, may well play a pivotal role, since they are likely to mature into adults and survive into the next season, so that they can provide the males needed by the virgins emerging as a result of last years' infections of new crabs. However, if few or no male larvae are available for emerging virgins the parasite population will easily become extinct. This may explain why *S. carcini* has only rarely accompanied *C. maenas*, when the latter has spread to numerous areas around the world as an unwanted marine immigrant (Grozholz and Ruiz 1995; Torchin et al. 2001). Even if adult *S. carcini* were ported to a new locality, the virgin parasites resulting from new infections would approximately one year after need to acquire male cyprids, but at this time most if not all the originally imported adults are likely to have perished from old age (Lützen 1984). Therefore, if the new population is far removed from a large source of male cyprids, it cannot be sustained. *S. carcini* is, therefore, a prime

example of the intimate links between sexual system, larval biology and geographical distribution. Still, a few examples are known, where species of immigrant crabs have carried their sacculinid rhizocephalan parasite with them to a new locality (see Øksnebjerg 2000) and these isolated examples are probably explained by the repeated introduction of new, parasitized crabs.

Males and juvenile development in rhizocephalans

It is frequently stated that female rhizocephalan parasites cannot reach sexual maturity unless they receive males (Brusca and Brusca 1990), but in reality, documentation exists only for a handful of species (see Høeg and Lützen 1995). For *S. carcini*, Lützen (1984) and Høeg (1987) were the first to demonstrate the role played by males, and we have verified and extended these observations by observing, for the first time, the growth of juveniles into adults following laboratory controlled implantation of males.

In the few investigated rhizocephalans, the time required from male implantation to sexual maturity varies from 1 week to 2–3 months, but this may largely be an effect of temperature. The cold water rhizocephalan *C. paguri* required 2–3 months to reach adulthood at 10°C, and a very large proportion of its external life span is spent as a non-reproducing juvenile (Høeg 1982). In contrast, *L. porcellanae* from S. California required only 1 week in the summer (24°C) and 3 weeks in the winter (13°C) to grow into adulthood following male implantation into the virgin externa (Høeg and Ritchie 1985), and this compares rather well with the 3 weeks presently observed for *S. carcini* kept at ca. 12°C. We also found that *S. carcini* required ca. 3 days from emergence to attaining the male attracting stage 2, and if we assume that externae generally live no longer than 1 year, they will spend only about 10% of the external life span in the juvenile phase. In lernaediscid and peltogastrid rhizocephalans, both infecting Anomura, the adult reproductive phase can be very long (several years) so the juvenile phase amounts to even less of the life span (Høeg and Lützen 1995).

Following Lützen (1984) we assume that *S. carcini* virgins that fail to recruit males soon after emergence will eventually perish. At both of our sampling localities, we found aged stage 2 virgins that were quite clearly in ill health and probably incapable of receiving males altogether due to accumulating epibionts that obstructed the mantle aperture area. This emphasizes how critical the ready availability of male cypris larvae is. Those failing to receive males soon

after emergence are unlikely to ever attain the adult stage and thus become lost from the breeding population. This is very different from the situation in *L. porcellanae*, where virgin externae can remain perfectly healthy for at least six months and still attract males and then grow to sexual maturity as quickly as if males were acquired soon after emergence. The reason is that the host, a porcelain crab, continuously groom its parasite, in effect mimicking the behaviour normally shown towards a brood of embryos (Ritchie and Høeg 1981; Høeg and Ritchie 1985).

Conclusion and perspectives

For the rhizocephalan parasite *S. carcini* we have demonstrated both seasonal and regional variation in the frequency of external developmental stages and in the success with which juvenile, virgin parasites acquire the male larvae necessary for reaching adulthood and thus enter the breeding population. We suggest that variations in the availability of male cypris larvae may contribute to differences in parasite prevalence and also in part explain the total absence of the parasite in some host populations. To arrive at a deeper understanding of population dynamics of this important parasite it would be vital to make long term studies of its prevalence in *C. maenas* and of variations in the frequency of external developmental stages and the sex ratio of the larvae to understand why these parameters vary both seasonally and regionally. In addition, methods to study the prevalence and dispersal capability of the parasite larvae would be very valuable.

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