Host-parasite interactions in the European shore crab *Carcinus maenas* and their implications for the invasion success of this introduced species.

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In memory of my father.

Abstract

As a consequence of globalisation and worldwide trade, invasive species increasingly pose a problem for ecologists and conservationists. In the invasive European shore crab *Carcinus maenas*, parasite loss during introduction was suggested to be a main reason for invasion success (parasite release hypothesis, PRH), based on observations of a correlation between crab mean size and biomass and the prevalence of parasitic castrators in Europe.

This study aimed at further investigating this claim by examining host-parasite interactions of *C. maenas* and their consequences on individual fitness, with a focus on reproductive potential and immunological factors. To accomplish this, I examined crabs from a number of European populations, as well as from invasive populations in South Africa and Australia.

My parasitological investigations showed that the source of invasive populations of this crab is an important factor for the consideration of the PRH, as parasite prevalence and intensity is highly variable, even on small scale geological distances, within Europe. However, my results on reproductive potential and on immune parameters of the hemolymph lend little further support to the PRH hypothesis. Parasitic castrators had a clear effect on reproduction, but apart from that the crabs were mostly unaffected by parasites, both on the individual level and the population level. There was weak evidence for an effect of microphallid parasites on hemocyte concentration under the influence of secondary stressors. Analysis of the age-intensity relation of helminths indicated that high intensities may lead to mortality in the crab hosts. Experimental infections with microphallids did not confirm these findings, but reduced hemolymph protein levels in infected crabs indicates that fresh infections strain the hosts metabolism and lead to a slight nutrient deficit.

Based on these results, I suggest that parasites are not the major factor controlling or regulating *C. maenas* populations in Europe. It is more likely that multiple factors act on them, including chemical and physical characteristics of the environment, as well as community interactions. A loss of parasites during the invasion process may therefore contribute to the success of the shore crab as an invader, but it is unlikely to play a major role. Resource availability, community interactions or temperature regime in the invaded ecosystems could also be of relevance. In addition, my study focused on the effect of macroparasites, but the role of microparasites and other pathogens for *C. maenas* is largely unknown. Research into these areas may yield more information about the invasion success and may help to find new control measures against this invasive species.

Zusammenfassung

Invasive Arten stellen zunehmend eine Gefahr für die weltweite Biodiversität dar. Häufig wird der Invasionserfolg solcher Arten dem Fehlen natürlicher Feinde im neuen Lebensraum zugeschrieben. Zu diesen gehören auch Parasiten, wie im Fall der invasiven europäischen Strandkrabbe *Carcinus maenas*. Für diese Art wurde ein Zusammenhang von Durchschnittsgröße und Biomasse einer Population mit der Prävalenz von parasitischen Kastratoren festgestellt.

Ziel meines Promotionsprojektes war es, die Rolle von Parasiten - und deren Fehlen - für den Invasionserfolg der Krabbe zu untersuchen. Zu diesem Zweck wurden Krabben aus Europa und aus den kolonisierten Gebieten in Australien und Südafrika auf Parasiten untersucht, sowie auf deren Auswirkungen auf die Fitness des Wirtes. Hierbei wurde besonderes Augenmerk auf das reproduktive Potential und auf Immunfaktoren gelegt, die als Fitnessindikatoren dienen.

Meine Untersuchungen zeigen eine starke Variation sowohl in Bezug auf die Prävalenz als auch die Intensität von Parasiten in Europa. Aus diesem Grund spielt die Ursprungspopulation eine große Rolle bei der Beurteilung der Bedeutung von Parasitenverlust für den Invasionserfolg. Ich konnte bestätigen, dass parasitische Kastratoren den erwarteten negativen Effekt auf die Wirtsreproduktion haben. Was jedoch andere Parasiten sowie andere Fitnessparameter betrifft, konnte ich im Großen und Ganzen keine weiteren negativen Auswirkungen feststellen, weder in individuellen betroffenen Krabben noch auf Populationsebene. Es gab jedoch schwache Hinweise auf Mortalität bei starken Infektionen mit microphalliden Digenea sowie Anzeichen für Veränderungen in der Hämocytenkonzentration unter dem Einfluss von sekundären Stressoren. Experimentelle Infektionen mit diesen Parasiten konnten diese Beobachtungen nicht bestätigen, erniedrigte Proteinkonzentrationen in der Hämolymphe deuteten hier jedoch darauf hin, dass die generelle Konstitution des Wirtes beeinträchtigt wird.

Zusammenfassend kann aus meinen Ergebnissen geschlossen werden, dass Parasitenbefall nicht der Hauptfaktor ist, der die Populationen von *C. maenas* in Europe kontrolliert. Es ist wahrscheinlich, dass zahlreiche Faktoren sowohl abiotischer als auch biotischer Art zusammenwirken. Daher ist davon auszugehen dass der Verlust von Parasiten im Verlauf des Invasionsprozesses nur bedingt zum Erfolg der Strandkrabbe in Übersee beiträgt. Andere Faktoren, die bisher noch kaum untersucht wurden, sind die Verfügbarkeit von Ressourcen und die unterschiedlichen Temperaturverhältnisse. Darüber hinaus beschränkt sich meine Arbeit auf die Auswirkung von Makroparasiten. Auch die Untersuchung anderer

Pathogene könnte daher Aufschluss geben über den Invasionserfolg der Art, sowie Möglichkeiten für die Kontrolle der invasiven Populationen eröffnen.

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Contents

1 Introduction

1.1 Biological invasions and marine ecosystems

Globalisation has become a key word over the last decades, gaining relevance not only in economic matters but also for our environment. While popular and media interest is often focused on climate change, biologists and conservationists also worry about a second implication of globalisation: the issue of introduced species (VITOUSEK et al. 1997). The worldwide transport of goods is accompanied by the intentional and unintentional transport of organisms – animals and plants including pathogens. These are becoming increasingly relevant in studies of ecosystem functioning and homeostasis (LOCKWOOD et al. 2007).

Intentional introductions by humans are common and include agricultural and ornamental plants, domestic animals and pets as well as species that have been introduced for biological pest control (Davis 2009). This mode of species transport may be past its peak now, due to an already globalized agricultural world and increased awareness of the consequences that the release of non-indigenous species (NIS) can have. However, unintentional transport of organisms is linked to the worldwide transport of goods, which is still increasing. Today, an estimated 480,000 NIS have been introduced into various ecosystems (COLAUTTI & MACISAAC 2004), the fact that only a fraction of the NIS arriving in a new area can establish stable populations notwithstanding (WILLIAMSON & FITTER 1996a). Despite this high number of introduced species, only those which are considered to pose an economic or ecological threat are typically termed invasive species (COLAUTTI & MACISAAC 2004).

This threat, however, can be substantial. Invasive species can have a significant impact on the respective ecosystems and are now one of the most important factors reducing worldwide biodiversity. They are either a major cause of or involved in the extinction of many native species (CLAVERO & GARCÍA-BERTHOU 2005). The damage caused by invasive species is hard to quantify and it is often only recorded in places where humans suffer direct negative effects, which is common in agriculture and fisheries. For example, BRIGHT (1999) calculated that NIS-caused yearly losses for the US agriculture of up to 248 billion dollars. The consequences for the affected ecosystems and native species can rarely be estimated in monetary terms, but examples like the South African Fynbos region may show the magnitude of the problem. In this unique ecosystem 80% of the endangered species earned their status due to the threat by invaders (ARMSTRONG 1995).

One reason for the serious impact of invasive species is the exceptional success some of them show in the invaded areas compared to their native habitats. John Elton (1958), often considered the founder of invasion biology, describes this process as an “ecological explosion”. A commonly cited example for such an explosion is the introduction of the zebra mussel (*Dreissena polymorpha*) to the Great Lakes of the United States. It was first observed in the late 1980s (GRIFFITHS et al. 1991), but within two decades the species spread over the complete eastern part of the US, including all Great Lakes and the Mississippi river system. The mussels cover any suitable substrate at very high densities, are very adaptable and cause substantial economic losses and alterations to ecosystems (MILLS et al. 1994).

Dreissena, despite being a freshwater species, was introduced to the United States with ballast water of transatlantic ships (GRIFFITHS et al. 1991) which demonstrates a specific aspect of marine invasions. These have a history that is closely connected to maritime navigation (CARLTON 1989). Fouling on wooden ships and boats was the first transport medium, both for the fouling organisms and for small fish and invertebrates that could use the fouling organisms as a substrate. In modern ships, huge volumes of ballast water are transported from port to port over great distances and with increasing speed, enhancing the survival chances of accompanying organisms. CARLTON (1989) estimates that over 10,000 different species are ferried across the globe in ballast water each day. In addition to this process, aquaculture and fisheries industries lead to large numbers of introductions. This is why marine and freshwater systems belong to the most affected systems on earth (GROSHOLZ 2002).

Despite this fact, such introductions have long been disregarded in invasion biology. It was only in the 1980s that they began to receive significantly more attention from scientists, partly fuelled by the conspicuous *Dreissena* invasion. Since then, it has been found that marine invasions – like terrestrial ones – can have substantial consequences for species of the same or adjacent trophic levels (GROSHOLZ 2002). Communities and ecosystems can be altered significantly, and invaders can even shape habitats (RUIZ et al. 1997).

NIS often show morphological adaption in newly colonized areas, among them are size changes. These are not typically directional in terrestrial organisms, deviations can occur both to smaller and bigger sizes. In marine and estuarine invertebrates however, researchers have found a clear trend to increased size in invasive populations (GROSHOLZ & RUIZ 2003). This indicates an unusual success of these species in the colonized areas.

1.2 Parasites in invasion biology and ecology

Reasons for the success of some invaders are complex, and gaining more knowledge in this field is crucial for the prevention and the control of biological invasions (WILLIAMSON & FITTER 1996b). The availability of resources as well as altered competition patterns can provide part of the explanation (CRAWLEY 1986; DOBSON 1988), as can the enemy release hypothesis (ERH) (ELTON 1958; COLAUTTI et al. 2004). This hypothesis suggests that the loss of natural enemies during the invasion process will increase invasion success. The ERH is a general model, applied to predators as well as pathogens or parasites. A more strict interpretation of the ERH is the parasite release hypothesis (PRH), based on the idea that invading populations generally have lower parasite diversity and loads (TORCHIN et al. 2001, 2002).

The colonization process represents a barrier for many parasites, resulting in lower parasite diversity and intensities. The colonizing organisms are only a small subset of the source population and in effect also carry only a subset of the original parasite diversity with them (DOBSON 1988). This bottleneck effect decreases parasite diversity. Many hosts are transported as juvenile stages, for example plant seeds or larval invertebrates, which are typically free of parasites (TORCHIN et al. 2002). At the onset of an invasion host density will generally be low, which may prevent parasite spread when it is under the persistance threshold (SWINTON et al. 2002). In addition to these factors, many parasites have complex life-cycles which require several obligatory hosts that might not always be present in the colonized area (TOMPKINS & POULIN 2006).

These mostly theoretical considerations are supported by parasitological studies on numerous invasive species. It was found that the parasite diversity in the source population was on average three times as high as in the invasive populations (TORCHIN et al. 2002). This is especially pronounced in marine or aquatic species which are transported by means of ballast water, usually as larval stages.

In the history of mankind, epidemic outbreaks of parasites and pathogens have played a crucial role (MCNEIL 1976). Despite the significance of diseases for us, a similar relevance for population ecology was long doubted (HUDSON et al 2002). Parasites were considered to be mostly benign and well-adapted to their respective host, so as not to cause high pathogenicity which would disturb the equilibrium between both (LACK 1954). This point of view changed only in the 1970s, when Roy Anderson and Robert May (ANDERSON & MAY 1978; MAY & ANDERSON 1978) developed a theoretical model that demonstrated the ability of parasites to regulate host populations.

Since then, a number of studies have proven them right, although the situation in the field is rarely as clear as in the case of the tree snail *Partula turgida*, whose last population was eradicated by microsporidian parasites (CUNNINGHAM & DASZAK 1998). Still, significant reductions in population density were observed in experimental populations, for example in mice and flour beetles (KEYMER 1981; SCOTT 1987). Nevertheless, definitive regulatory effects are very hard to verify in wild populations, although there are examples for this as well. In reindeer (*Rangifer tarandus*), gastrointestinal nematodes have a strong negative impact on fecundity to the point of being the main regulatory factor for population density (ALBON et al. 2002). Anthelminthic treatment prevents periodic population crashes and fluctuations in red grouse, caused by the nematode *Trichostrongylus tenuis* (HUDSON et al. 1998).

In light of the importance of parasites for host population dynamics, it is quite possible that they may have a similar impact on the success of invasive species in their new habitat. Parasites, parasitoids and pathogens actually have a long tradition as biological control agents. However, these efforts are almost exclusively limited to terrestrial systems. With the growing awareness of marine invasions, parasites have now also been considered for application in this area (THRESHER 1997; KURIS et al. 2005). But even today, our knowledge on host-parasite interactions – which is essential for any practical applications – is fragmentary at best, resulting in a need for more basic research in this field (TOMPKINS et al. 2002).

1.3 Thesis goals

The ERH is a popular hypothesis because it is intuitive. However, despite this it is not without controversy, as was demonstrated by COLAUTTI et al. (2004). The authors found conflicting results in two types of studies on invasive species. Biogeographical studies observe a clear reduction in natural enemies in colonized habitats compared to the source population. When investigating invaders within their new biocoenosis, however, it became obvious that they did not generally have less enemies than native species. Frequently the ERH is assumed as soon as a loss of enemies coincides with exceptional invasion success, but the causal connection is not always clear. It is therefore necessary to further investigate the impacts of enemies in general, and in particular that of parasites on invasive hosts (COLAUTTI et al 2004).

The European shore crab *Carcinus maenas* is an important research object for invasion biologists, as it is one of the most significant invaders in the often neglected marine environments (LOWE et al. 2004). Besides its home range in Europe it can now be found on

the coasts of five continents where it is generally considered a pest species with substantial negative impacts on native invertebrates (CARLTON & COHEN 2003). In Europe, it is one of the most commonly observed crabs along the northern and western coasts, and it is host to numerous parasites. However, a large percentage of its parasites were lost during the colonization process and only few new parasites were gained in the colonized areas (see Figure 1). This coincides with a significant increase of almost 20% in mean crab size in the colonized areas, compared to Europe (GROSHOLZ & RUIZ 2003). An investigation of the relationship between parasite prevalence and crab mean size and biomass found that only a specific type of parasite is correlated negatively with these two factors: parasitic castrators. As a consequence, *C. maenas* was one of the first organisms that was suggested to be subject to the parasite release hypothesis (TORCHIN et al. 2001). Nevertheless, high parasitic castrator prevalence is by no means common in European populations, and this is therefore not sufficient to explain the observed size differences between European and invasive populations (GROSHOLZ & RUIZ 2003).

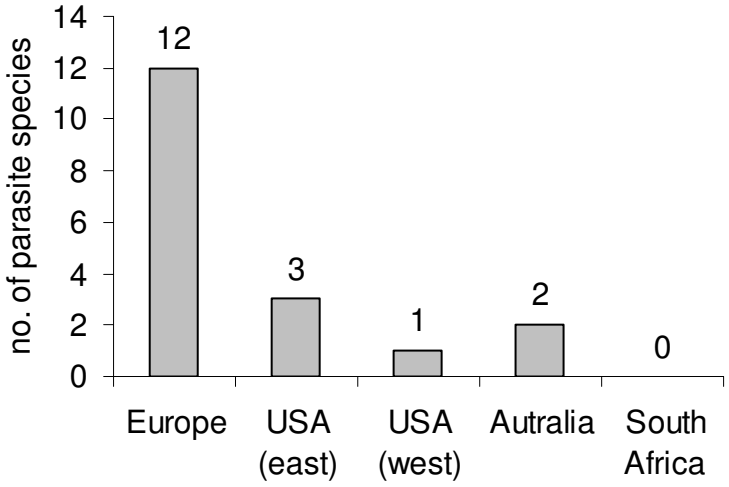

Figure 1: Number of macroparasite species confirmed for different distribution areas (TORCHIN et al. 2001; THIELTGES et al. 2008a; H. GLENNER, personal communication; present study).

Trophically transmitted helminth endoparasites that use shore crabs as intermediate hosts are ubiquitous in many if not most European populations of *C. maenas* (e.g. THIELTGES et al. 2008a) but knowledge on host-parasite interactions for these species is very rare. LAUCKNER (1986, 1987) found increased mortality in juvenile crabs infected with very high numbers of helminth larvae, but unfortunately this approach was never continued. It is therefore still

largely unknown if and in what way crab fitness is impaired by different parasites, aside from the obvious effects of parasitic castrators. The influence of parasites and their loss is yet to be determined and this problem gains even more weight in the light of recent efforts to investigate the possible use of parasites for the biological control of *C. maenas* (THRESHER et al. 2000; KURIS et al. 2005).

It is the goal of this thesis to further investigate host-parasite interactions in *C. maenas* and to test the parasite release hypothesis for this crab. I hypothesized that parasites cause morphological or physiological changes at the individual level of the shore crab that may have an impact on crab fitness and thus modify the population dynamics of this species. My main points of interest were reproductive fitness and immunocompetence.

By investigating resource allocation into reproductive effort, I was able to monitor two possible outcomes of reduced fitness caused by parasites. Firstly, a reduction in reproductive effort is possible by direct or indirect nutrient competition (HURD 2001). Secondly, parasite-induced mortality may lead to increased, or earlier, investment into reproduction to offset the shorter life-span (AGNEW 2000).

I used gonad weight as an indicator for resource allocation in reproductive effort. In females, ovary size is directly correlated with clutch size (THESSALOU-LEGAKI 1992; D'UDEKEM-D'AKOZ 1994; LATHAM & POULIN 2002a). In males, this approach is possible because testes weight in *C. maenas* is linked to reproductive success: STYRISHAVE et al. (2004) showed that the two colour morphs encountered in this crab actually represent two life-history strategies. Red crabs are stronger, more successful in mating competitions and also have comparatively larger gonads (KAISER et al. 1990; MCGAW et al. 1992; REID et al. 1997). For this benefit over green colour morph crabs there is a trade-off in growth and an increased susceptibility towards hypoxic and salinity stress (REID & ALDRICH, 1989; REID et al. 1989). This and other studies (e.g. SCHÄRER et al. 2004) indicate that gonad weight and allocation to reproductive effort are linked, providing me with an easily measured indicator for field samples.

The second main focus of this study was the crab immune system. Endoparasites invariably find themselves in close contact with the immune defences of the host which they can influence in different ways. While a number of parasites have learned to avoid the host immune defence completely (LOKER 1994), others actively manipulate it, for example by destroying cellular components or by releasing regulatory substances (UBELAKER et al. 1970; SHELBY et al. 2000). Where this is not the case, the parasite may initiate an immune response, therefore both binding resources that can not be put to use for growth or reproduction as well as making the host more vulnerable to secondary infections.

Haemocytes form one basis of the invertebrate immune system, fending off pathogens and foreign bodies by phagocytosis, cytotoxic activity and encapsulation (SMITH 1996; YOSHINO & VASTA 1996). The enzyme cascade of the phenoloxidase system plays a crucial role in these mechanisms, as it locally produces cytotoxins and encapsulates pathogens by melanisation (SÖDERHÄLL & SMITH 1986). For this reason I used total haemocyte counts (THC) as well as the activity of phenoloxidase in the haemolymph plasma as indicators for the condition of the crab immune system.

The investigation of these parameters was conducted on two levels. In a field survey, a number of crab populations were investigated both in their native distribution in Europe and in colonized areas in overseas. Experimental infections with helminth larvae in a controlled laboratory environment allowed me to monitor haemolymph parameters directly after an infection.

In summary, my thesis aimed at studying the impact of parasites on reproductive potential and immunocompetence of individual crabs in the field and in the laboratory, as well as the influence of parasite pressure on these parameters on the population level.

2 Materials and methods

2.1 Study animals

2.1.1 The European shore crab *Carcinus maenas*

Taxonomy:

Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Decapoda
Infraorder: Brachyura
Family: Portunidae

Along the coasts of its native and introduced distribution, the European shore crab *Carcinus maenas* Linnaeus, 1758 is one of the most commonly encountered decapods (e.g. CARLTON & COHEN 2003). Like all crustaceans of the infraorder Brachyura its pleon is short, flat and folded under the body. The pleopods are involved in brood care in female crabs, in male crabs they are reduced to gonopods. Despite the lack of characteristically flattened swimming legs, *C. maenas* belongs to the Portunidae or swimming crabs (Figure 2).

Figure 2: The European shore crab, *Carcinus maenas*.

The carapace can reach up to 80mm width and 60mm length, with females being smaller than males. Five spikes next to the eyes and 3 rounded spikes between the eyes are species characteristic. Although it is also called the “green crab”, both colour and markings can be highly variable, but usually range between brown and green. The first pair of legs is

transformed to strong claws that are mainly used for feeding but are also involved in mating competitions (KAISER et al. 1990).

The shape of the abdomen allows for easy sex determination. In females, the abdomen is urn-shaped and lined with hair. The segment borders are clearly visible. The male abdomen is pyramid-shaped, with fusing segment borders and no hair-lining (Figure 3).

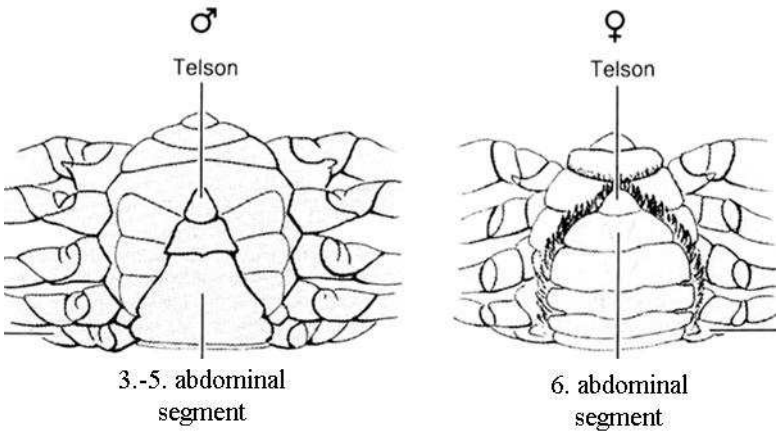

Figure 3: Abdomen shape in male and female shore crabs (modified from: KÜKENTHAL 1999)

2.1.1.1 Native range and invasions

The European shore crab is native to the Atlantic coasts of Europe and the Baltic Sea (Figure 4). Along the coast of Norway its distribution reaches the 70th parallel and it is also found on Iceland (CHRISTIANSEN 1969). Towards the south, it can be found along the Atlantic coasts of Spain, Portugal and Morocco (FOREST & GANTES 1960).

Originating from these native populations, *C. maenas* has successfully colonized a number of other areas (Figure 4): the Atlantic and Pacific coasts of North America, Argentina, South Africa, Australia and Japan (see CARLTON & COHEN 2003 for a review; HIDALGO et al. 2005).

The first successful invasion of the shore crab was observed in New England at the beginning of the 19th century (SAY 1817). The following decades saw a continuous spread of this species and today it can be found from New York in the south to Prince Edward Island and Nova Scotia in the north. Invasion biologists presume that its introduction and the following expansion were promoted by transport on the fouling of ships' hulls and in ballast water (CARLTON & COHEN 2003).

Towards the end of the 19th century the crab was also introduced to Australia. In 1900 it was already common in Port Phillip Bay, Victoria (FULTON & GRANT 1900). However, not until the 1970s did the shore crab spread to other coasts in southern Australia (see CARLTON &

COHEN 2003). It was probably introduced recently to Tasmania, where it was first observed in 1993 (GARDNER et al. 1994).

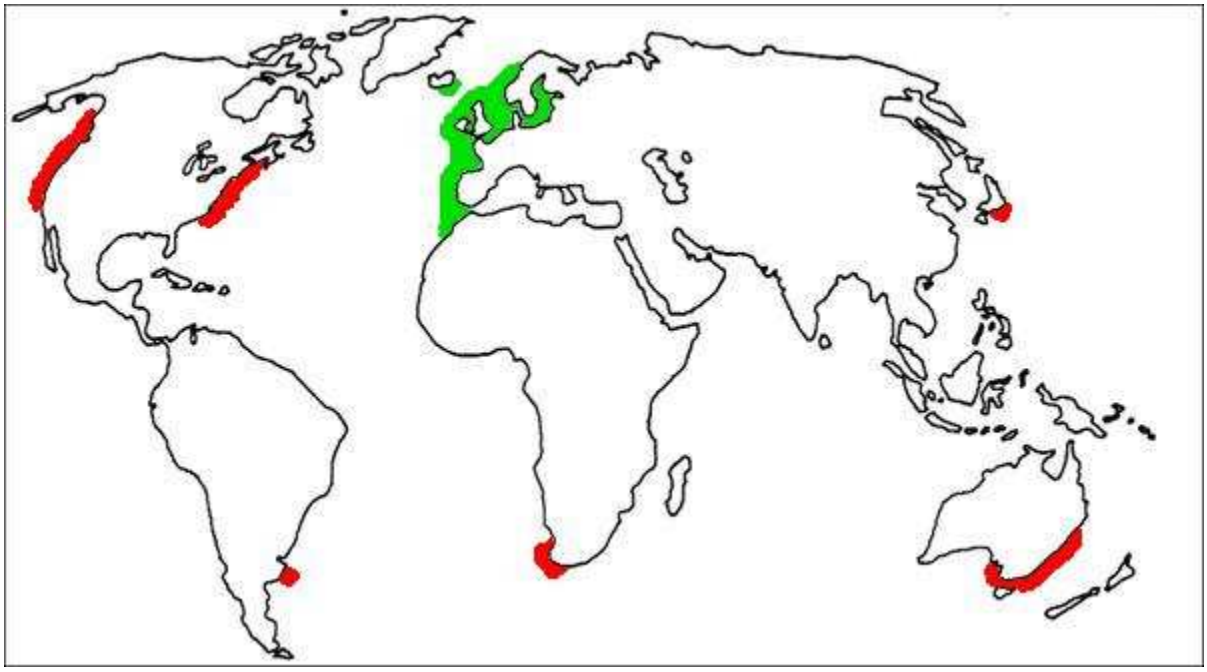

Figure 4: Distribution of the European shore crab *Carcinus maenas*. Green: Native distribution in Europe. Red: Colonized areas with established invasive populations.

Introduction events became significantly more common at the end of the 20th century, possibly due to the increased worldwide transport of planktonic larvae in the ballast water of ships, as a side effect of globalisation. In 1983 it was found along the South African coast around Cape Town where it now has established populations (LE ROUX et al. 1990). Only one year later the Mediterranean sibling species *C. aestuarii* was observed in Tokyo Bay, Japan. Genetic analysis, however, revealed a mixed invasion of both crab species (GELLER et al. 1997). Between 1989 and 1990 *C. maenas* also settled on the Pacific coast of the USA, where it is now very common and has expanded its range north as far as Canada (COHEN et al. 1995; GROSHOLZ & RUIZ 1995). The most recent discovery was that of an established shore crab population in Patagonia, which was described in 2005 (HIDALGO et al. 2005).

2.1.1.2 Ecology and life cycle

In *C. maenas*, both males and females reach maturity at a size of approximately 20-30 mm carapace width (CW). Mating can only take place directly after the female has moulted, and males will carry a female under their body for several days prior to this to guard her. Several

months later the eggs are deposited under the female's abdomen, where they are fixed to the setae of the pleopods. Female *C. maenas* care for their brood by cleaning it and by ventilating it with oxygen-rich water. The first larval stage, the zoea, spawns in the following spring. Three moults lead to the second larval stage, the megalopa. Both are part of the plankton. The megalopa sinks to the bottom and moults to the first juvenile crab stage and becomes part of the benthic community. Until the crabs reach sexual maturity, the moulting frequency is high and gradually slows down afterwards (CROTHERS 1967).

The European shore crab occurs on sheltered coastal areas (HAMPTON & GRIFFITHS 2007) and utilizes both sandy and rocky surfaces. They can survive a broad range of salinities (4-52 ppm) and temperatures (0-30°C) and are therefore well adapted to the life in estuaries and brackish water. Juvenile crabs are typically found in the intertidal zone, whereas adult crabs migrate with the tides and are also present in sublittoral areas of up to 50 m depth (HUNTER & NAYLOR 1993). The daily activity patterns of green crabs are determined by both tidal and diurnal factors, reaching their peak during nightly high tides (CROTHERS 1968).

C. maenas individuals can be separated in two different groups based on the ventral colour of the cephalothorax. Generally we distinguish between "green" and "red" colour morphs, although the colour can range from shades of yellow and green over orange to deep red. These colours are closely connected with the moulting cycle and are best visible in male crabs. Freshly moulted crabs are green or a shade of yellow. The orange and red colour develops after a longer time without moult (STYRISHAVE et al. 2004). These colour morphs appear to be connected to two different life history strategies, which have mainly been investigated in male crabs (WOLF 1998). In their own investigations and in a review of other studies STYRISHAVE et al. (2004) found that red males have stronger and larger chelae, a stronger carapace and are more successful in grasping receptive females and in defending them against competitors. These benefits, however, come with a trade-off. Green males are significantly more tolerant towards salinity extremes, hypoxic environments and other stressors such as heavy metals.

Although little work has been conducted on the role of colour morph for female crabs, first results showed a difference in salinity tolerance comparable to the situation in males (LEE et al. 2003).

European shore crabs are omnivores but feed most commonly on other invertebrates like bivalves. In Europe it is a major predator and a key species in coastal ecosystems. In beds of young mussels (*Mytilus edulis*) its presence is the main reason for the high mortality of 70 to 85% (DARE & EDWARDS 1976), and indeed the shore crabs have a high impact on the distribution and abundance of this mussel (EBLING et al. 1964). It has similarly decimating

effects on small size classes of hard clams and oysters (WALNE & DEAN 1972, 1977). They will also consume sea urchins as well as different kinds of snails (e.g. MUNTZ et al. 1965). However, it is the predation on bivalves that has made this crab unpopular even in its native range, and the shellfish industry will typically consider it a pest species (DARE & EDWARDS 1976).

Nevertheless, the situation is more severe in the invaded biocoenoses, where some native invertebrates have experienced a reduction in abundance by up to 90% (GROSHOLZ 2000). The crab is considered to be responsible for the collapse of the US east coast soft-shell clam (*Mya arenaria*) fishery (GLUDE 1955; ROPES 1968). It has also greatly reduced populations of scallops (*Argopecten irradians*), hard shelled clams or quahogs (*Mercenaria mercenaria*) as well as a number of species without commercial relevance (MORGAN et al. 1980; MENGE 1983, 1985; TETTLEBACH 1986; GROSHOLZ & RUIZ 2002).

2.1.2 Parasites of the European shore crab

Carcinus maenas is final or intermediate host to a substantial number of parasites from 8 different taxa (Table 1). Common endoparasites in Europe are the cirriped *Sacculina carcini* and helminths like microphallids and acanthocephalans (CROTHERS 1968; THOMPSON 1985a). Helminths use *C. maenas* as intermediate host and consequentially the larval stages of metacercariae and cystacanths are found in the crabs. *S. carcini* and the much less frequently found isopod *Portunion maenadis* are so-called parasitic castrators, both of which use the shore crab as definitive host. The nematodes and copepods found in the European range of *C. maenas* are generally monoxeneous ectoparasites. *Fecampia erythrocephala* is the only known parasite of the shore crab, which acts like parasitoids typically encountered in insects. A single specimen develops in a juvenile shore crab and kills it on emergence (KURIS et al. 2002).

Contrary to the European situation where parasites are diverse and span a wide range of taxa, the parasite diversity is significantly reduced in the invaded areas (reviewed in TORCHIN et al. 2001). So far there is no confirmed case of a European parasite following the crab to the new areas. However, along the east coast of North America the trematode *Microphallus similis* was found in introduced shore crabs, a species which is also present in Europe (STUNKARD 1957). In the same area acanthocephalans of the genus *Polymorphus* were found, but as the species has not been definitively identified, it is not known wether it is identical to the European acanthocephalans (BRATTEY et al. 1985; TORCHIN et al. 2001). In both cases we do not know if these parasites are native to these coasts or if they were introduced, possibly in the wake of the *C. maenas* introduction event.

Table 1: Parasites of the European shore crab *Carcinus maenas* in Europe and overseas (based on TORCHIN et al. 2001; THIELTGES et al. 2008a; present study).

	Species	Comments
	Europe	
Trematoda	*Microphallus similis* *Microphallus claviformis* *Maritrema subdolum* *Microphallus* spp.	infected with metacercariae
Fecampiida	*Fecampia erythrocephala*	parasitoid
Acanthocephala	*Profilicollis botulus* *Polymorphus* sp.	infected with cystacanths
Cirripedia	*Sacculina carcini*	parasitic castrator, monoxeneous
Isopoda	*Portunion maenadis*	parasitic castrator
Nematoda	*Carcinonemertes carcinophila* unknown nematode	ectoparasite, egg predator endoparasite
Copepoda	*Lecithomyzon menaedis*	monoxeneous
	North America (Pacific)	
Nematoda	*Carcinonemertes epialti*	ectoparasite, egg predator
	North America (Atlantic)	
Trematoda	*Microphallus similis* *Microphallus* spp.	infected with metacercariae
Acanthocephala	*Polymorphus* sp.	infected with cystacanths
Copepoda	*Chioniosphaera cancrorum*	monoxeneous
	Australia	
Cestoda	*Dolfusiella martini* *Trimacracanthrus aetobatidis*	infection with plerocercoids
Nematoda	*Proleptus* sp.	

In several cases native parasites of the colonized areas have made a host-switch and now also utilize *C. maenas*. This happened for the trypanorhynch cestodes *Dolfusiella martini* and *Trimacracanthrus aetobatidis* as well as a nematode of the genus *Proleptus* in Australia (GURNEY et al. 2004). The former are unusual in that they are the only tapeworms that infest *C. maenas*. Their final hosts are rays and sharks (pers. communication IAN BEVERIDGE), contrary to other helminth parasites of the crab which have sea bird final hosts.

In California, the local nematode *Carcinonemertes epialti* fills a similar niche to the European egg predator *C. carcinophila* (TORCHIN et al. 1996), and in New England the copepod *Chioniosphaera cancrorum* has been found in shore crabs (JOHNSON 1957).

2.1.2.1 Sacculina carcini

Sacculina carcini (THOMPSON 1836) is a cirriped (Rhizocephala: Kentrogonida) completely adapted to a parasitic mode of living. Possibly due to the easily identifiable external signs of parasitism it has been the focus of numerous studies, resulting in a well-known life-cycle (HØEG et al. 2005).

Larval development of this parasite is very similar to other crustaceans, beginning with the free-swimming nauplius. The nauplius moults to a cypris larva, which - if female - settles on an appropriate crustacean host, with apparently only little host specificity (ØKSNEBJERG 2000). In a second moult, the cypris develops to a kendrogon, which penetrates the host cuticle and injects the vermigon tissue into the host haemolymph (WALKER 1985; GLENNER 2001). Within the crab an internal root system ("interna") is formed (Figure 5) that absorbs nutrients and has a significant influence on host behaviour and physiology (RUBILIANI 1983; HØEG 1995).

Following a phase of internal growth, the parasite penetrates the abdomen of the crab host and forms a brood chamber, the so-called externa (Figure 5). A maximum of two male cypris larvae can settle in the receptaculi of the female externa to fertilize the eggs. The nauplius larvae of the next generation are released from this brood chamber.

S. carcini, like most rhizocephala, has a significant influence on morphological, physiological and behavioural aspects on the host, to the point where it controls it completely (HØEG 1995). Among the first targets of the parasite are the nervous and endocrine systems of the host crab. It denatures the androgenic gland, which leads to a feminisation of male hosts and an inhibition of spermatogenesis (RUBILIANI 1983). The male feminisation is reflected both in a broadening of the abdomen and in behavioural changes (GIARD 1887; RASMUSSEN 1959). Although the host is effectively sterilized as a consequence of these factors, the commonly

used term "parasitic castration" is misleading because the gonads are not reduced completely (HØEG 1995).

Figure 5: A European shore crab infested with *Sacculina carcini*. It carries mature externa (left side) and the internal root system (right side) is clearly visible in the connective tissue of the body cavity. Photos: Judith Hermann.

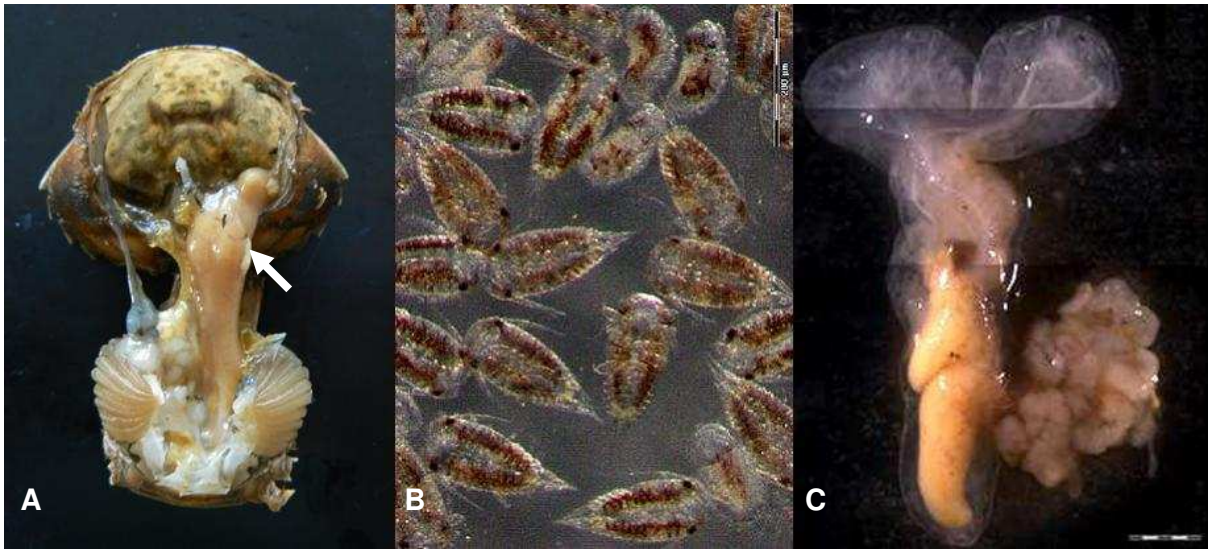

Figure 6: A - *Portunion maenadis,* female with brood sack in the opened body cavity of a shore crab. **B** - Larvae from a female brood sack (scale bar=200 µm). **C** - Female *P. maenadis* dissected from a shore crab (scale bar=2 mm). Photos: Judith Hermann.

2.1.2.2 Portunion maenadis

The isopod *Portunion maenadis* belongs to the family Entoniscidae, which is the only endoparasitic family among the isopod parasites. However, the life cycle of these unusual

animals is still largely unknown. LESTER (2005) suggests that copepods serve as intermediate hosts for an ectoparasitic stage in the life-cycle of *P. maenadis*. While males of this species retain a morphology that is characteristic for isopods, females undergo an extensive transformation (Figure 6). In the crab host they form a brood sack in which eggs and larvae grow until they are released by means of a connection to the gill chamber (LESTER 2005). The shore crab is therefore considered the final host of this parasite. Like *S. carcini*, it is a parasitic castrator with very similar effects on male crabs in terms of morphology and gonad size. However, the mechanisms involved in this process are unknown at this time.

2.1.2.3 Profilicollis botulus

One of the helminth parasites of *C. maenas* is the acanthocephalan *Profilicollis botulus* (RAYSKI & GARDEN 1961). In this case the crab is the intermediate host, with eider ducks (*Somateria molissima*) as final hosts of the adult worm (Figure 7). The final host sheds eggs with its faeces, which are ingested by the crabs. The first larval stage, the acanthor, emerges and penetrates the intestinal wall. This encysts in the body cavity of the crab to become the cystacanth (Figure 7), a dormant stage which stays infectious for the whole life span of the intermediate host (TARASCHEWSKI 2000). The parasite is transmitted trophically to the final host when an eider duck ingests an infected crab. The parasite can then develop to the adult worm in the bird's intestines.

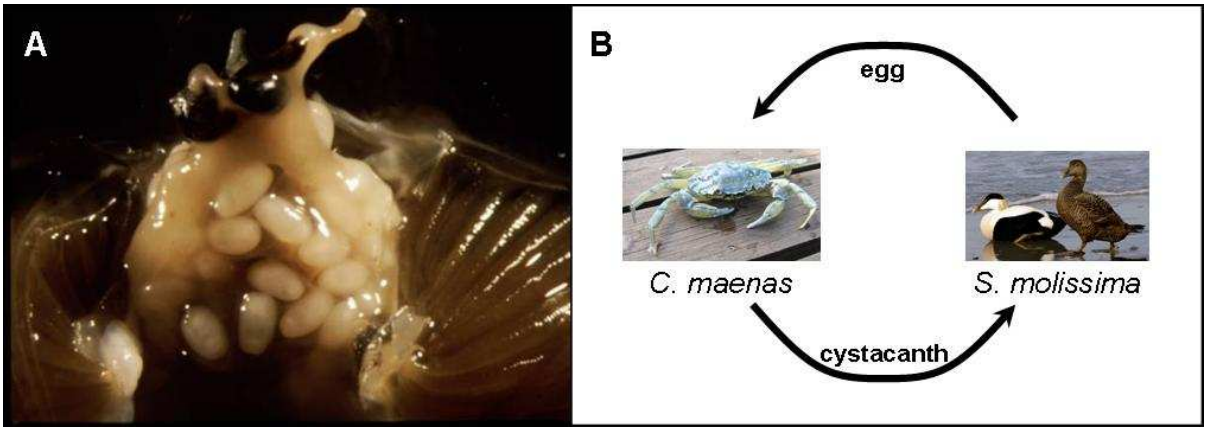

Figure 7: A - Cystacanths of *Profilicollis botulus* in the opened body cavity of a shore crab (Photo: Judith Hermann). **B** - Life cycle of *P. botulus*.

2.1.2.4 Microphallid trematodes

Microphallid trematodes are the second common helminth parasite of *C. maenas*. Species found in the shore crab are *Maritrema subdolum*, *Microphallus claviformis* and *Microphallus similis* (TORCHIN et al. 2001; THIELTGES et al. 2008a). Like most digeneans they have a complex life-cycle, involving several different hosts as well as free-swimming stages (Figure 8).

The vertebrate final hosts in this case are various sea birds, where sexual reproduction takes place in the host intestines. The eggs are passed to the environment with the faeces and ingested by the first intermediate host, *Hydrobia ulvae*, a small intertidal snail (MOURITSEN et al. 1997). Asexual reproduction takes place within the snail, where the eggs develop to sporocysts that shed cercariae. The cercariae actively leave the snail and penetrate the cuticle of the second intermediate host with the help of penetration glands and a stylet (CRIBB 2005). This second intermediate host is either *C. maenas* – which is penetrated via the gills (SAVILLE & IRWIN 2005) - or the much smaller crustacean *Corophium volutator* (MOURITSEN 2002). Upon invading the host the cercaria sheds its tail and encysts to become a metacercaria (Figure 8). Similar to the acanthocephalan cystacanths, this is a dormant stage that can stay infectious for the whole life-span of the crustacean host. The transmission to the final host is trophic and development to an adult worm takes place in the bird's intestines.

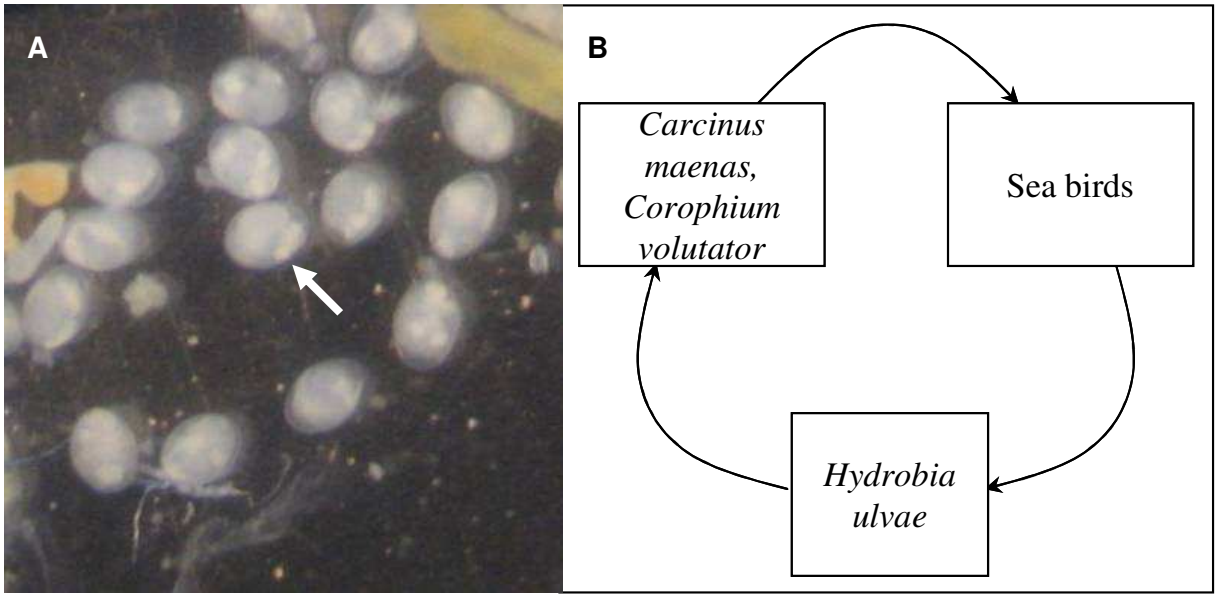

Figure 8: A – Metacercariae ofmicrophallids from the hepatopancreas of *Carcinus maenas*. **B** – Life cycle of microphallids of *C. maenas*.

2.2 Examination of crabs

2.2.1 Preparation of study animals

Prior to dissecting them, study animals were anesthetized by cooling at -20°C for 20 to 30 min, depending on crab size. For the removal of haemolymph this time was reduced to approximately 10 min to achieve a slight sedation and easier handling. The anaesthesia time was completed after the haemolymph sampling. For the dissection of the animals the legs were removed and the carapace was carefully lifted off to avoid injury to the gonads.

2.2.2 Morphology and external characteristics

Crab size was measured with callipers to the nearest 0.5 mm at the widest point of the carapace, between the tips of the two outermost carapace spines (Figure 9). Size is a covariate for gonad weight and an important factor for reproductive success (D'UDEKEM-D'ACOZ 1994). Size distributions can also give hints to size-dependent effects that could be connected to parasite-induced mortality.

Colour morph is an important trait, especially for male shore crabs, since it has implications for fitness parameters like reproductive success but also stress tolerance (see 2.1.1). The proportion of red crabs in a given size class can also give hints to the frequency of moults in that class, and therefore growth rate. A low proportion of green crabs suggests that moulting is rare and that growth has slowed down considerably in that size class. The colour morph was determined following the method of MCKNIGHT et al. (2000). *C. maenas* with green, whitish or yellowish ventral sides were considered "green", whereas crabs ranging from orange to red were considered "red" (Figure 9).

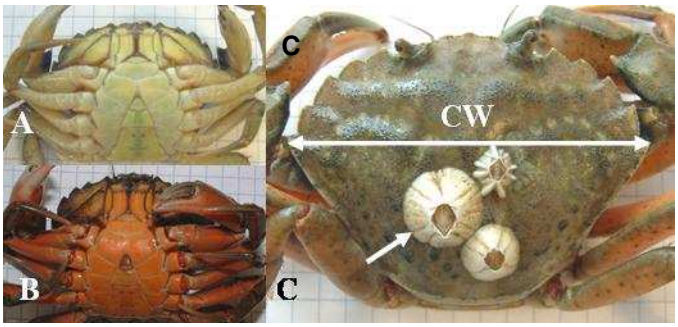

Figure 9: A – Green colour morph. **B** – Red colour morph. **C** – Measurement of carapace width (CW) and typical fouling caused by barnacles.

When faced with an immediate threat by a predator, such as being attacked by a bird, *Carcinus* crabs can shed a leg at a predetermined breaking point (SMITH & HINES 1991). This autotomy can be used to gain information on the frequency of predator attacks on the crabs. Therefore I recorded missing legs and chelae in all examined crabs.

I also observed and recorded fouling by three different general types of epibionts: barnacles, bryozoans and polychaetes. They were not determined in more detail. Similar to colour morph, the amount of fouling can give additional information on the time passed since the last moult (Figure 9).

2.2.3 Feminisation

Feminisation is often found in male crabs infected with parasitic castrators (see 2.1.2). To determine the degree of feminisation and to identify a possible hyperfeminisation effect in females I recorded the length of the abdomen as well as the width in the middle of the abdominal segments (Figure 10).

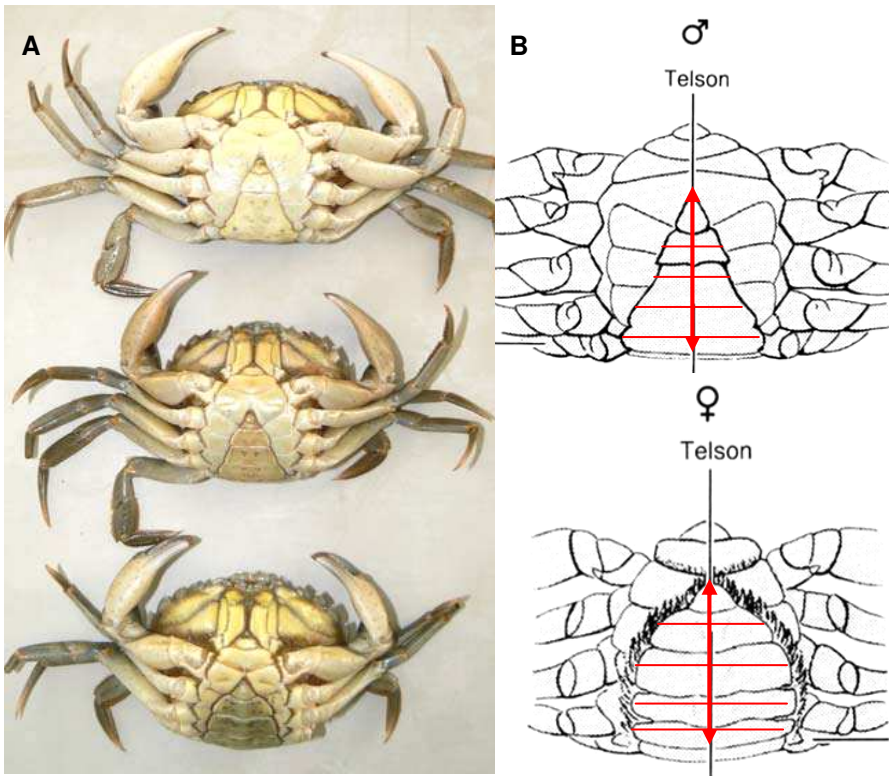

Figure 10: A – Feminisation: male crabs in different stages of feminisation. From top to bottom: normal abdomen – slightly broadened abdomen with distinct segment border – feminised abdomen with distinct segment borders and lined with hair. Photo: Judith Hermann. **B** – Measured abdomen proportions of male and female shore crabs (red lines and arrows). Modified from KÜKENTHAL 1999.

I calculated a feminisation index based on the method described by WERNER (2001), using the ratio of the 3rd and the 6th abdominal segment.

2.2.4 Parasitological examinations

After lifting the carapace I removed the heart and the gonads. The body cavity and gills were examined meticulously under a dissecting microscope to identify parasites. I also recorded noticeable factors, like melanized structures that can indicate bacterial infections. The hepatopancreas was then removed to examine it more closely. This was done either by squeezing it between two glass petri dishes or by digestion of the crab tissue. Both methods allow a good view on parasite stages in the digestive gland, but tissue digestion is especially suited to quantify large amounts of parasites.

Digestion with pepsin was conducted on the hepatopancreas tissue of all European crabs, where microphallids can reach very high densities (THIELTGES et al. 2008a). Pepsin is a digestive enzyme of vertebrates that breaks down the crab tissue. Parasitic helminths that use *C. maenas* as intermediate host are adapted to the passage through the intestinal tract of the final host and they can resist pepsin. Approximately 25 ml filtered or artificial sea water were added to the crab hepatopancreas along with 6 drops of hydrochloric acid for acidification (32%, Carl Roth GmbH, Karlsruhe). With the addition of approximately 600 mg pepsin (Carl Roth GmbH, Karlsruhe) I created conditions similar to a vertebrate stomach, leading to the digestion of the crab tissue. After an incubation period of one day at room temperature, the remaining contents were filtered through a 200 µm sieve to retain only larger particles including parasites. These could easily be quantified with a dissecting microscope.

In crabs infected with *S. carcini*, the internal root system was classified into three "age classes", based on observed differences in density, dimensions and colour of the interna. Isolated, fine translucent threads indicated a young infection (class 1), frequent and slightly thicker threads were considered an infection of medium age (class 2), and a dense root system with strong threads taking on a yellowish colour was classified as old infection (class 3).

2.2.5 Indicators for reproductive potential

Reproductive fitness is one of the main factors determining the population dynamics of a species. A common means of measuring the individual reproductive potential is the weight or

dry weight of gonads. This method is common in sperm competition research in both vertebrates and invertebrates, dealing with evolutionary and life history aspects of energy investment in reproduction (e.g. HARCOURT 1981; GAGE 1994, 1995; HOSKEN 1997). These studies showed that gonad weight is in many cases a good indicator for the amount of resources invested into reproduction. Bigger testes were also found to have a higher rate of cell proliferation and thus to "work more" in a free-living plathelminth (SCHÄRER et al. 2004).

In *C. maenas*, it was the research on life-history differences between red and green individuals that shed some light on the role of testes weight for resource allocation. Red colour morph crabs have a distinct advantage when grasping and defending females, which goes along with higher testes weight (STYRISHAVE et al. 2004). This confirms that testes weight is indeed an appropriate measure of resource allocation into reproductive fitness in *C. maenas*. Consequently, I used this parameter when investigating parasite effects on crab reproductive fitness.

2.2.5.1 Gonad dissection and preparation

In male crabs I removed the ventral and posterior part of the testes. This is clearly separate from the anterior part which is small and hard to dissect out completely. In females I only dissected out mature ovaries, which are characterized by a deeply orange colour (stage 3). Here both the anterior and the posterior part could be removed completely. I also distinguished between three other ovarial stages (CHEUNG 1966), separating transparent (stage 0) and white ovaries (stage 1) from yellow ovaries (stage 2).

All gonads were transferred to 1.5 ml Eppendorf tubes that had been weighed (Mettler Toledo, AB204) previously. They were dried (Heraeus drier) at 60°C until a constant weight was reached, which was then used for further analyses.

2.2.5.2 Statistical analysis of gonad weight

The two species of microphallid trematodes found, *Microphallus claviformis* and *Maritrema subdolum*, are difficult to distinguish at the metacercarial stage and were pooled. The isopod *Portunion maenadis* typically occurs in low prevalences. For this reason it was pooled with *Sacculina carcini* for a number of tests, resulting in the group "parasitic castrators".

Log-testes weight is a log-linear function of crab size. Expected testes weight could be determined from the linear regression equation of log-testes weight on log-carapace width. I

then calculated a reproduction index (RI) as the ratio of observed testes weight to expected testes weight.

STYRISHAVE et al. (2004) found that testes weight is higher in red colour morph crabs, and our own preliminary tests showed that sampling site influences testes weight significantly (ANCOVA, $F=32.459$, $p<0.001$). Both factors were accounted for in the statistical analysis of parasite effects by calculating RI separately for sample sites and colour morphs. Subgroups with insufficient sample size for this procedure were not included in the analysis.

The RI for ovary weight was calculated in a similar way. However, there was no consistent difference between females of different European populations. For this reason RI was only calculated separately for different morphs, when examining the effects of parasite infection.

2.3 Haemolymph parameters

Vertebrates have a well-known and extensively investigated immune system, but the ability of invertebrates to fend off pathogens, parasites and contaminants is often underestimated. YOSHINO & VASTA (1996) reviewed the immune system of invertebrates, which consists of both cellular and humoral components. Phagocytic activity of haemocytes in the haemolymph is a major factor in the cellular immune system. Larger antigens can also be encapsulated and rendered harmless. Both cellular receptors and humoral components acting as opsonins are involved in the recognition of pathogens. The humoral immune system is based on the production of cytotoxins, agglutinins and parts of the phenoloxidase (PO) system. The latter is an enzyme cascade with many similarities to the vertebrate complement system. The intricately regulated reaction chain leads to a serine protease which cleaves the inactive prophenoloxidase, resulting in active PO (CERENIUS & SÖDERHÄLL 2004). PO transforms phenols into cytotoxic quinones, with melanine as the final product. The melanization of pathogens and parasites, as well as injuries, is crucial to invertebrate immune systems.

With the paramount role of the PO system and of haemocytes for the arthropod and crustacean immune system it is not surprising that both are commonly used to gauge the efficiency of immune defence.

Total haemocyte counts (THC) can act as an indicator for immune defence that can be recorded with comparatively little effort. The concentration of haemocytes is a major component of invertebrate resistance against both parasites and bacteria. Higher THC offers protection against parasites in *Drosophila melanogaster* (ESLIN & PREVOST 1996), but this comes at the cost of a trade-off in larval competitiveness (KRAAIJEVELD et al. 2001). Similarly,

a connection between THC and parasite resistance was found in the signal crayfish, *Pacifastacus leniusculus*, by PERSSON et al. (1987). In *C. maenas* itself, THC levels are known to drop significantly while the immune system fends off bacterial infections. Numerous studies have shown that fitness losses caused by stressors are often reflected in THC level in crustaceans. Environmental factors like salinity, hypoxia or metal stress all lead to reduced THC in shrimp (HAUTON et al. 1997; LE MOULLAC et al. 1998; Le MOULLAC & HAFFNER 2000; YEH at el. 2004; WANG & CHEN 2005).

Total haemolymph protein concentration: In *C. maenas*, total protein concentration is negatively correlated with starvation and as such a parameter for overall fitness (UGLOW 1969). It was found to reliably predict survival in crickets infected with a number of bacterial pathogens (ADAMO 2004), possibly due to the effects of nutritional status or the presence of antimicrobial peptides which may be indicated by high protein concentrations (BACHÈRE et al. 2004). Infections of different kinds were observed to cause various effects on protein concentration in decapods, which are reviewed in DEPLEDGE & BJERREGAARD (1989).

PO activity is a commonly used parameter in the immunology of invertebrates, both in the haemolymph and within haemocytes. It is positively correlated with resistance to bacterial pathogens in *P. leniusculus* (LIU et al. 2007). Experiments on *Drosophila* showed that PO activity is also associated with resistance to parasites (VASS & NAPPI 2000). In *C. maenas*, bacterial infections lead to significant changes in this parameter (HAUTON et al. 1997), indicating that it may be relevant for parasite infections as well.

2.3.1 Total haemocyte counts

The crabs were slightly sedated by cooling them down in a freezer for 10 to 20 minutes depending on size. 200 µl haemolymph were removed with a syringe containing 200 µl of 20% seawater-formalin (20% formalin, 80% sea water, filtered or artificial), which ensured a fast fixation of the cells (TRUSCOTT & WHITE 1990). This was inserted between the carapace and first abdominal segment and haemolymph was removed from the visceral cavity above the heart. The haemolymph-formalin mix was then applied to a Neubauer hemocytometer (Neubauer Assistent, 0.1 mm x 0.0025 mm^2) and haemocytes were counted in 4x16 predefined squares of the counting grid, using a microscope (Zeiss, Axiolab) at 400x magnification (Figure 11). Based on the known dilution within the syringe the cell concentration in the haemolymph could be calculated.

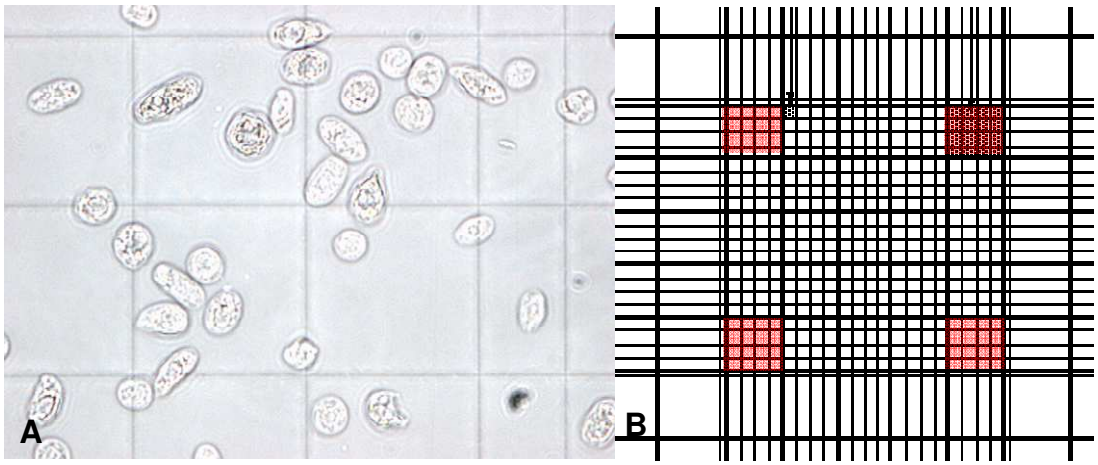

Figure 11: A - Haemocytes in the counting grid of the haemocytometer (400x). **B** - Predefined squares in which haemocytes were counted (marked in red); modified after www.wikipedia.com.

2.3.2 Total haemolymph protein concentration

The crabs were slightly sedated by cooling them down in a freezer for 10 to 20 minutes depending on individual size. Haemolymph was removed using a sterile syringe (0.9x40mm needles) filled with 200 µl ice cold 0.01M cacodylate buffer (cacodylic acid sodium salt trihydrate [$C_2H_6AsNaO_2*3H_2O$] [Roth, Karlsruhe] in aqua dest., adjusted to pH7 with hydrochloric acid [HCl] [Roth, Karlsruhe]). All following steps were conducted on ice to avoid proteinase reactions. The buffered haemolymph sample was transferred to a 1.5 ml Eppendorf tube and centrifuged at 1000 g and 4°C (Hettich Zentrifugen, Universal 16R). The supernatant containing the haemolymph plasma was transferred to a new tube and frozen at -20°C for later analysis of both the total haemolymph protein concentration and phenoloxidase activity.

Total protein in a sample was determined with a BCA Protein Assay Kit (Pierce, Rockford) suitable for use with microplates. Samples were diluted 1:10 with cacodylate buffer and two samples per dilution were measured (Tecan Safire 2). Preliminary tests showed that this dilution is well within measuring range of the kit. The methods followed the instructions for the BCA kit.

2.3.3 Phenoloxidase activity

PO activity in the haemolymph plasma samples (see 2.3.2) was determined by absorption measurements in microplates. PO converts a suitable substrate, in this case L-dopa, to a quinone and ultimately to melanin. Each well was filled with 50 µl haemolymph plasma in

cacodylate buffer (see 2.3.2). 50 µl 0.1% trypsin solution (trypsin [Roth, Karlsruhe] in cacodylate buffer) was added to cleave inactive proPO and transform it to active PO. After incubation for 15 min at room temperature 50 µl 20mM L-dopa solution (3,4-dihydroxy-L-phenylalanin [$C_9H_{11}NO_4$] [Carl Roth, Karlsruhe] in aqua dest.) was added and absorption measurements at 490 nm were immediately started and repeated in intervals of 90 sec (Tecan Safire 2). The absorption changes reflect the increase in melanin concentration and thus the amount of active PO in the haemolymph sample. The measurement was repeated 3 times for each sample.

Absorption increase was approximately linear over the first 5 min and the slope of the increase was used to determine absorption per minute. I then described PO-activity in units, defined as absorption per minute and per µg total protein in the sample.

2.4 Field study: Investigation of *Carcinus maenas* populations in Europe and in overseas

The aim of my field study was to examine *C. maenas* from different populations in both Europe and along colonized coasts overseas (Figure 12). Parasite prevalence was recorded and possible parasite effects on host fitness were investigated both at the individual and population levels. In order to do so I sampled a total of 10 populations in Europe, 3 populations in South Africa (Cape Town) and 1 population in Australia (Port Phillip Bay).

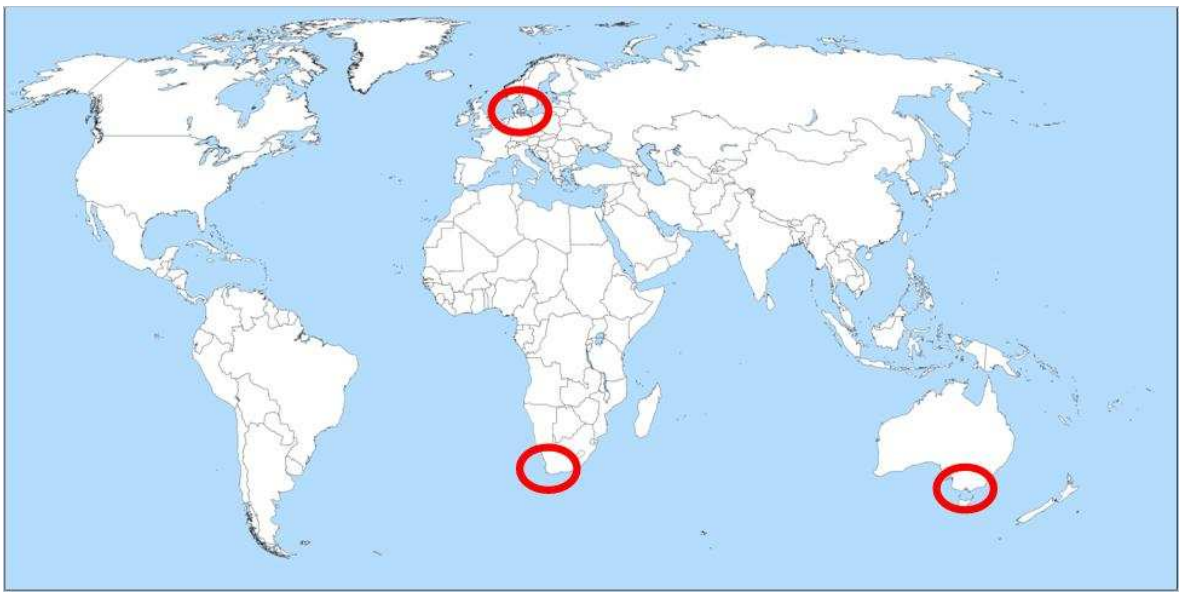

Figure 12: General sample areas in Europe, Africa and Australia.

Table 2: Populations examined in Europe and overseas. Location numbers refer to Figure 13.

	Sample area	Loc	Sample time	Coordinates	Method	Depth [m]	Salinity [ppm]
Germany	Sylt	1	10/2008	55°01′17"N, 08°27′16"E	dredge	8	32
	Helgoland	2a	08/2008	54°11′03"N, 07°54′11"E	traps	6	32
		2b	08/2008	54°11'19"N, 07°54'63"E	manual	0	32
		2c	08/2008	54°10'52"N 07°54'63"	manual	0	32
		2a	05/2009	54°11′03"N, 07°54′11"E	traps	6	32
Denmark	Limfjorden	3	06/2006	56°38′53"N, 08°41′19"E	dredge	5	30.3
	Limfjorden	4	10/2006	56°45'23"N, 08°50'52"E	traps	4.5	29.5
			09/2007		traps	4.5	29.5
	Limfjorden	5	06/2006	56°47'16"N, 08°52'36"E	dredge	5	29.5
			09/2007		traps	5	29.5
	Limfjorden	6	09/2007	56°47'18"N, 08°52'47"E	traps	1.8	29.5
	Limfjorden	7	09/2006	57°03'16N, 9°44'28"E	traps	1.5	26
	Kattegat	8	09/2005	55°37'12"N, 10°07'48"	traps	5	21.7
	Kattegat	9	06/2008	54°52'12"N, 09°38'29"E	traps	1.8	21.7
	Kattegat	10	09/2006	55°10'07"N 11°35'39"E	traps	1.5	21.7
South Africa	Sea Point	11	03/2007	33°54'46"S 18°23'11"E	manual	0.5	35
			03/2008	33°54'46"S 18°23'11"E		0.5	35
	Water Front	12	03/2007	33°54′29"S, 18°25′08"E	traps	8	35
			03/2008	33°54′29"S, 18°25′08"E		8	35
	Hout Bay Harbor	13	03/2007	34°03′14"S, 18°20′52"E	traps	5	35
			03/2008	34°03′14"S, 18°20′52"E		5	35
Australia	Altona	14	04/2008	37°52′56"S, 144°50′41"E	traps	1	34.1

2.4.1 Sampling and maintenance of *C. maenas*

2.4.1.1 Denmark

In Denmark, eight sites were sampled between 2005 and 2008 (Table 2, Figure 13). Five of these were situated in the Limfjord, three of them in different areas of the Kattegat.

Figure 13: Sample sites in Europe. Numbers refer to Table 2.

In most cases crabs were caught with the help of traps that were put out over night in water depths of 5-10 m. Typically, commercial eel and shrimp traps were used. While the trap constructions varied, certain parameters were constant over all traps. Mesh size was a maximum of 1.5 cm and the opening at least 5 cm in diameter (Figure 14). In rare cases (sample location 3, 5 and 8) populations were sampled with dredges (Table 2).

Crabs were maintained in an overflow system with filtered sea water. They were fed at least twice weekly with crushed mussels or fish *ad libitum*.

Figure 14: Trap types used in Denmark. **A** – Prawn and shrimp trap. **B** – "Operahouse" trap. **C** – Eel trap. Photos A and C: DANSK SKALDYRCENTER 2005

2.4.1.2 Germany

Two populations of *C. maenas* were investigated on the German islands of Sylt and Helgoland (Figure 13, Table 2) in 2008 and 2009. Crabs that were collected from the harbour of the island of Düne, Helgoland (location 2a) were caught with the help of traps. These were used for all examinations of crab fitness, as this method was comparable to the one used in other areas. Crabs were also sampled manually in the intertidal area of the Düne (location 2b), as well as the main island (location 2c).

All crabs from Germany were kept in aerated sea water basins until transported to the Karlsruhe Institute of Technology. Here crabs were kept in aerated aquariums with artificial sea water (33 ppm, Red Sea, Israel). They were fed twice weekly with fish *ad libitum*, followed by a change of water to avoid the accumulation of protein.

2.4.1.3 South Africa

Three populations were investigated in 2007 and 2008 in the Cape Town area (Figure 15), with a focus on the heavily populated inner harbours of Cape Town itself and Hout Bay (ROBINSON 2005).

Crabs in the intertidal area of Sea Point/South Point were caught by turning stones and examining tidal pools at low tide. In the harbours I used two different trap types (Figure 16). These were equipped with baitfish and placed on the harbour bed in 5-10 m depth, for 1 to 2 h each. The traps had an opening of 5 cm and a mesh size of 1cm and 1.5 cm.

Until examination, the crabs were kept in an overflow system with filtered sea water. They were fed twice weekly with sardines.

Older investigations found a sister species of *C. maenas* in South Africa, the Mediterranean shore crab *C. aestuarii* (GELLER et al. 1997). Although by 2005 no specimen of this species was found (ROBINSON et al. 2005), all crabs sampled in my study were examined for typical distinguishing traits (BEHRENS YAMADA & HAUCK 2001), mainly the shape of the male pleopods and the shape of the frontal area between the eyes. The carapace width to length ratio was measured in randomly chosen crabs. All methods confirmed the findings of ROBINSON (2005): all sampled crabs were therefore considered to be *C. maenas.*

Figure 15: Sample sites on Cape Peninsula, South Africa. Numbers refer to Table 2.

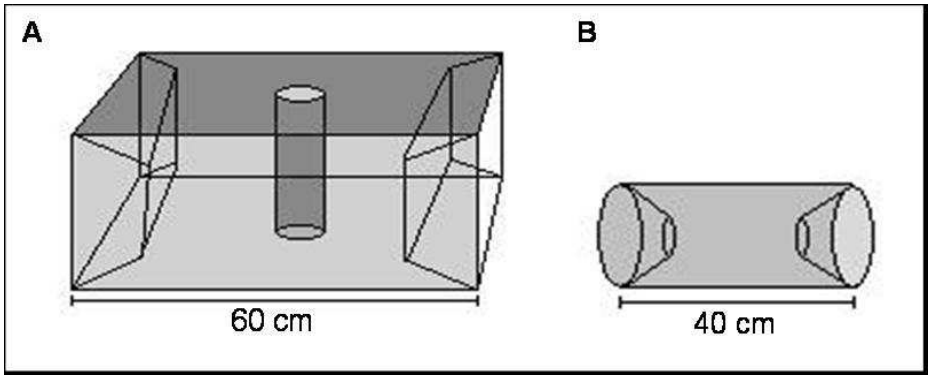

Figure 16: Trap types used in the Cape Town area. **A** – A robust metal frame covered with a flexible plastic net (mesh size 1 cm). **B** – Made from wire mesh with a mesh size of 1.5 cm.

2.4.1.4 Australia

European shore crab populations in Port Phillip Bay, Australia, were investigated as a part of a study on possible biological control options several years ago (GURNEY 2006). I chose this area for the known populations of *C. maenas* and because both parasite species found in Australian *C. maenas* were observed in Port Phillip Bay. However, in this study I found an unexpectedly low abundance of crabs with only one sample site yielding sufficient crab numbers for investigation.

Figure 17: Sample site in Port Phillip Bay, Australia. The number refers to Table 2.

This population was located in a small estuary in Altona and was sampled with the help of tuna-baited opera house traps that were left in the shallow water over night (Figure 14). Crabs were kept in sea water and examined within two days of capture.

2.4.2 Parasite-induced mortality

Microphallid trematodes and the acanthocephalan *P. botulus* use *C. maenas* as intermediate host only, forming durable cysts within the crab tissue (MOURITSEN 2002; SAVILLE & IRWIN 2005). These cysts survive in the host until it dies, leading to an accumulation of metacercariae and cystacanths over time. In populations where these parasites are common, a positive correlation between the number of cysts and the age of the crab is therefore expected. In crustaceans, size is a good measure of age. Accordingly, the mean number of cysts per crab should increase with size class. However, if parasite accumulation leads to mortality, the increase in mean number of cysts is expected to level out in larger size classes, possibly even leading to a decrease (ANDERSON & GORDON 1982). Similarly, when the rate of parasite acquisition is variable between hosts, the dispersion of parasites, described as the variance to mean ratio, should increase with size class. However, if parasite-induced mortality is present, the maximum dispersion is expected in intermediate size classes.

To test for this, European crab populations where helminths were prevalent were divided into 5 to 9 size classes, depending on the number of available crabs and the size range covered. Due to low numbers of crabs at the extreme ends of the size spectrum, some pooling was conducted for the lowest and highest size classes. The number of cysts was log(x+1) transformed, following the method of LATHAM & POULIN (2002a). The mean number of cysts was set in relation to size class and a subsequent curve fitting was conducted for linear, quadratic and polynomial models. Where either the quadratic of the polynomial model proved to be a better fit for the curve, parasite induced mortality was assumed. I also explored the variance to mean ratio for a decrease in higher size classes.

2.5 Experimental infection of *Carcinus maenas*

In the field a multitude of factors influence the fitness and behaviour of host organisms. To complement the field study it was necessary to conduct a laboratory study in a controlled environment. It was the goal of this study to investigate the effect of a nascent parasite infection on *C. maenas*, mainly with regards to immunocompetence. Both acanthocephalan

and microphallid parasites exist as encysted and mostly dormant stages within the shore crab. This makes the initial phase of an infection especially interesting, since the parasite is moving actively through host tissue, which should lead to higher immune system involvement.

For this study, previously uninfected crabs were experimentally infected with both microphallid and acanthocephalan parasites and the immune reaction was monitored over ten weeks.

2.5.1 Origin and maintenance of crabs

Carcinus maenas for the experimental infections were obtained from populations in South Africa, as these provided study animals that are guaranteed to be free of macroparasites. The crabs were caught in June 2009 and only male individuals were used to avoid sex bias in the results. They were transported to Karlsruhe, Germany by plane. The animals were kept in humid and cool conditions during transport. In Germany, they were transferred to aquariums filled with aerated artificial sea water (Red Sea, 33 ppm). Two types of aquariums were used (28x28x48 cm and 25x22x35 cm), which were filled with 27 l and 15 l water, respectively. Crabs were provided clay plant pots for hiding and were allowed to adapt to the new environment and lose tidal rhythms for three weeks. They were fed three times a week with fish, followed by an exchange of half of the water to avoid protein accumulation in the water. Each crab received its piece of fish individually to minimize feeding competition and the resulting stress.

2.5.2 Experimental infections

Study animals were split into 5 groups, one group for strong and medium infections with microphallids and acanthocephalans, respectively, as well as an uninfected control group (Table 3). Due to availability issues it was not possible to enforce strict size limitations to the study animals. However, I spread size classes evenly over the experimental groups. Prior to the experiments, natural markings were recorded for all crabs, including size, colour morph, carapace colour and markings, fouling and injuries. These allowed for an individual identification of each crab in an aquarium. Two types of aquariums were used, filled with a volume of 15 l and 25 l respectively. Small aquariums housed 4 to 5 small crabs, while larger aquariums housed up to 6 bigger crabs. Crabs from different experimental groups were kept seperate.

Table 3: Experimental groups for infections with microphallids (*Maritrema subdolum* and *Microphallus claviformis*) and an acanthocephalan *(Profilicollis botulus*).

Experimental group	Parasite	Infection strength	Number of crabs	Size range (CW)
1	microphallids	medium	14	49.5-79 mm
2	microphallids	strong	14	50.5-81 mm
3	*P. botulus*	medium	15	54-83.5 mm
4	*P. botulus*	strong	15	50.5-76 mm
5	control	-	15	51-78 mm

2.5.2.1 Infection with *Profilicollis botulus*

The final host of *P. botulus* is the eider duck *Somateria mollissima*, which sheds acanthocephalan eggs with its faeces. It was not possible to obtain adult worms from bird intestines, which would provide the best source of high amounts of eggs. Eider duck faeces was therefore collected on Sylt and sent to the University of Karlsruhe where it was kept humid and cool (8°C).

Faeces samples were first examined with a formalin-ethyl acetate sedimentation technique based on ASH et al. (1994) to detect small amounts of acanthocephalan eggs. The formalin-fixed faecal sample was filtered through a 400 µm polyamide sieve. The pellet was transferred to a 10 ml centrifuge tube and 8 ml formalin was added along with 2 ml ethyl-acetate. The suspension was mixed for 1 min and centrifuged at 500 g for 10 min. The pellet, now free of detritus, was resuspended in 0.5 ml formalin. 100 µl of this suspension was applied to a microscope slide and examined with a Zeiss microscope at a magnification of 400x. The concentration of eggs was very low (under 5 eggs per slide), requiring a concentration of living eggs to obtain infectious material for the experimental infections.

For this, 20 g of the faeces was suspended in 40 ml sea water and filtered through a 0.4 mm sieve to retain larger particles like sand grains as well as crushed mussel shells and crab carapaces. The remaining suspension contained fine silt and acanthocephalan eggs which were centrifuged for 5 min at 500 g. The resulting sediment was used to cover the normal

fish ration each crab received. All crabs of group 3 and 4 were fed individually to make sure each received a similar amount of both food and infectious material. The faeces coating of the fish pieces did not influence acceptance by the crabs. Group 3 was provided with the infectious food once a week, while group 4 received it twice a week (Table 3). This procedure started during week 31 of 2009 and it was continued over 7 consecutive weeks to ensure infection despite the low amount of eggs present.

2.5.2.2 Infection with microphallids (*Maritrema subdolum, Microphallus claviformis*)

Cercariae of microphallids that are infectious for *C. maenas* were obtained from *Hydrobia ulvae* snails. These were caught in Sylt and sent to Karlsruhe, where they were kept in a sea water aquarium and fed with standard fish food flakes (Tetra). For the infection, the snails were crushed in a petri dish filled with a small amount of sea water. The emerging cercariae were examined closely with a microscope and microphallids were transferred to a clean petri dish with a pipette. Microphallid cercariae could be distinguished based on the stiletto that is characteristic for this genus.

For the infection process, crabs were transferred to glass beakers. The beakers were filled with just enough sea water to cover the crabs, while still allowing them to rise over the water surface to aerate their gills. Microphallids were added to this water and the crabs were left in the beakers over night. Group 1 crabs received a minimum of 100 microphallids, while group 2 crabs received a minimum of 500 cercariae. However, while group 1 infections could be conducted during the first week of the experiment, group 2 infections had to be delayed until the third week. This was caused by an unexpected drop in cercarial prevalence in the snails, which made a new sampling of snails necessary.

2.5.3 Immune parameters throughout the infection process

At several points during the experiments haemolymph samples for THC measurements and PO activity analysis were taken to monitor the development of immune factors. The blood sampling and analysis was conducted as described in section 2.3. The method deviated from the previously described methods only in using 0.1 ml of haemolymph and the same amount of buffer and fixative to minimize the negative impact on the crabs. Weekly or biweekly sampling was not possible as the study animals were not able to regenerate haemocytes sufficiently rapidly; samples were therefore taken at increasing time gaps (Table 4).

Table 4: Time table for the experimental infections.

	haemolymph sample	microphallids	acanthocephalans
week 1	yes	group 1 infection	---
week 2	yes	---	group 3 +4 infection
week 3	---	group 2 infection	group 3 +4 infection
week 4	yes	---	group 3 +4 infection
week 5-8	---	---	group 3 +4 infection
week 9	---	---	---
week 10	yes	---	---

2.5.4 Dissection of crabs

Prior to dissection, the crabs were anesthetized by cooling at -20°C for 20 to 30 minutes, depending on crab size. For the dissection of the animals the legs were removed and the carapace was carefully lifted off to avoid injuries of the gonads. The dissection was proceeded as described in section 2.2, including the recording of morphological and physiological characteristics, the quantification of parasites and the removal of gonads. No further haemolymph samples were taken at this point in the experiments.

3 Results

3.1 Results of the field study

Overall, more than 1500 crabs from 15 different general sampling locations were collected and examined for parasites. In most cases, populations were heavily biased towards males (Table 5). Crab size ranged from 12 mm carapace width up to 88 mm, which was observed in South Africa. However, mean size was typically between 45 and 66 mm carapace width.

Table 5: Sample data for all locations in Europe and overseas. Location numbers ("loc") refer to Figure 13. CW = carapace width.

		Sex		Colour morph		CW [mm]		
Loc	**N**	**Female**	**Male**	**Green**	**Red**	**Min**	**Max**	**Mean±SE**
1	49	2	47	26	23	36	63.5	49.7±1.1
2	158	63	95	93	65	17	76	46.9±1.4
3	129	49	80	77	52	27	72.5	49.5±0.9
4	179	62	117	75	103	29	70	48.8±0.7
5	76	11	65	28	48	28.5	68.5	50.6±1.2
6	100	15	85	45	55	33.5	66.5	52.1±0.7
7	115	34	81	51	63	29.5	64	45.2±0.8
8	67	20	47	47	19	12	72	44.7±2.1
9	109	7	102	49	60	34	73	57.6±0.7
10	179	5	174	52	123	42	77	65.9±0.4
11	35	18	17	27	8	38	80	55.9±1.9
12	180	51	129	131	49	35	88	62.1±0.7
13	100	57	43	59	41	21	83	66.3±1.0
14	107	76	31	82	25	30.5	72.5	50.2±0.7
Total	1583	470	1113	842	734	29.5	73.3	53.3

3.1.1 Parasites in Europe

3.1.1.1 Survey of parasites in Europe

In European *C. maenas*, I found cystacanths of the acanthocephalan *Profilicollis botulus* as well as metacercariae of microphallid trematodes. Besides helminths, I also encountered two parasitic castrators, the cirriped *Sacculina carcini* and the comparably rare isopod *Portunion maenadis* (Table 6)

It was not possible to determine the microphallids to species level, but both *Microphallus claviformis* and *Maritrema subdolum* are commonly found in the areas which I investigated (THIELTGES et al. 2008a). However, since it is rarely possible to determine the metacercarial stages of microphallids, which also occur in mixed infections, to species, they were pooled for this work. Both parasitic castrators only occur as single infections, their intensities are therefore not displayed in Table 6.

Parasite prevalence was highly variable, with significant (or near significant in the case of *P. maenadis*) differences between European sample sites for all four parasite species and groups (Table 7, Appendix table 1 to Appendix table 4). While this is expected in samples that cover a large area, differences can also be found in the parasite prevalences in smaller scale geographical areas (see 3.1.1.2).

P. botulus was typically most common in the open sea areas of the Kattegat and the North Sea, but it was found in less than 5% of all crabs sampled in the Limfjord. Microphallids were present in most Limfjord populations, but at comparably lower prevalences than in most open sea populations of the Kattegat and the North Sea (Table 6).

The distribution of the parasitic castrator *S. carcini* showed an opposing trend. It was almost exclusively found in Limfjord areas, where it showed considerable variation. It was absent from Helgoland and one Kattegat population near the island of Fyn, and only occured rarely in crabs of other North Sea and Kattegat populations. The second parasitic castrator, *P. maenadis* was rare or absent in all investigated populations, with no obvious preference for fjord or open sea habitats (Table 6).

Table 6: Prevalences and intensities of endoparasite infections found in European populations of *Carcinus maenas*. **N**=sample size, ***SC***=*Sacculina carcini*, ***PM***=*Portunion maenadis*, **MP**=microphallids, ***PB***=*Profilicollis botulus*, **P**=prevalence, **I**=intensity, **Loc**=sampling location (Figure 13).

Sample site	Loc	Time & method	*SC* P [%]	*PM* P [%]	*MP* P [%]	*MP* I	*PB* P [%]	*PB* I	**N** (total)
Sylt	1	10/2008 dredge	6.1	0	71.4	433.7	44.9	2.1	**49**
Helgoland	2a	08/2008 traps	0	0	47.2	97.3	61.1	1.8	**36**
	2b	08/2008 manual	0	0	97.6	131.0	24.3	2.1	**41**
	2c	08/2008 manual	0	0	25.7	180.9	40.0	2.8	**35**
	2a	05/2009 traps	0	0	42.9	387.7	64.3	3.3	**42**
Limfjorden	3	06/2006 dredge	41.9	2.3	2.3	1742.0	3.1	1.5	**129**
Limfjorden	4	10/2006 traps	65.0	3.4	11.7	47.3	1.7	1	**60**
		09/2007 traps	26.9	3.5	18.5	26.5	0	-	**119**
Limfjorden	5	06/2006 dredge	50.9	0	0	-	0	-	**55**
		09/2007 traps	9.5	0	9.5	9.0	0	-	**21**
Limfjorden	6	09/2007 Traps	10.0	2	20.0	49.5	1.0	1.0	**100**
Limfjorden	7	09/2006 traps	4.3	4.3	21.7	512.9	2.6	1.7	**115**
Kattegat	8	09/2005 traps	13.4	1.5	3.0	18.0	68.7	9.2	**67**
Kattegat	9	06/2008 traps	1.8	6.4	45.9	219.0	22.9	1.7	**109**
Kattegat	10	09/2006 traps	0	0	49.7	98.6	18.4	1.2	**179**

In most populations, neither sex nor colour morph had a significant effect on parasite prevalence. However, in isolated cases differences could be observed (Appendix table 5 & 6). An overall survey of all sampled crabs provided a higher sample size and showed that red crabs are significantly more common among crabs infected with *S. carcini* and microphallid

trematodes (Fisher's exact test: *S. carcini* χ^2=5.060, p=0.028; microphallids χ^2=5.226, p=0.023). This was not the case for crabs infected with *P. botulus* (Figure 18).

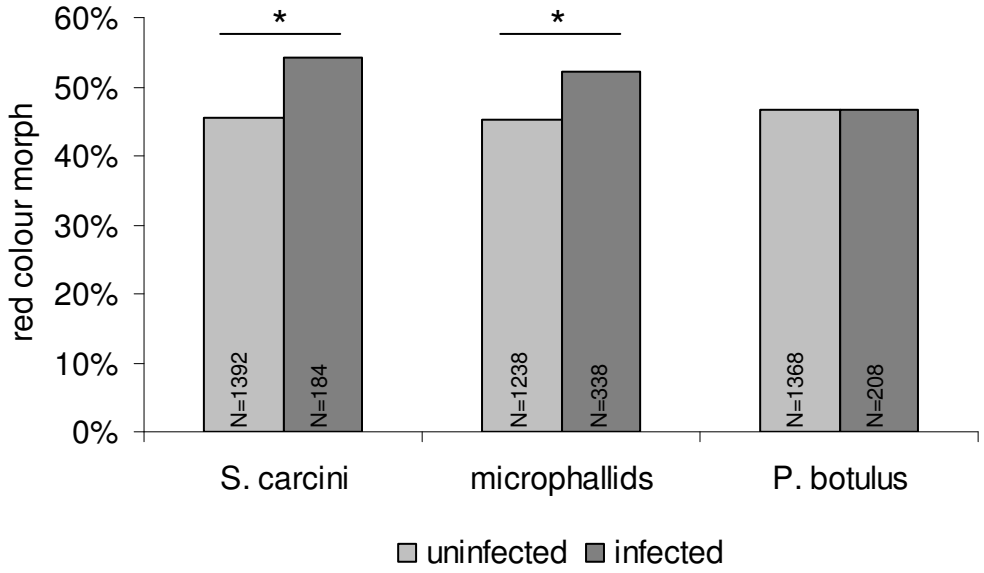

Figure 18: Fraction of red colour morphs in uninfected crabs and crabs infected with *S. carcini*, microphallid trematodes and *P. botulus*. * marks significant differences (Fisher's exact test on absolute numbers: *S. carcini* χ^2=5.060, p=0.028; microphallids χ^2=5.226, p=0.023).

3.1.1.2 Prevalence differences in European sub-localities

Both helminth groups displayed significantly different prevalences between the North Sea sample sites, and even between the sample sites of Helgoland itself which lie in close proximity to each other (Table 7). Microphallid prevalence was significantly higher in the samples collected in the intertidal areas of the main island of Helgoland, compared to both sample sites on the dune island (Fisher's exact test: χ^2=25.263, p<0.001 and χ^2=42.552, p<0.001). However, even for the two samples from the dune island a trend to different microphallid prevalences was displayed (Figure 19).

Acanthocephalan prevalence was significantly higher in the subtidal sample than in the intertidal sample from the main island (Fisher's exact test: χ^2=10.642, p≤0.001). A similar trend could be observed compared to the crabs from intertidal areas of the dune island (Figure 19). As opposed to microphallid parasites, no differences between the two intertidal samples from the different islands were found for *P. botulus*.

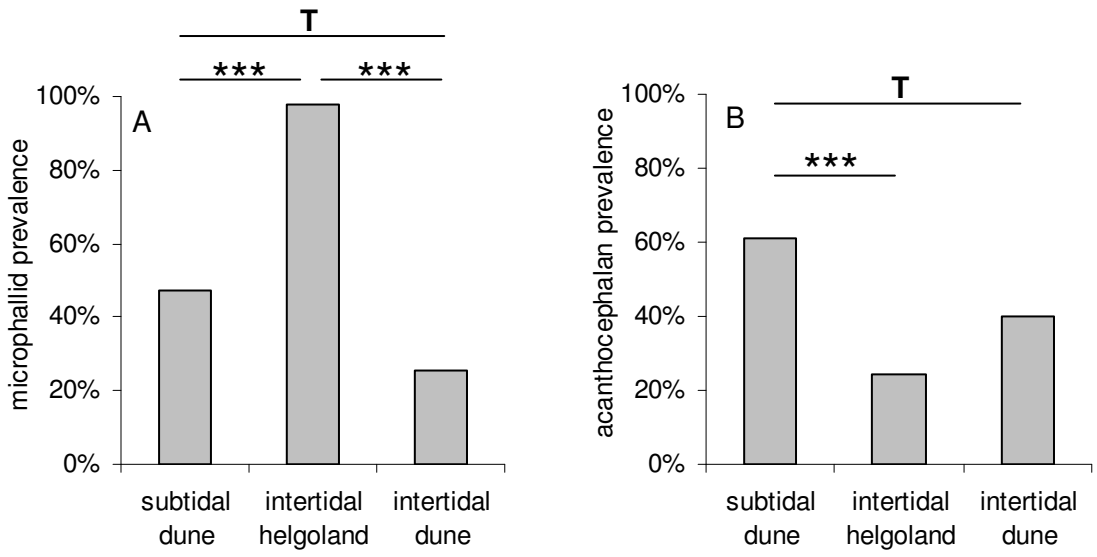

Figure 19: Parasite prevalence in different sampling locations of Helgoland and the dune island (location 2). **A** - microphallid prevalence: *** = significant differences: Fisher's exact test: χ^2=25.263, p<0.001; χ^2=42.552, p<0.001; T = trend: Fisher's exact test: χ^2=3.537, p=0.051. **B** - acanthocephalan prevalence (*P: botulus*): *** = significant differences: Fisher's exact test: χ^2=10.642, p≤0.001; T = trend: Fisher's exact test: χ^2=3.164, p=0.061. Sample sizes are given in Table 6.

When examining the crabs collected in the Limfjord, close to Nykøbing (Mors) I found significant differences in prevalences of the parasitic castrator *S. carcini* (χ^2_2=11.547, p=0.003). Despite close proximity, the southernmost sample site (location 4) was found to have a significantly higher prevalence than the northern site (location 6) (Fisher's exact test: χ^2=10.002, p=0.001). There was a similar trend for location 5, which lies between the other two sample sites and for which only a smaller sample size of crabs was available for examination (Fisher's exact test: χ^2=2.928, p=0.069)(Figure 20).

The three Kattegat populations investigated were spread over large distances, with one sample site on the Danish mainland and sites on Fyn and Sjaelland, respectively. Accordingly, there were significant differences in parasite prevalence between the sites for all four parasites that are common in *C. maenas* (Table 7).

Crabs from location 8 near the island of Fyn had a significantly different helminth fauna then crabs from the other two locations, with lower microphallid and higher acanthocephalan prevalence (Fisher's exact test: χ^2=56.401, p<0.001 and χ^2=36.041, p<0.001) (Figure 21).

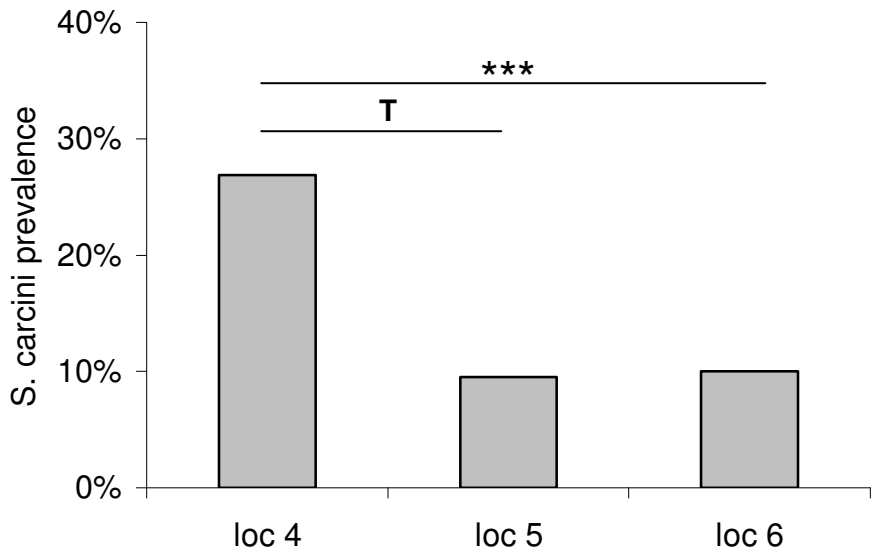

Figure 20: *Sacculina carcini* prevalence in three different locations of the Limfjord, close to Nykøbing, Mors. Location numbers refer to Figure 13. * = significant difference (Fisher's exact test: χ^2=10.002, p=0.001; T = trend: Fisher's exact test: χ^2=2.928, p=0.069). Sample sizes are given in Table 6.

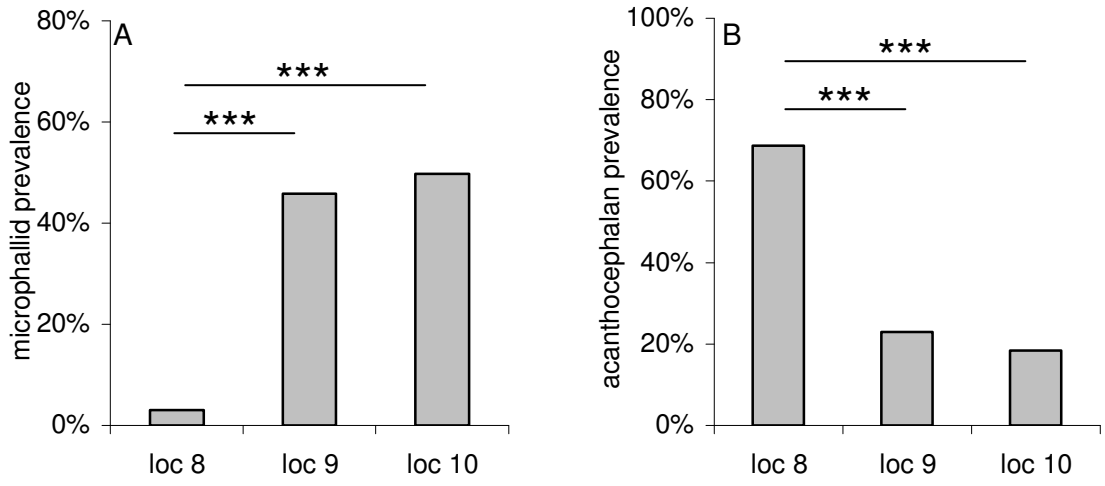

Figure 21: Helminth prevalences in different subpopulations of the Kattegat. **A** - Microphallid prevalence. Location numbers refer to Figure 13. * = significant differences (Fisher's exact test: χ^2=45.686, p<0.001 and χ^2=36.663, p<0.001). **B** - Acanthocephalan (*P. botulus*) prevalence. * = significant differences (Fisher's exact test: χ^2=56.401, p<0.001 and χ^2=36.041, p<0.001). Sample sizes are given in Table 6.

A similar pattern, with location 8 being different, was observed for *S. carcini*, which was more common in this area than elsewhere (Fisher's exact test: χ^2=24.958, p<0.001 and χ^2=9.526, p=0.003). *P. maenadis,* on the other hand, generally occured in low prevalences or was completely absent from the Kattegat populations. It was most common in the mainland population at location 9 (Figure 22).

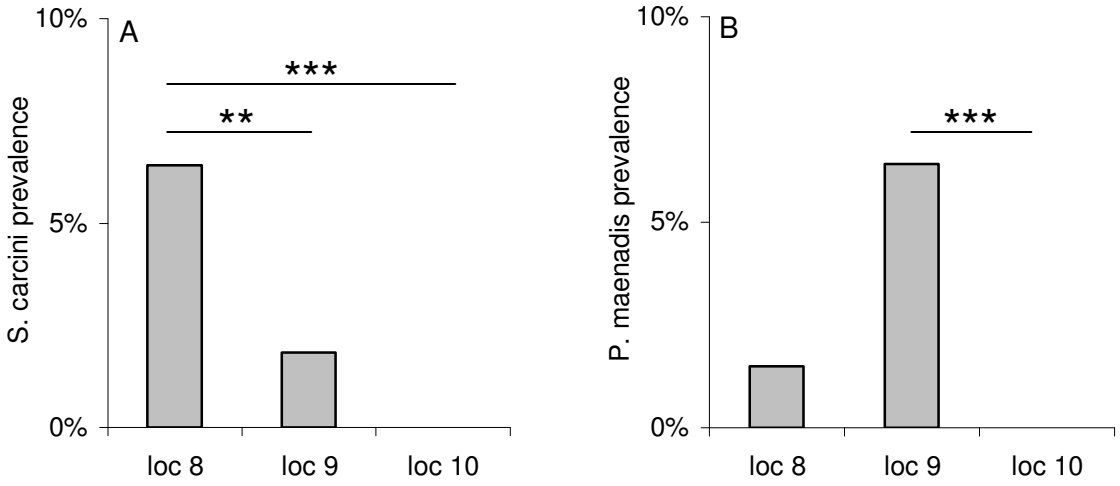

Figure 22: Parasitic castrator prevalence in different subpopulations of the Kattegat. **A -** *S. carcini* prevalence. Location numbers refer to Figure 13. * = significant differences (Fisher's exact test: χ^2=24.958, p<0.001 and χ^2=9.526, p=0.003). **B** - *P. maenadis* prevalence. * = significant differences (Fisher's exact test: χ^2=11.782, p=0.001). Sample sizes are given in Table 6.

Table 7: Statistical differences between parasite prevalence of subpopulations on a European scale and in smaller geographical areas. Europe: loc 1-10, North Sea: loc 1-2, Helgoland: loc 2a-2c, Nykøbing: loc 4-6, Kattegat: loc 8-10. Fisher's exact tests were used to determine differences. Significant p-values are printed bold. n.a.: parasite species or group not present. Sample sizes are given in Table 6. Location (loc) numbers refer to Figure 13.

	S. carcini		***P. maenadis***		**microphallids**		***P. botulus***	
	χ^2	**p**	χ^2	**p**	χ^2	**p**	χ^2	**p**
Europe	333.594	**0.000**	24.599	0.056	319.824	**0.000**	373.663	**0.000**
North Sea	9.816	0.081	n.a.	n.a.	53.888	**0.000**	17.124	**0.004**
Helgoland	n.a.	n.a.	n.a.	n.a.	46.490	**0.000**	10.807	**0.013**

	S. carcini		*P. maenadis*		microphallids		*P. botulus*	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Nykøbing	11.547	**0.003**	1.092	0.579	1.276	0.528	1.406	0.495
Kattegat	30.134	**0.000**	12.901	**0.002**	46.962	**0.000**	62.434	**0.000**

3.1.1.3 Helminth intensity

Like prevalences, helminth intensities showed marked variation between sample sites in Europe. Infection intensities of microphallid trematodes were highest on the island of Sylt and in the western part of the Limfjord (Figure 23, Table 6). However, there were no significant intensity differences in sub-localities of Helgoland, the Nykøbing area or the Kattegat.

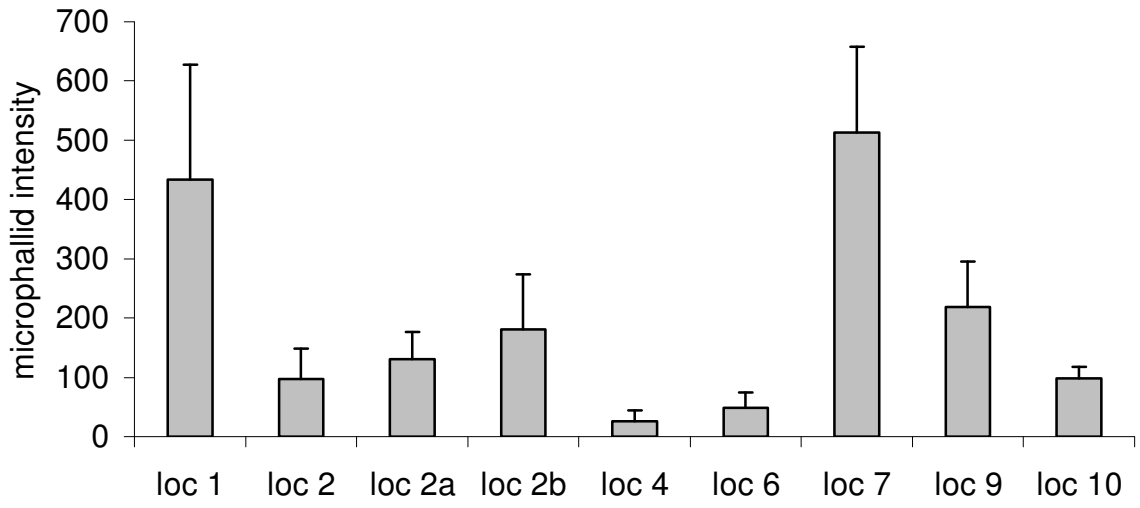

Figure 23: Mean microphallid intensities (+/-SE) in different locations of Europe. Location numbers refer to Figure 13. Kattegat. Location 2a and 2b are Helgoland samples that were caught manually in intertidal areas. Only data for sample sizes of n>5 is shown.

In microphallids, there was no correlation between prevalence and intensity, however, these factors showed a significantly positive correlation for *P. botulus* (Figure 25).

Acanthocephalan intensity ranged between an average of 1 and 3 cystacanths per host in most European populations (Table 6). One exception was location 8 on the northern coast of Fyn (Figure 13), where mean intensity reached a peak of over 9 cystacanths per infected crab (Figure 24). Not surprisingly, this lead to significant differences between the three

populations sampled in the Kattegat (Kruskal-Wallis test: χ^2_{12}= 52.194, p<0.001). No such differences were found within the other sub-localities, the North Sea and the Nykøbing area.

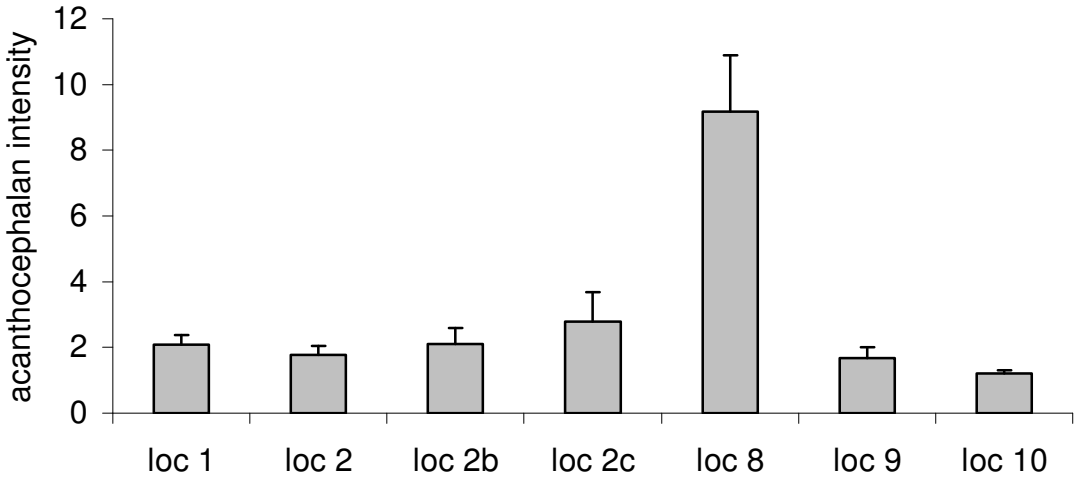

Figure 24: Mean acanthocephalan intensity (+/-SE) at different European locations. Location numbers refer to Figure 13. Location 2a and 2b are Helgoland samples that were caught manually in intertidal areas. Only data for sample sizes of n>5 is shown.

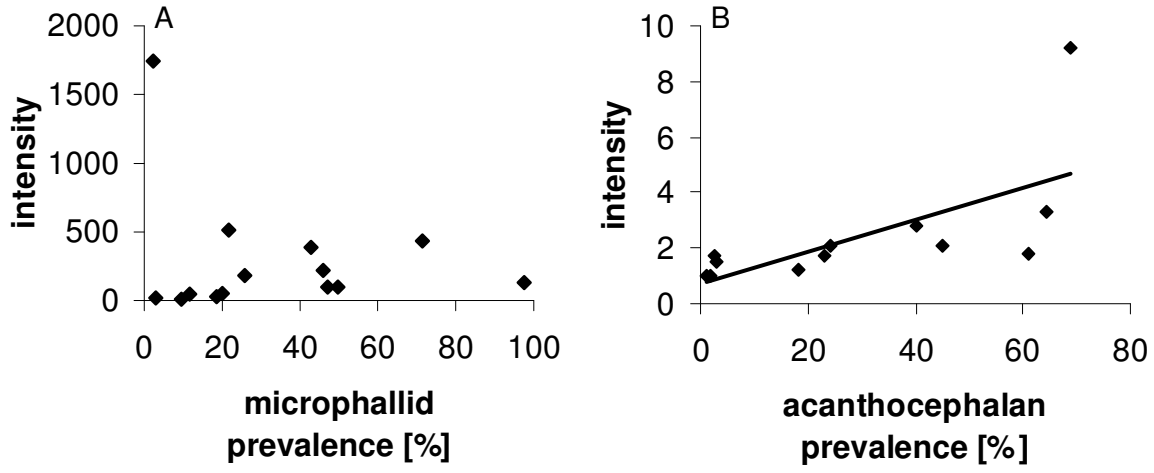

Figure 25: Correlation between helminth prevalence and mean intensity. **A** - Microphallid trematodes: no significant correlation. **B** - Acanthocephalans (*P. botulus*): R_s=0.907, p<0.001.

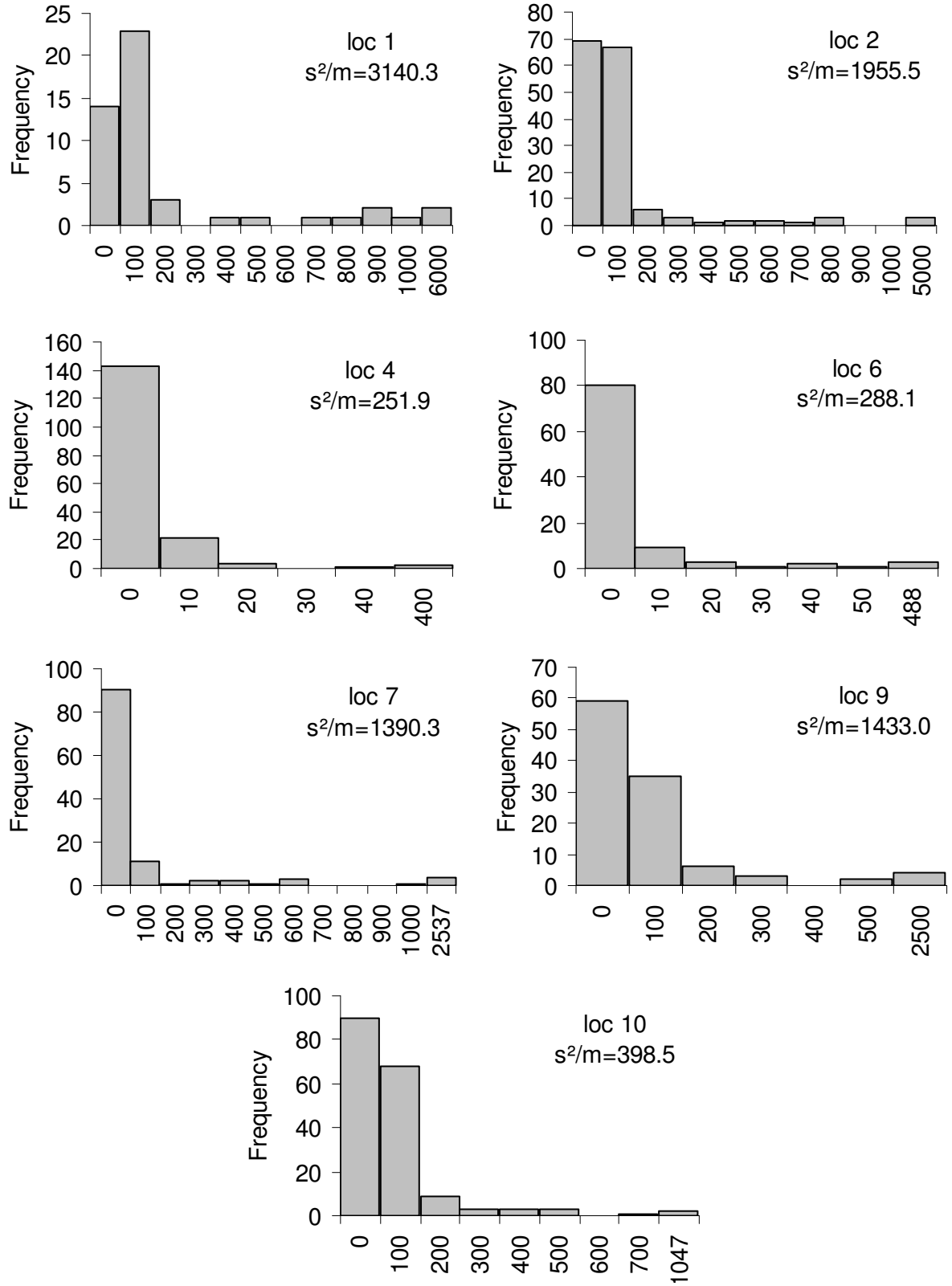

Figure 26: Frequency distribution of metacercariae in crab hosts. X-axis desdribes the maximum number of metacercariae per infected crab in each category. Variance to mean ratio (s^2/m) is given to describe the degree of aggregation.

Both helminths were aggregated in populations where they were common. Most crab hosts were infected only with moderate amounts of metacercariae and cystacanths, while a few individuals suffered from severe infections. This was much more pronounced with regards to microphallid trematodes (Figure 26). Here the variance to mean ratio ranged between 250 and well over 3000, indicating a strong overdispersion. In the case of acanthocephalans, the cystacanth frequency was aggregated to a much smaller degree (Figure 27).

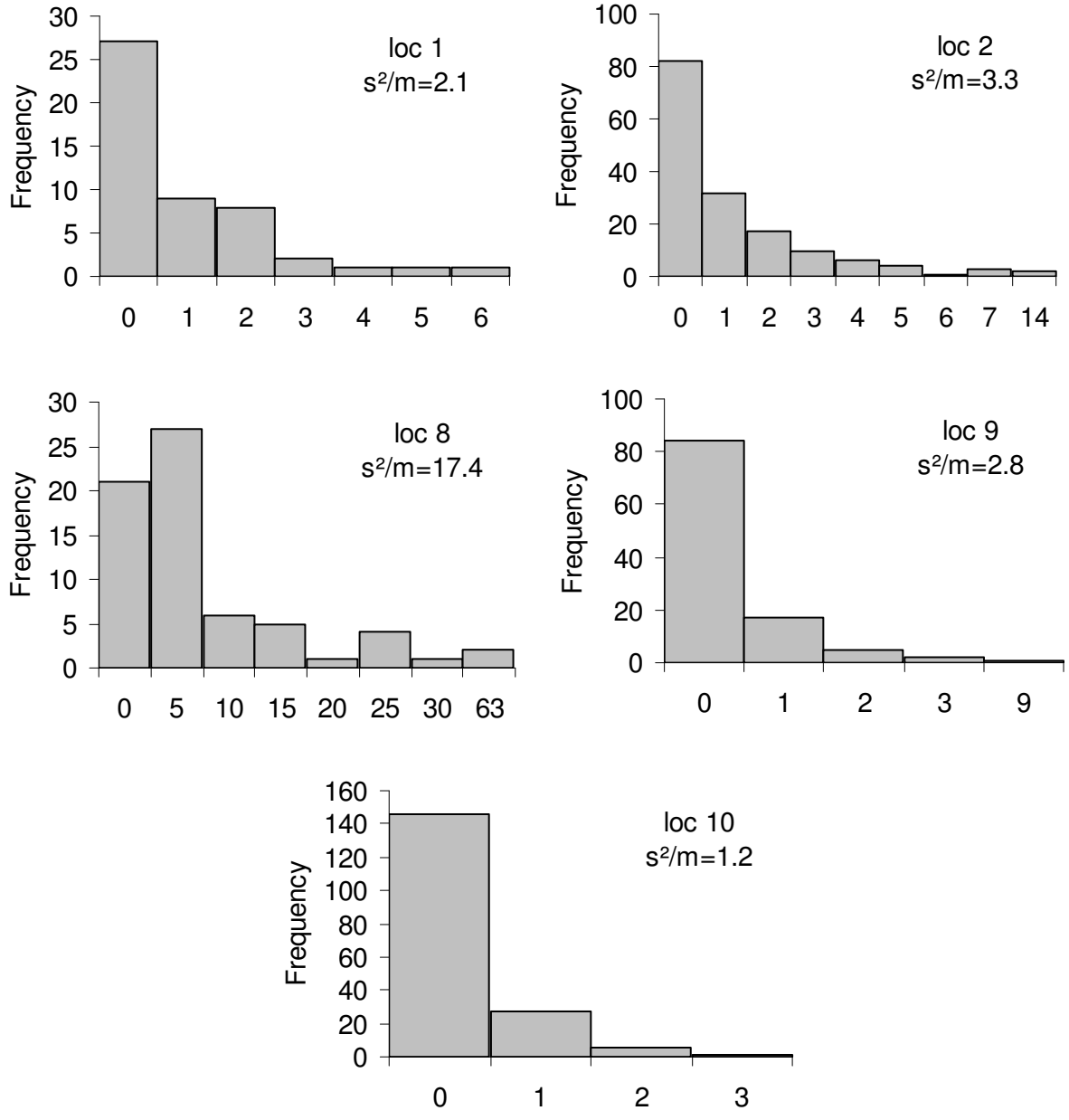

Figure 27: Frequency distribution of cystacanths in crab hosts. X-axis desdribes the maximum number of cystacanths per infected crab in each category. Variance to mean ratio (s^2/m) is given to describe the degree of aggregation. Location (loc) numbers refer to Figure 13.

Values of variance to mean ratio ranged from 1.2 to 3.3, which supports a distribution of cystacanths approaching random. The one exception to this was location 8, where variance to mean ratio was $s^2/m=17.4$, which coincides with the high prevalences and intensities in this specific location. The only other population with a similar prevalence of acanthocephalans in *C. maenas* was location 2, which had a considerably lower mean intensity, however.

3.1.1.4 Annual variation in prevalence and intensity

In two sample locations in the Nykøbing area it was possible to sample crabs in September and October of two consecutive years, 2006 and 2007. The Helgoland site was sampled in August 2008 and again in May 2009. This allowed for a comparison of prevalence and intensity of the parasites found in these areas over two years.

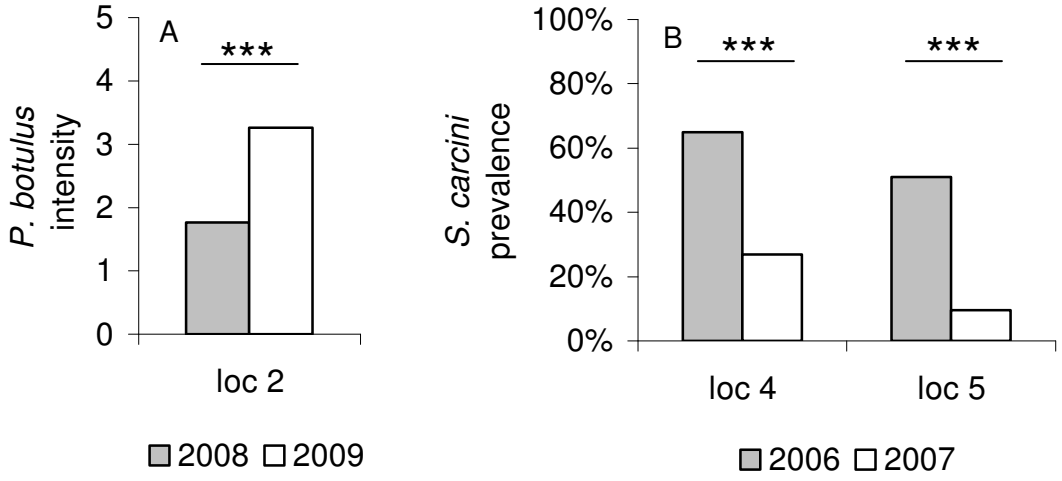

Figure 28: A - Differences in *P. botulus* intensity in Helgoland between 2008 and 2009. * = significant differences (Mann-Whitney-U test: Z=-2.548, p=0.01). **B** - Differences in *S. carcini* prevalence in two sample locations near Nykøbing, Mors between 2006 and 2007. * = significant differences (Fisher's exact test: location 4: χ^2= 24.206, p<0.001; location 5: χ^2= 10.895, p=0.001). Location (loc) numbers refer to Figure 13.

In Helgoland, there were no prevalence differences between 2008 and 2009, for either helminth group. However, there was a significant difference in *P. botulus* intensity in infected crabs, which was higher in spring compared to the previous summer (Mann-Whitney-U test: Z=-2.548, p=0.01) (Figure 28).

In the Nykøbing area, the main difference was found in *S. carcini* prevalence, which was significantly reduced in the second year, in both populations (Fisher's exact test: location 4: χ^2= 24.206, p<0.001; location 5: χ^2= 10.895, p=0.001) (Figure 28).

3.1.2 Parasites in South Africa and Australia

In South Africa, a total of 325 European shore crabs were examined. None of them carried macroparasites, indicating that this invasive population is free of parasites.

In Australia, the sampling of crabs was considerably harder than expected, as they were very rare in areas where they had previously been sampled successfully (GURNEY 2006). Anecdotal reports of fishers and divers indicate that *C. maenas* populations in Port Phillip Bay may be declining in general. It was therefore not possible to sample more than one population in sufficient numbers. Of the 107 crabs caught in this area, 5 individuals were found to carry one encysted plerocercoid of the cestode *Trimacracanthus aetobatidis*, resulting in a prevalence of 4.7%. Only one crab (0.9%) was infected with a nematode of the genus *Proleptus* sp.

3.1.3 Size distribution

For the analysis of size distributions between different populations only crabs from overseas as well as from the populations sampled in Denmark in 2007 were used. This includes two populations from the harbour areas sampled in South Africa (location 12-13, Figure 15) and three neighbouring populations close to Nykøbing in the Limfjord (location 4-6, Figure 13). These were sampled with a standardized method, including the use of similar traps and the same sampling season in late summer and early autumn. All crabs of a catch were examined to avoid non-random sampling.

Statistical analysis of carapace width (CW) showed significant differences between these six locations (ANOVA: males: F=26.346, p=0.000; females: F=55.211, p=0.000). A subsequent post-hoc test (Tukey-HSD) revealed that the South African populations form a separate group with a significantly higher mean CW than in Europe and Australia (Table 8). Male mean CW in Europe was 53.8 mm, in contrast to 65 mm in South Africa. The size difference was even more pronounced in females, which show a 44.6 mm mean CW in Europe and 61.3 mm in South Africa. This equals a 21% and 37% size increase in males and females, respectively. With mean CWs of 55.5 mm (males) and 48.0 mm (females), *C. maenas* in Australia was well in line with European size ranges.

Table 8: Comparison of mean size (carapace width) between 6 examined subpopulations. Homogenous subgroups were determined with a Tukey-HSD post-hoc test. Location (loc) numbers refer to Figure 13.

Sample location		Homogenous groups 1	2	ANOVA p	F
		Males			
6	Europe	53.2mm			
4	Europe	53.8mm			
14	Australia	55.5mm		0.000	26.346
5	Europe	56.1mm			
12	South Africa		63.4mm		
13	South Africa		69.7mm		
		Females			
4	Europe	44.2mm			
6	Europe	45.5mm			
14	Australia	48.0mm		0.000	55.211
12	South Africa		58.7mm		
13	South Africa		63.7mm		

To examine crab size more closely crabs were separated into size classes (Figure 29) and the distribution over these size classes was compared between Europe and the two invasive populations. For this, the three subpopulations in Denmark and the two subpopulations of South Africa were pooled, respectively.

There was a significant difference in size distribution between South Africa and Europe for both males (χ^2_5=94.0, p<0.001) and females (χ^2_4=70.9, p<0.001). This was caused by an above average representation of larger size classes (Figure 29).

The situation in Australia was different, with no differences in size distribution between European and Australian males. However, in females there was a significant difference with an increased number in the larger size classes in Australia (χ^2_4=20.6, p<0.001) (Figure 29).

Colour morph is correlated with crab size, as moult frequency slows down in older and larger crabs. A high percentage of green crabs in a size class indicates that moult, and therefore further growth is common. This was investigated for male crabs caught in the Limfjord and in South Africa in 2007. Due to the comparatively low number of males found in Australia in the following year, this location could not be investigated. Colour morphs in females are much less pronounced; therefore I limited this analysis to males.

There were significant differences in the distribution of red individuals over different size classes (χ^2_9=69.785, p<0.001). In South Africa, males were much less likely to be red than males in comparable size classes in Europe (Figure 30).

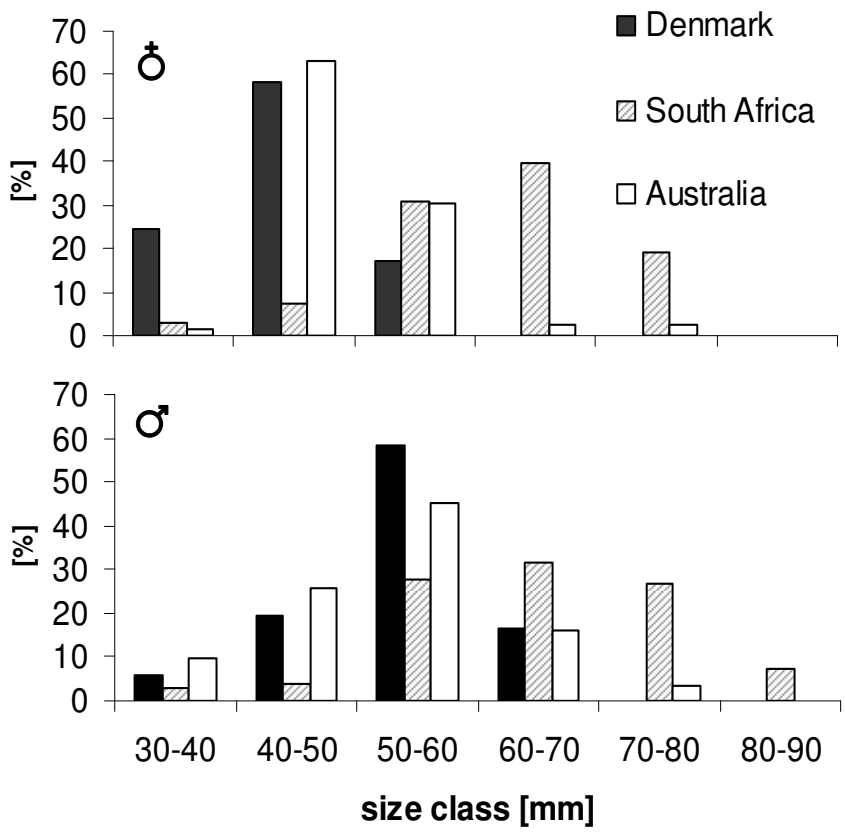

Figure 29: Distribution over size classes in Denmark (location 4-6), South Africa (location 12-13) and Australia (location 14). Location numbers refer to Figure 13, 15 & 17.

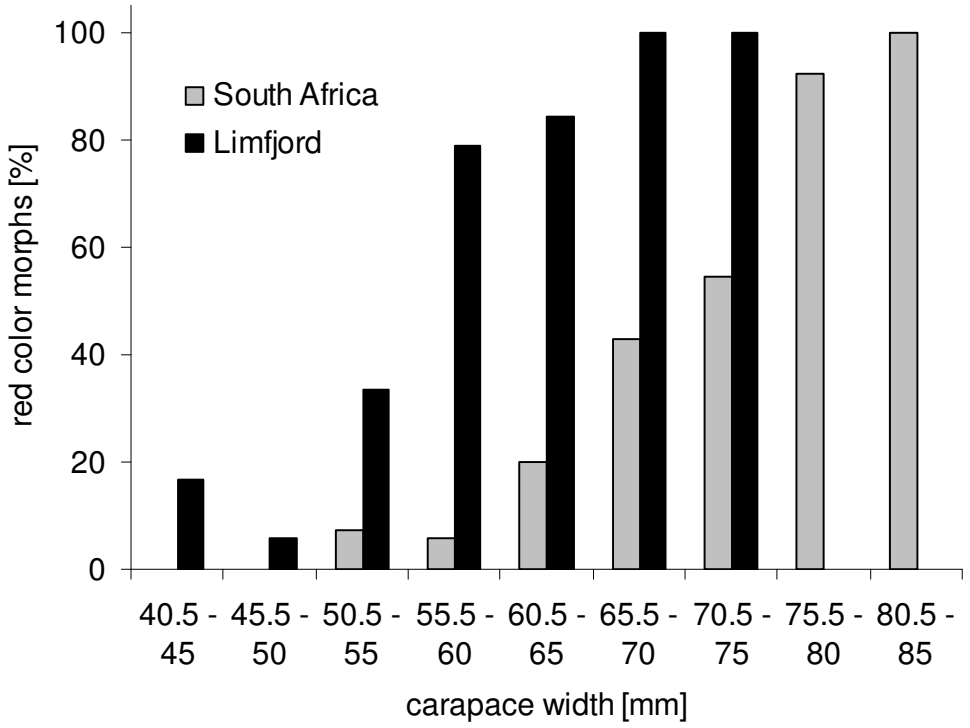

Figure 30: Fraction of crabs with red colour morph in different size classes. Populations from South Africa (2007) and the Limfjord (2007) were pooled (χ^2_9=69.785, p<0.001).

3.1.4 Predation

Autotomy in *C. maenas* was analysed for certain populations caught from 2007 to 2008 (for sample locations see Figure 13, 15 & 17). For these specific samples, an autotomy bias caused by captivity or the catching process could be excluded.

I found that autotomy was highly variable between populations, ranging from under 5% of Australian female crabs to 38% in male crabs from one European population (Figure 31). In Europe there were significant differences between populations, with the highest frequency of autotomy found in crabs from location 5 (χ^2_9=12.391, p=0.006). There was no significant difference between South African populations, but a trend was present as well (χ^2_2=5.546, p=0.062). Here, crabs from intertidal location 11 generally showed less cases of autotomy.

There was typically no influence of crab sex on the occurrence of autotomy, with the exception of Australia, where male crabs lack limbs significantly more often (Fisher's exact test: χ^2=6.785, p=0.009) (Figure 31).

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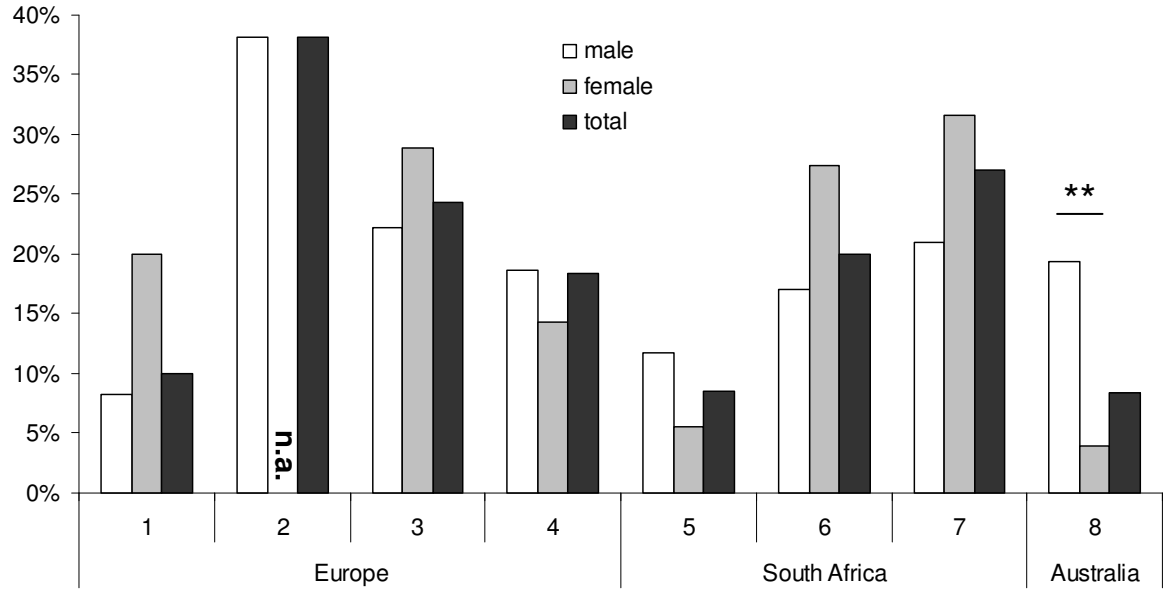

Figure 31: Occurrence of walking leg autotomy in crab populations of Europe, South Africa and Australia. Location numbers refer to Figure 13, 15 & 17. No female data available for location 2 (n.a.). * = significant differences between male and female crabs (Fisher's exact test: χ^2=6.785, p=0.009).

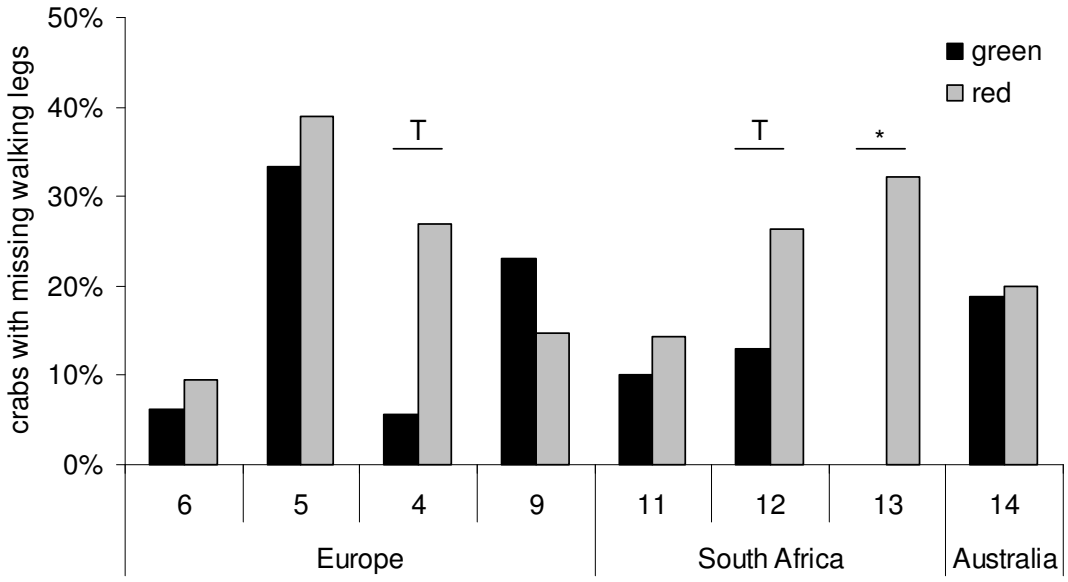

Figure 32: Occurrence of autotomy in male crabs of green and red colour morph. Numbers refer to sample locations marked in Figure 13, 15 & 17. * = significant differences between morphs (Fisher's exact test: χ^2=6.098, p=0.014). T = trend (Fisher's exact test: p<0.08).

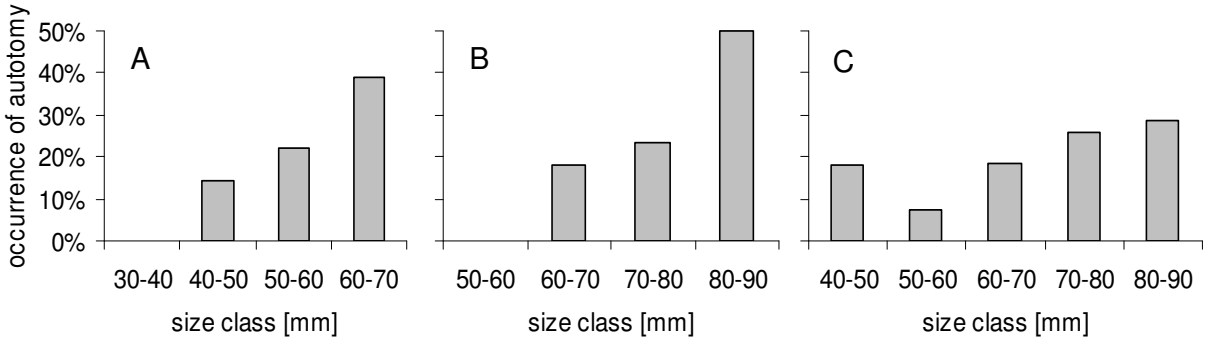

Figure 33: Size was correlated with the frequency of walking leg autotomy in two sample locations, with a trend in a third location. **A** - location 4, Europe (R_s=0.258, p=0.02). **B** - location 12, South Africa (R_s=0.335, p=0.028). **C** - location 11, South Africa (R_s=0.166, p=0.06). Location numbers refer to Figure 13 & 15.

In male crabs, red colour morph was connected with a higher amount of autotomy in location 13 of South Africa (Fisher's exact test: χ^2=6.098, p=0.014) with a similar trend in two additional populations (Fisher's exact test: p<0.08) (Figure 32). However, these latter two also show a significant correlation between crab size class and the frequency of autotomy (location 4, Europe: R_s=0.258, p=0.020; location 12, South Africa: R_s=0.335, p=0.028) (Figure 33). Yet this was not the case for location 13 in South Africa, indicating that the differences between morphs cannot be attributed to size or age effects in this population.

Sampling depth had a very clear impact on the incidence of autotomy. The mean number of missing legs was lowest in shallow water and increased with water depth (R_s=0.499, p=0.025) (Figure 34).

In a comparison of the average frequency of autotomy between all crabs of the major three areas Denmark, South Africa and Australia, a significant difference was only found for Australia (Fisher's exact test: χ^2=8.619, p=0.013) (Figure 35). However, this effect is mainly caused by the high number of female crabs that was sampled in this population which showed a significantly reduced occurrence of autotomy compared to males.

Neither helminth parasites nor the castrator *S. carcini* had any significant influence on the frequency of autotomy or on the number of missing legs in their host (Appendix table 7 to 9). There is therefore no indication for higher or lower predation rates on these crabs.

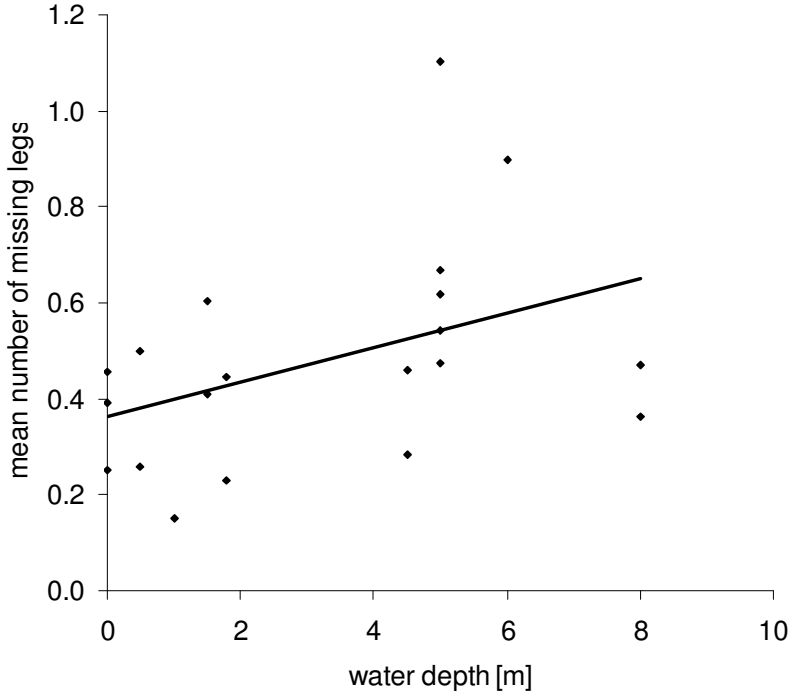

Figure 34: Correlation between water depth of the sampling and mean number of missing legs in a population (R_s=0.499, p=0.025).

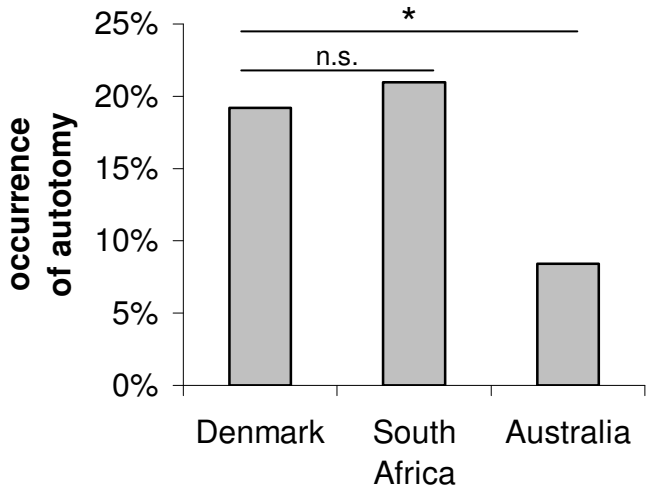

Figure 35: Overall occurrence of walking leg autotomy in Europe, South Africa and Australia. * = significant difference (Fisher's exact test: p=0.013, χ^2=8.619).

3.1.5 Epibionts

I typically found three different groups of epibionts colonizing the carapace of *C. maenas*: bryozoans, barnacles and the tubes of polychaete worms (Figure 36). Epibionts were not determined further and simply grouped into these three categories. In isolated cases other fouling organisms were observed, mainly algae and mussels. While mussel spat was the more common occurrence of these two, the number of affected crabs was so low as to not warrant analysis. Mussel spat was mostly observed in the branchial chamber or around the eye stalks, while all other epibionts preferably colonized the carapace.

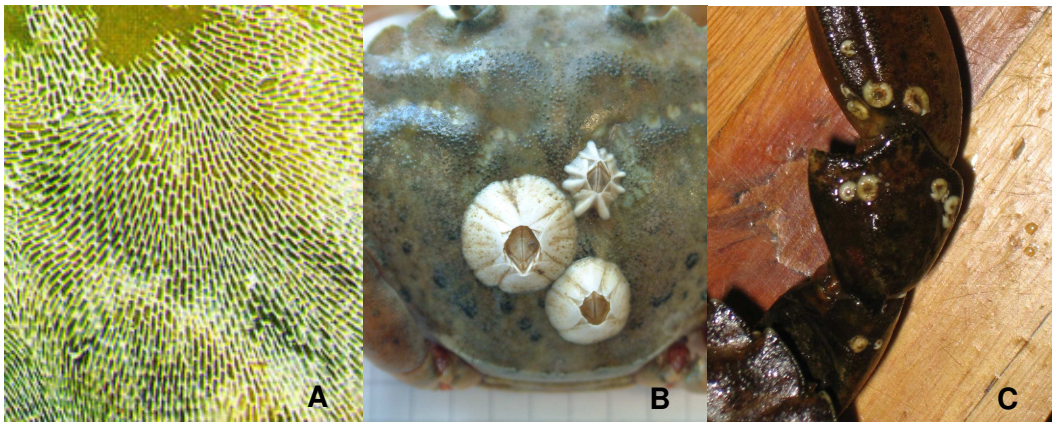

Figure 36: Epibionts found on the carapace of *C. maenas*. **A** - Bryozoans. **B** - Barnacles. **C** - Polychaetes.

3.1.5.1 Epibiont prevalence

Similar to parasite prevalence, epibiont prevalence was highly variable between different sampling locations in Europe (Figure 37). The two North Sea locations showed a comparably low percentage of crabs infected with bryozoans and polychaetes. The prevalence of barnacles, however, was significantly higher in Sylt (location 1) (Fisher's exact test: χ^2=41.988, p<0.001), where the maximum of all sampled locations was reached at over 40%.

In the Limfjord, there was a significant difference between the sampling locations for all epibionts (polychaetes χ^2_4=17.633, p=0.001; bryozoans χ^2_4=22.307, p<0.001; barnacles χ^2_4=33.844, p<0.001). Especially location 7 in the eastern Limfjord was almost free of epibionts. In the Kattegat, only barnacle prevalence varied between the three locations (χ^2_2=14.183, p=0.001), while bryozoans showed an evenly low prevalence and polychaetes were completely absent.

In general, bryozoan and polychaete prevalence on *C. maenas* individuals was under 10% in most areas and under 20% in the Limfjord. Barnacles were found in all populations and showed a considerably higher variance in prevalence (Figure 37)

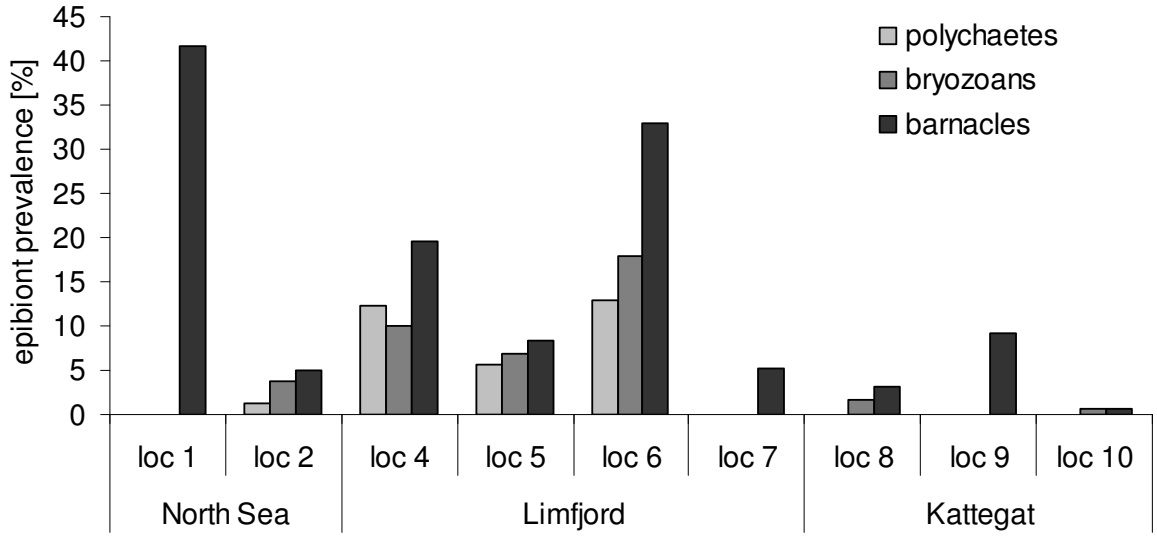

Figure 37: Prevalence of three groups of epibionts on *C. maenas* in different European locations. Location (loc) numbers refer to Figure 13.

In the invasive areas of South Africa and Australia, no bryozoans were found on *C. maenas*. While the crabs that I examined in Australia were completely free of epibionts, both barnacles and polychaetes were commonly encountered in South Africa (Figure 38). Prevalence of both types of epibionts varied significantly between sampling locations (polychaetes χ^2_2=7.127, p=0.028; barnacles χ^2_2=17.941, p<0.001), mainly due to exceptionally low values at location 11 (South Point).

In most areas, epibionts were significantly more prevalent on crabs of the red colour morph (Table 9 to 11). Sex was irrelevant for polychaete prevalence (Table 9). For bryozoans and barnacles, however, females and males differed significantly in some areas, while being similar in others (Table 10 & 11). Overall, there was no clear trend indicating that one sex is preferentially settled by epibionts, as the results vary based on sampling location.

Epibionts have the potential to alter predator-prey interactions. Barnacles were the most conspicuous and most common types of epibionts found on *C. maenas* in our samples, but in almost all cases there was no correlation between the number of barnacles and the occurrence of autotomy. There was, however, a single exception to this. In area 12 of South

Africa I observed a weak but significant positive correlation between autotomy and number of barnacles (R=0.155, p=0.038), indicating increased predation on crabs settled by barnacles in this area.

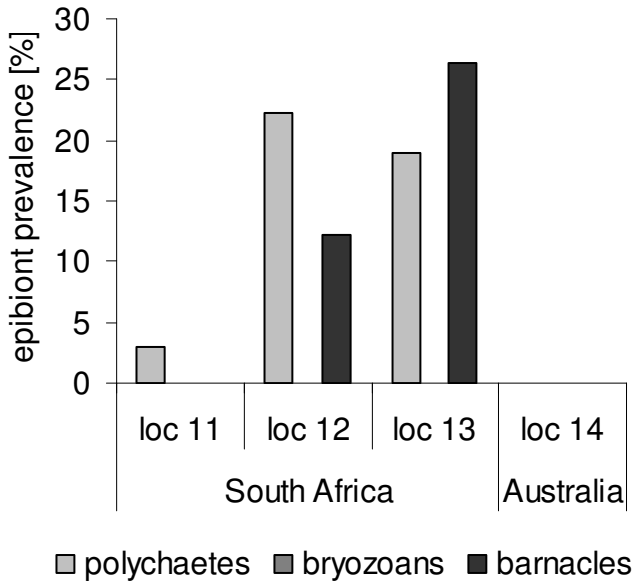

Figure 38: Prevalence of three groups of epibionts on *C. maenas* in different invasive locations. Location numbers refer to Figure 15 and Figure 17.

Table 9: Prevalence (prev.) of polychaete epibionts on crabs from all sampled populations. Prevalence differences between male and female crabs are given, as well as differences between red and green colour morphs. Significance levels (p) for these differences are given where sample sizes were sufficient (Fisher's exact tests). Significant differences are printed bold. N_{total}: all sampled crabs. N_{Poly}: Sample size of crabs colonized by polychaetes. Location (loc) numbers refer to Figure 13, 15 & 17**.**

	total			**prev. [%]**			**prev. [%]**		
	prev. [%]	**N_{total}**	**$N_{Poly.}$**	**male**	**female**	**p**	**green**	**red**	**p**
				North Sea					
loc 1	0.0	49	0	0.0	0.0	---	0.0	0.0	---
loc 2	1.3	158	2	2.1	0.0	---	2.2	0.0	---
				Limfjord					
loc 4	12.3	179	22	13.7	9.7	n.s.	6.7	16.5	0.064
loc 5	5.6	72	4	6.5	0.0	---	0.0	9.1	---
loc 6	13.0	100	13	14.1	6.7	n.s.	4.4	20.0	**0.020**
loc 7	0.0	115	0	0.0	0.0	---	0.0	0.0	---
				Kattegat					
loc 8	0.0	65	0	0.0	0.0	---	0.0	0.0	---
loc 9	0.0	109	0	0.0	0.0	---	0.0	0.0	---
loc 10	0.0	178	0	0.0	0.0	---	0.0	0.0	---
				South Africa					
loc 11	2.9	35	1	5.9	0.0	---	0.0	12.5	---
loc 12	22.2	180	40	20.2	27.5	n.s.	14.5	42.9	**0.000**
loc 13	19.0	100	19	16.3	21.1	n.s.	13.6	26.8	n.s.
				Australia					
loc 14	0.0	107	0	0.0	0.0	---	0.0	0.0	---

Table 10: Prevalence (prev.) of bryozoan epibionts on crabs from all sampled populations. Prevalence differences between male and female crabs are given, as well as differences between red and green colour morphs. Significance levels (p) for these differences are given where sample sizes were sufficient (Fisher's exact tests). Significant differences are printed bold. N_{total}: all sampled crabs. $N_{Bryo.}$: Sample size of crabs colonized by bryozoans. Location (loc) numbers refer to Figure 13, 15 & 17.

	total			**prev. [%]**			**prev. [%]**		
	prev .[%]	**N_{total}**	**$N_{Bryo.}$**	**male**	**female**	**p**	**green**	**red**	**p**
				North Sea					
loc 1	0.0	49	0	0.0	0.0	---	0.0	0.0	---
loc 2	3.8	158	6	6.3	0.0	---	0.0	9.2	---
				Limfjord					
loc 4	10.1	179	18	15.4	0.0	**0.000**	0.0	17.5	**0.000**
loc 5	6.9	72	5	8.1	0.0	---	0.0	11.4	---
loc 6	18.0	100	18	20.0	6.7	n.s.	8.9	25.5	**0.028**
loc 7	0.0	115	0	0.0	0.0	---	0.0	0.0	---
				Kattegat					
loc 8	1.6	65	1	0.0	5.0	---	2.2	0.0	---
loc 9	0.0	109	0	0.0	0.0	---	0.0	0.0	---
loc 10	0.6	179	1	0.6	0.0	---	0.0	0.0	---
				South Africa					
loc 11	0.0	35	0	0.0	0.0	---	0.0	0.0	---
loc 12	0.0	180	0	0.0	0.0	---	0.0	0.0	---
loc 13	0.0	100	0	0.0	0.0	---	0.0	0.0	---
				Australia					
loc 14	0.0	107	0	0.0	0.0	---	0.0	0.0	---

Table 11: Prevalence (prev.) of barnacles on crabs from all sampled populations. Prevalence differences between male and female crabs are given, as well as differences between red and green colour morphs. Significance levels (p) for these differences are given where sample sizes were sufficient (Fisher's exact tests). Significant differences are printed bold. N_{total}: all sampled crabs. $N_{Barn.}$: Sample size of crabs colonized by barnacles. Location (loc) numbers refer to Figure 13, 15 & 17.

	total			**prev. [%]**			**prev. [%]**		
	prev .[%]	**N_{total}**	**$N_{Barn.}$**	**male**	**female**	**p**	**green**	**red**	**p**
				North Sea					
loc 1	41.7	48	20	39.1	100.0	---	19.2	68.2	**0.001**
loc 2	5.1	158	8	7.4	1.6	n.s.	0.0	12.3	**0.001**
				Limfjord					
loc 4	19.6	179	35	24.8	9.7	**0.017**	2.7	32.0	**0.000**
loc 5	8.3	72	6	9.7	0.0	---	3.6	11.4	---
loc 6	33.0	100	33	37.6	6.7	**0.018**	17.8	45.5	**0.005**
loc 7	5.2	115	6	7.4	0.0	---	0.0	9.5	---
				Kattegat					
loc 8	3.1	65	2	2.2	5.0	---	4.4	0.0	---
loc 9	9.2	109	10	7.8	28.6	n.s.	2.0	15.0	**0.022**
loc 10	0.6	178	1	0.6	0.0	---	0.0	0.8	---
				South Africa					
loc 11	0.0	35	0	0.0	0.0	---	0.0	0.0	---
loc 12	12.2	180	22	14.0	7.8	n.s.	4.6	32.7	**0.000**
loc 13	26.3	99	26	37.2	17.9	**0.039**	13.8	43.9	**0.001**
				Australia					
loc 14	0.0	107	0	0.0	0.0	---	0.0	0.0	---

3.1.5.2 Epibiont intensity

Within Europe, epibiont intensity was generally similar between the sampling locations (Table 12). However, for barnacles there was a trend to higher variation (Kruskal-Wallis test: χ^2_8=15.229, p=0.055). Contrary to prevalence values, there were no significant differences in intensity between males and females and the two colour morphs (Table 13).

Mean epibiont coverage in crab populations was significantly positively correlated to water depth at which the crabs were sampled (R_s=0.768, p=0.001)(Figure 39).

Table 12: Mean intensities of epibiont growth on *C. maenas* in European locations. Total cover and bryozoan values are given in mean coverage of the carapace [%], while polychaete and barnacles are given as mean numbers of individuals per crab carapace. Location (loc) numbers refer to Figure 13.

	N	total cover		polychaete number		bryozoan cover		barnacle number	
		[%]	SE	No.	SE	[%]	SE	No.	SE
loc 1	21	18.57	4.77					5.35	1.04
loc 2	16	5.44	1.95	1.00	0.00	4.17	3.17	2.00	0.76
loc 3	17	25.29	4.86						
loc 4	48	21.10	3.45	10.23	4.76	1.00	0.00	2.43	0.43
loc 5	18	20.61	5.29	22.00	19.34	1.00	0.00	3.67	1.31
loc 6	43	11.07	2.01	2.00	0.38	1.00	0.00	2.36	0.45
loc 7	6	8.00	0.00					1.50	0.34
loc 8	5	35.80	18.45			100.00	.	5.50	4.50
loc 9	10	10.40	5.70					5.10	1.86
loc 10	2	8.00	0.00					1.00	.

Table 13: Epibiont intensity differences between sexes and colour morphs of *C. maenas*. Polychaete and barnacle intensity is given as mean number of tubes/individuals per crab carapace. Bryozoan intensity is given as the percentage of the carapace that is covered. Intensity differences between male and female crabs are shown, as well as differences between red and green colour morphs. Significance levels (p) for these differences are shown where sample sizes were sufficient (Mann-Whitney tests). Location (loc) numbers refer to Figure 13.

	male	female	p	green	red	p
			mean number of polychaetes			
loc 2	1.0	---	---	1.0	---	---
loc 4	4.4	25.7	n.s.	16.6	8.4	n.s.
loc 5	22.0	---	---	---	22.0	
loc 6	2.1	1.0	n.s.	1.0	2.2	n.s.
loc 11	1.0	---	---	---	1.0	
loc 12	1.8	4.2	n.s.	1.4	3.7	n.s.
loc 13	4.7	2.4	n.s.	2.1	4.1	n.s.
			mean bryozoan coverage [% of carapace]			
loc 2	4.2	---	---	---	4.2	---
loc 4	1.0	---	---	---	1.0	---
loc 5	1.0	---	---	---	1.0	---
loc 6	1.0	1.0	---	1.0	1.0	---
loc 8	---	100.0	---	100.0	---	---
			mean number of barnacles			
loc 1	5.7	2.0	n.s.	2.4	6.3	0.063
loc 2	2.1	1.0	n.s.	---	2.0	---
loc 4	2.6	1.7	n.s.	3.5	2.4	n.s.
loc 5	3.7	---	---	8.0	2.8	n.s.
loc 6	2.0	13.0	0.061	2.8	2.2	n.s.
loc 7	1.5	---	---	---	1.5	---
loc 8	1.0	10.0	n.s.	5.5	---	---
loc 9	3.8	10.5	n.s.	6.0	5.0	n.s.
loc 10	1.0	---	---	---	1.0	---
loc 12	2.4	5.3	n.s.	1.8	3.4	n.s.
loc 13	12.6	8.0	n.s.	6.1	12.9	n.s.

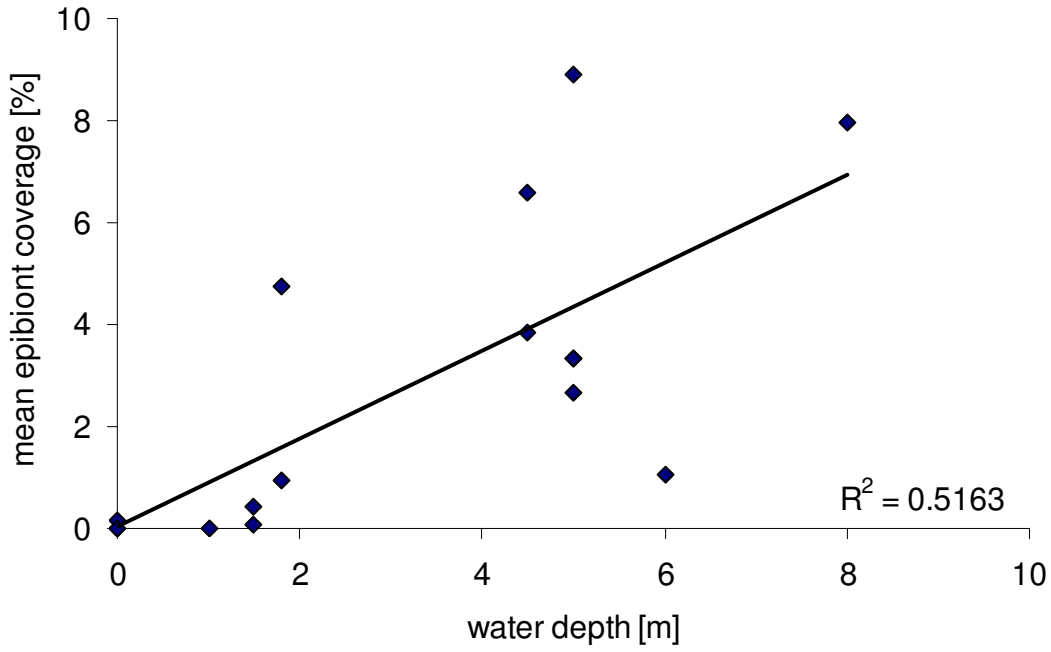

Figure 39: Mean epibiont coverage on crab carapaces in relation to water depth (R_s=0.768, p=0.001).

3.1.5.3 Epibionts and parasite infection

In general crabs infected by parasites did not carry more or less epibionts than uninfected crabs. There were only very few exceptions to this observation, which were mainly focused on location 8 where parasite infection and epibiont presence were positively correlated (Appendix tables 10 to 12).

Sample sizes were mostly too small to determine correlations between parasite and epibiont intensities. Where they were sufficient however, no correlations were found. If abundance rather than intensity was examined (which only includes crabs that harbour both parasites and epibionts), there are a number of notable effects indicating a relationship between *S. carcini* interna intensity and abundance of different epibionts (Table 14).

These effects are confirmed by comparing mean epibiont growth on crabs with and without parasite infection. Again I found significantly higher amounts of epibionts on crabs infected with *S. carcini*, in location 6 (Mann-Whitney-U test: barnacles Z=-2.187, p=0.029) and location 8 (Mann-Whitney-U test: bryozoans Z=-2.669, p=0.008; barnacles Z=-3.804, p<0.001).

Table 14: Correlation between *S. carcini* intensity (ranked 0 to 4) and abundance of epibionts in different European populations. Location (loc) numbers refer to Figure 13.

location	correlation with abundance of	Pearson correlation	significance level	N
loc 4	polychaetes	R=0.171	p=0.025	172
loc 5	polychaetes	R=0.242	p=0.041	72
loc 6	barnacles	R=0.241	p=0.016	100
loc 8	barnacles	R=0.355	p=0.003	65
loc 8	bryozoans	R=0.334	p=0.007	65

3.1.6 Parasite-induced mortality

3.1.6.1 Microphallid trematodes

Six crab samplings provided sufficient sample sizes and microphallid prevalences for an evaluation of parasite-induced mortality. These were samples taken in Helgoland (several sample sites in 2008) and Sylt, one sample from the eastern and the western Limfjord, respectively, and two samples from the Kattegat (Figure 40 and Figure 13 for location numbers).

At location 6 there was no increase in mean cyst number with size class (Figure 40). This was also the population with both the lowest prevalence and lowest intensity in infected crabs. While the graph shows a marked increase in mean cyst number with size class for both Kattegat populations (locations 9 and 10), there was no significant correlation. None of the tested models were significant for these populations and there is no drop in mean cyst number for higher size classes (Table 15).

For the remaining three sample sites a significant or near significant model was found (Table 15). However, in all three cases either the quadratic or the polynomial curve proved to be the best fit for the data. This is supported by the graphs, which show reduced mean cyst numbers in the higher size classes (Figure 40).

Variance to mean ration was not universally correlated with size class. Indeed, a significant correlation was only present in the two Limfjord populations (location 6 - R=0.938, p=0.018; location 7 - R=0.844, p= 0.035).

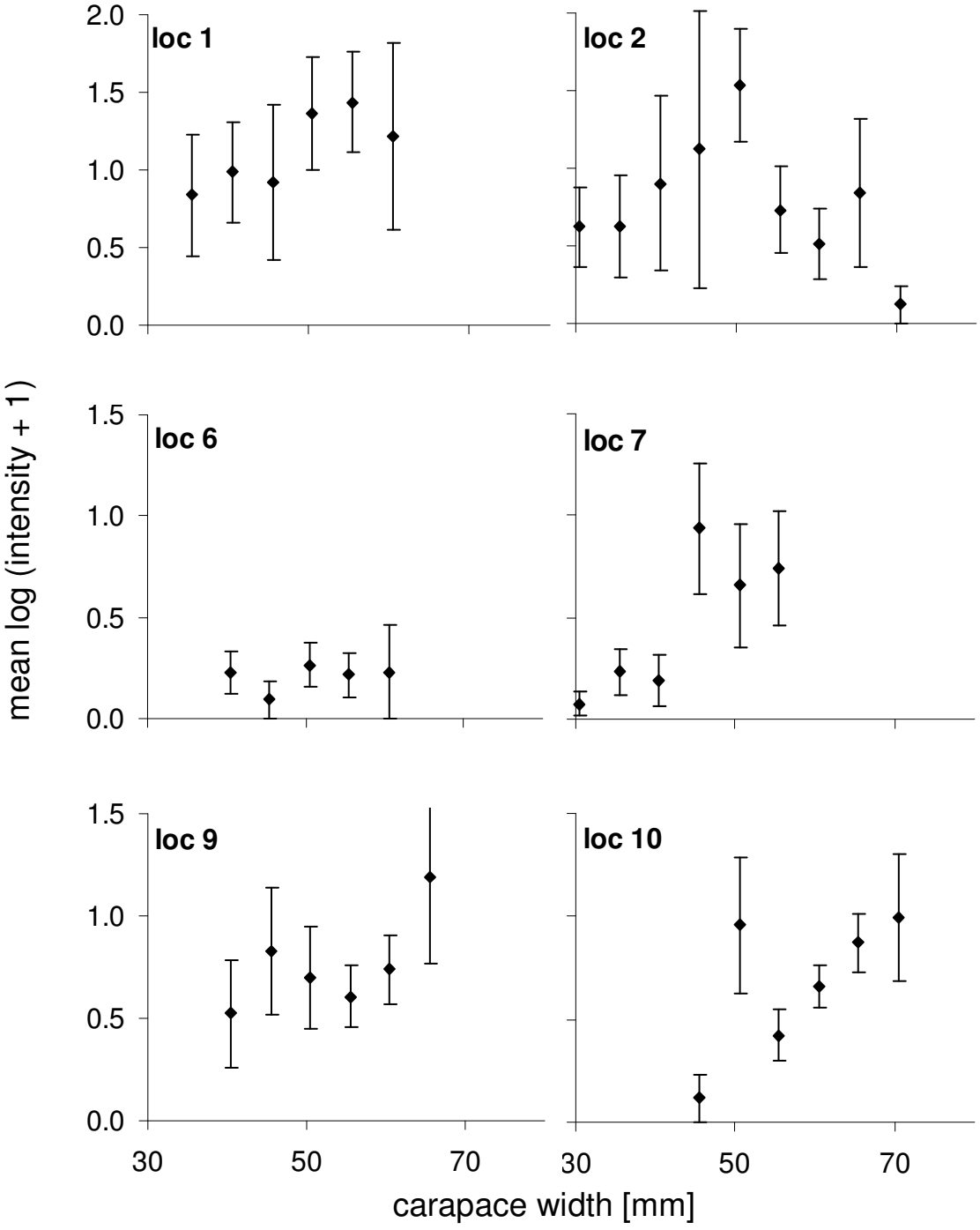

Figure 40: Mean number of log(x+1) transformed microphallid metacercariae per crab in relation to host size classes (+/- SE). Location (loc) numbers refer to Figure 13 (loc 1-2: North Sea; loc 6-7: Limfjord; loc 9-10: Kattegat).

Table 15: Result of the regression analysis for mean number of metacercariae per crab (log(x+1) transformed) in relation to crab size class. Regression analysis was performed for a linear, quadratic and curvilinear model. Models with the best fit are printed bold for all locations. * = significant correlation. Location (loc) numbers refer to Figure 13.

	linear		**quadratic**		**polynomic**		
	R^2	**p**	**R^2**	**p**	**R^2**	**p**	**N**
loc 1	0.64	0.056	0.70	0.162	**0.67**	**0.046***	49
loc 2	0.08	0.447	**0.60**	**0.065**	0.21	0.211	72
loc 6	0.09	0.627	0.11	0.885	**0.10**	**0.600**	100
loc 7	0.66	0.049	0.69	0.170	**0.77**	**0.022***	115
loc 9	0.46	0.136	**0.60**	**0.250**	0.47	0.133	109
loc 10	0.46	0.141	0.46	0.393	**0.50**	**0.116**	179

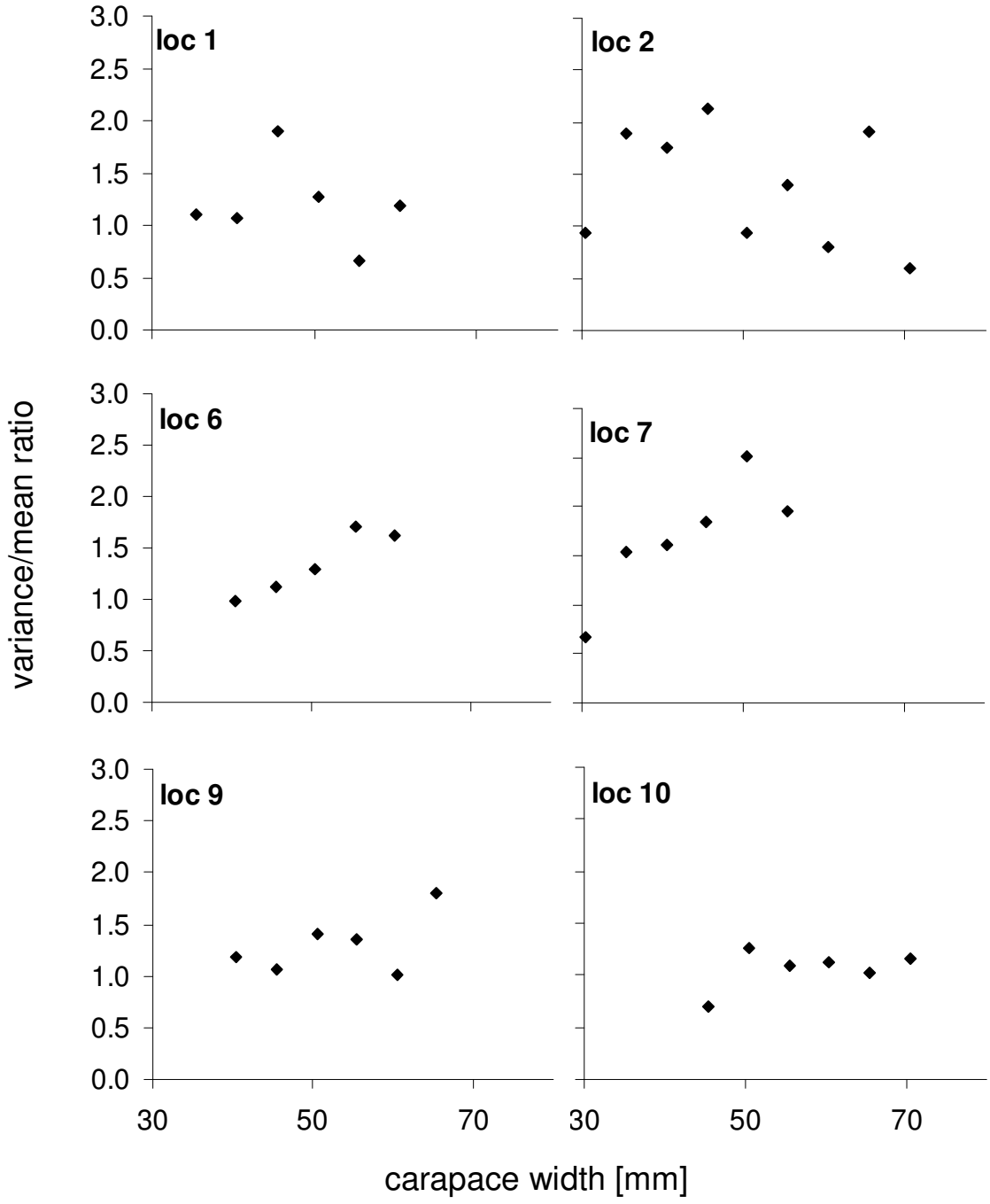

Figure 41: Ratio of variance to mean number of microhallid metacercariae (log(x+1) transformed), in relation to crab size class. Significant correlations in location 6 (R=0.938, p=0.018) and 7 (R=0.844, p= 0.035). Location (loc) numbers refer to Figure 13.

3.1.6.2 Profilicollis botulus

Five crab sampling areas provided sufficient sample sizes and acanthocephalan prevalences for an evaluation of parasite-induced mortality. These were samples taken in Helgoland (several sample sites in 2008) and Sylt, and three samples from the Kattegat (Figure 42 and Figure 13 for location numbers).

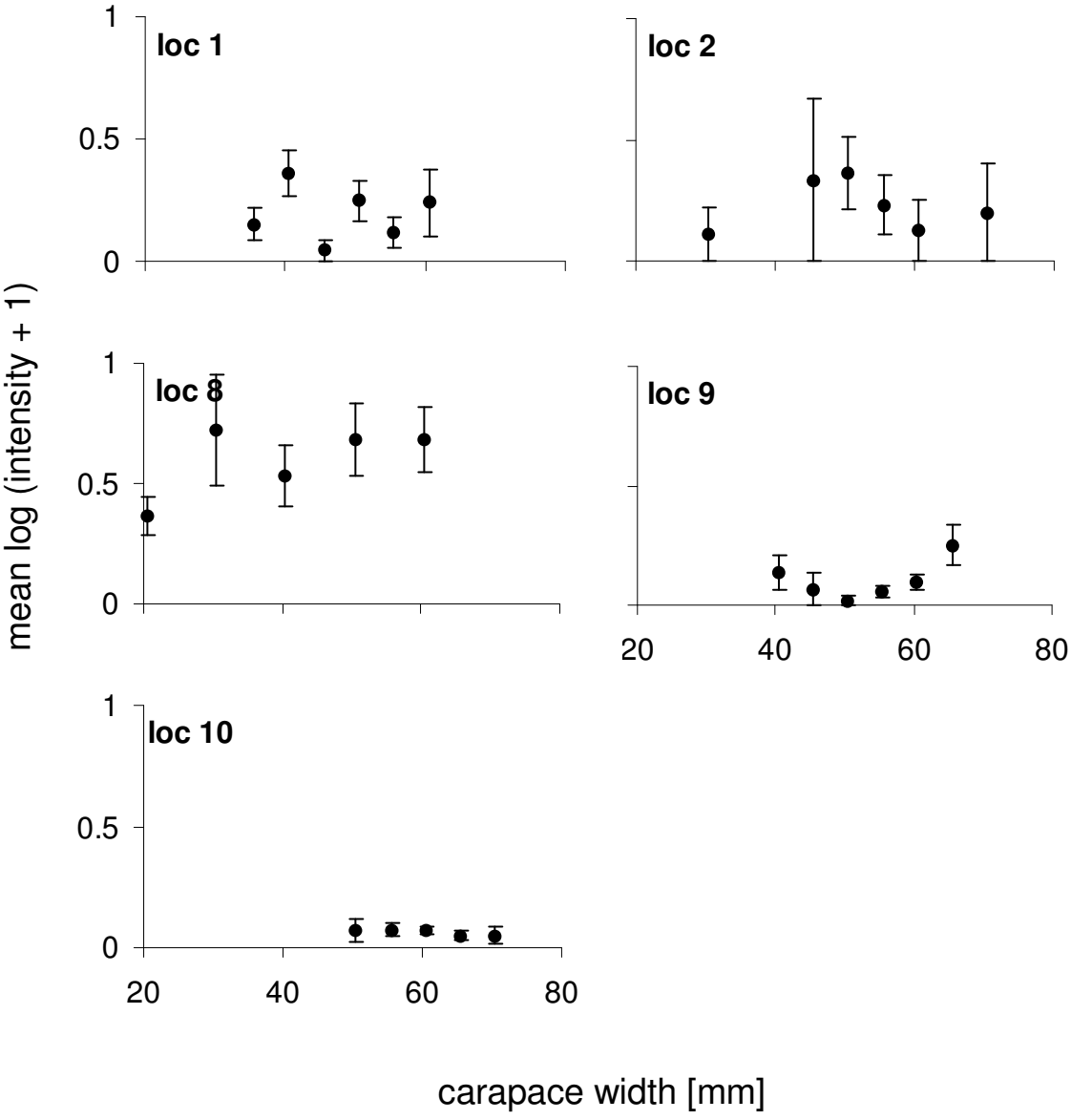

Figure 42: Mean number of log(x+1) transformed acanthocephalan cystacanths per crab in relation to host size classes (+/- SE). Location (loc) numbers refer to Figure 13 (loc 1-2: North Sea; loc 8-10: Kattegat).

The mean number of *P. botulus* cystacanths was not correlated to host size class in any of these populations, regardless of crab size (Figure 42). Regression analysis could therefore not be conducted. This may be caused by general low intensities of cystacanths in most of these populations, but it cannot explain the very similar behaviour in location 8 of the Kattegat, where almost 70% of the crabs harboured an average of 9 cystacanths. There was therefore no sign of accumulation of this parasite.

Like mean cystacanth numbers, the variance to mean ratio was not correlated with size class in any way (Figure 43).

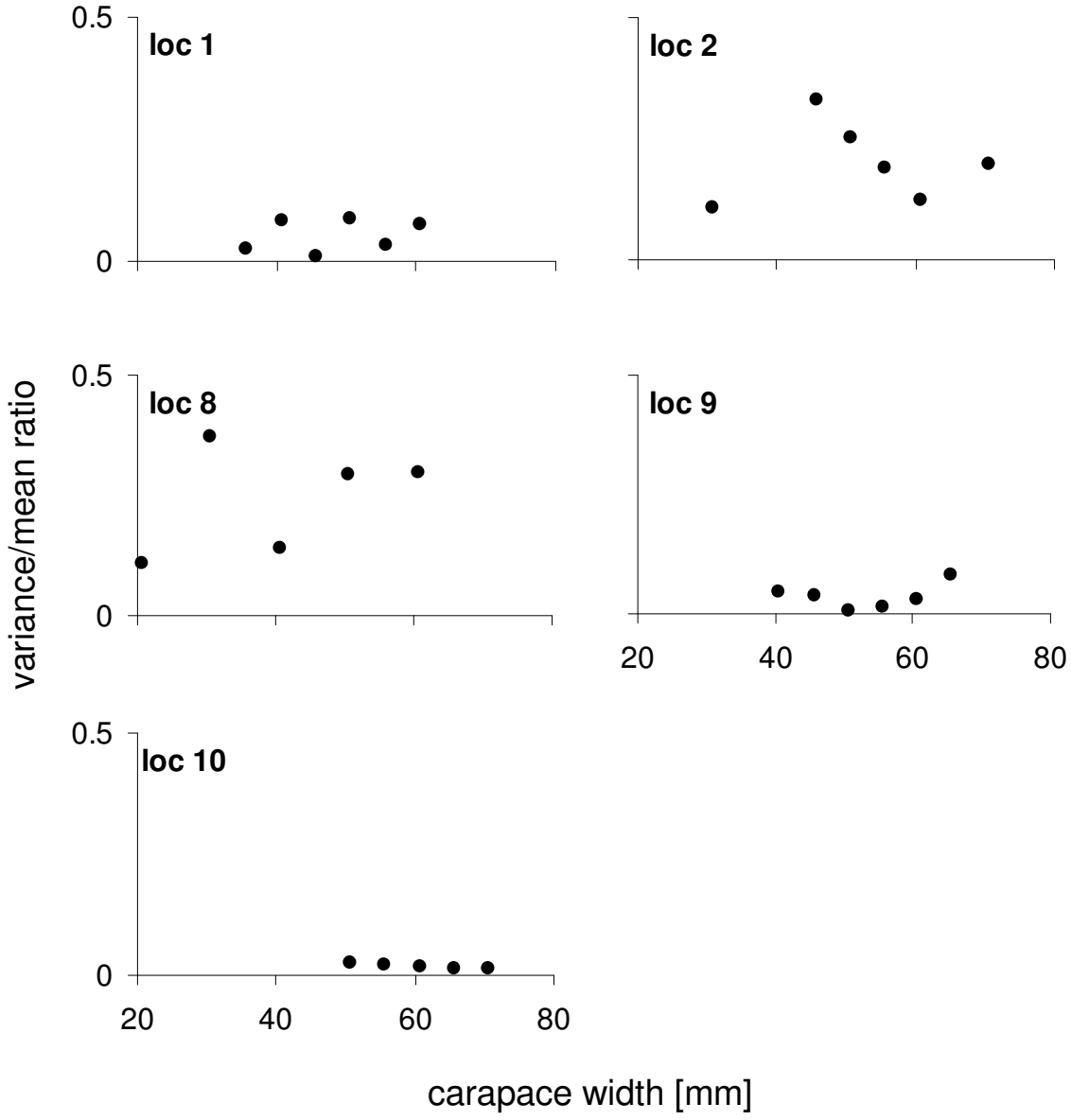

Figure 43: Ratio of variance to mean number of acanthocephalan cystacanths (log(x+1) transformed), in relation to crab size class.

3.1.7 Gonads

3.1.7.1 Dry weight of testes

An analysis of testes weight was conducted for most areas in which I sampled *C. maenas.* For statistical purposes, crabs that were sampled in close geographic proximity were combined, resulting in pooled locations for Helgoland (loc 2), Nykøbing (loc 5) and Cape Town (loc 12) (Figure 13 & 15).

Overall, reproduction index (RI) was significantly different for the two colour morphs of *C. maenas*, reflecting larger testes in red individuals (Mann-Whitney test: Z=-12.113, p<0.001). Nine sample sites yielded sufficient crabs of both morphs for a statistical analysis on the basis of location (Figure 44). However, only 5 of these displayed significant RI differences between colour morphs, with a sixth population showing a clear trend (see Appendix table 13 for detailed statistical results).

RI was also significantly influenced by sampling location (Figure 45), indicating that environmental factors may play a role for testes weight in male crabs (Kruskal-Wallis test: χ^2_9=229.157, p<0.001).

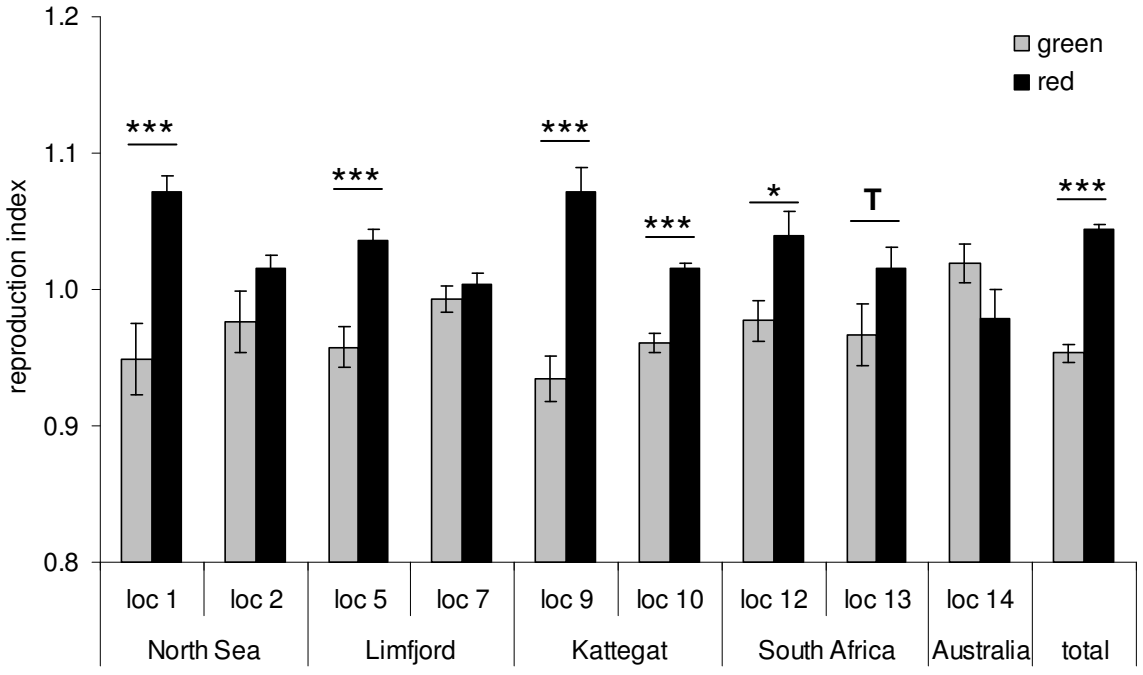

Figure 44: Mean reproduction index (+/-SE) in colour morphs of different sampling locations and for all sampled crabs (total). * = significant difference between morphs in the respective population, T marks a trend (p<0.07). Location (loc) numbers refer to Figure 13, 15 & 17. See Appendix table 13 for statistical results.

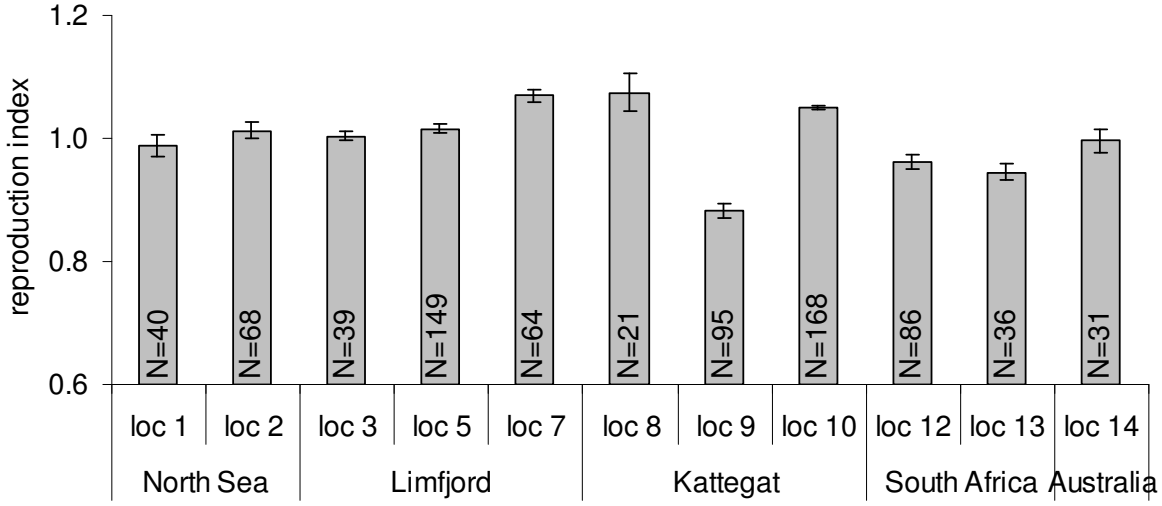

Figure 45: Mean reproduction index (+/-SE) in different populations of *C. maenas*. Populations differ significantly from each other (Kruskal-Wallis test: χ^2_9=229.157, p<0.001).

Pair-wise tests (Table 16) revealed that RI was significantly smaller in the two South African populations than in most European ones, except for locations 1 and 9 from the North Sea and the Kattegat. Location 9 was unique in that RI there was smaller than in all other sampled populations by a significant margin. In contrast to the invasive population of South Africa, Australian crabs had a testes weight that was well in line with most European and also one South African population. However, they still fell within the lower end of the spectrum of RIs.

These differences, based on morph and location, were controlled for statistically to allow testing for possible parasite influence with pooled data (see 2.2.5.2). I found that both *S. carcini* (Mann-Whitney test: Z=-9.389, p<0.001) and *P. maenadis* (Mann-Whitney test: Z=-4.854, p<0.001) infected crabs had considerably smaller testes than uninfected crabs. However, neither infection with microphallids nor with the acanthocephalan *P. botulus* lead to differences in testes weight compared to uninfected crabs (Figure 46).

Larvae of both helminth groups can occur at variable intensities. It is therefore possible that they affect the host only when present in high numbers. Yet there was no correlation between microphallid and acanthocephalan intensity and RI. However, there was a correlation between microphallid numbers and RI at high intensity levels of over 1000 metacercariae per crab. In this range a significantly positive correlation was observed (R_s=0.827, p=0.002). No comparable effect was found for the cystacanths of *P. botulus*.

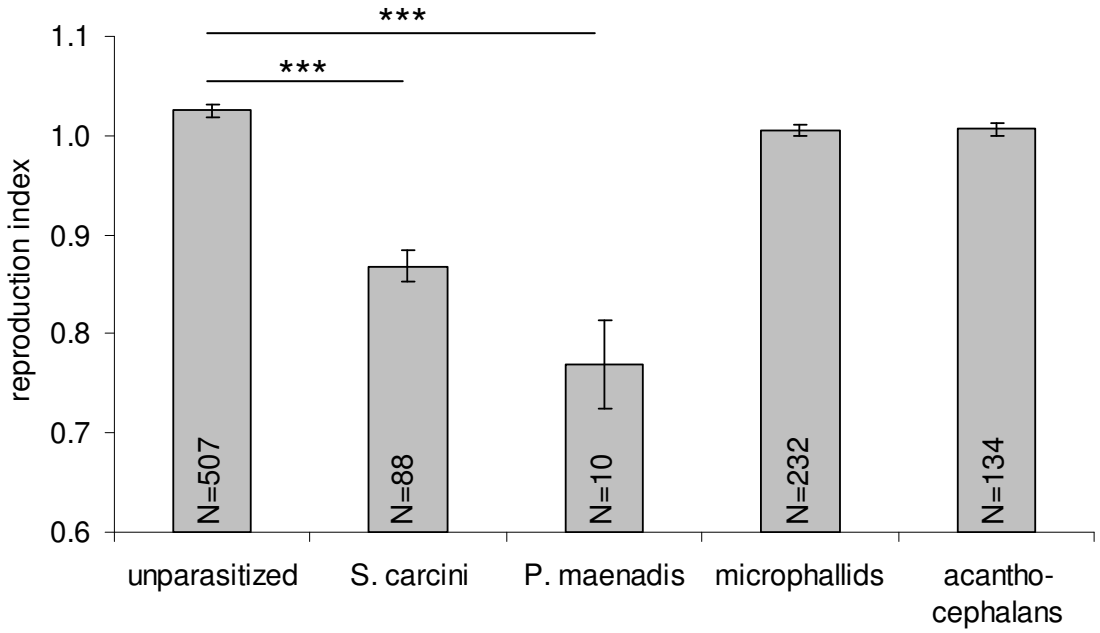

Figure 46: Mean reproduction index in crabs parasitized by different parasites (+/-SE). * marks significant differences compared to uninfected crabs (*S. carcini*: Mann-Whitney test: Z=-9.389, p<0.001; *P. maenadis*: Mann-Whitney test: Z=-4.854, p<0.001).

Table 16: Results of pair wise Mann-Whitney-U tests, indicating the differences in reproduction index between the sample locations. P-values for each tests are given where p<0.1, significant differences are printed bold, n.s. = not significant with values of p>0.1. Location (loc) numbers refer to Figure 13.

loc	1	2	3	5	7	8	9	10	12	13	14
1	-	n.s.	n.s.	n.s.	**0.000**	0.076	**0.000**	**0.000**	n.s.	**0.022**	n.s.
2	n.s.	-	**0.033**	n.s.	**0.012**	n.s.	**0.000**	**0.015**	**0.000**	**0.000**	n.s.
3	n.s.	**0.033**	-	n.s.	**0.000**	0.060	**0.000**	**0.000**	**0.035**	**0.000**	n.s.
5	n.s.	n.s.	n.s.	-	**0.000**	n.s.	**0.000**	**0.000**	**0.000**	**0.000**	n.s.
7	**0.000**	**0.012**	**0.000**	**0.000**	-	n.s.	**0.000**	n.s.	**0.000**	**0.000**	**0.002**
8	0.076	n.s.	0.060	n.s.	n.s.	-	**0.000**	n.s.	**0.002**	**0.000**	0.085
9	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	-	**0.000**	**0.000**	**0.001**	**0.000**
10	**0.000**	**0.015**	**0.000**	**0.000**	n.s.	n.s.	**0.000**	-	**0.000**	**0.000**	**0.009**
12	n.s.	**0.000**	**0.035**	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	-	n.s.	n.s.
13	**0.022**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	n.s.	-	**0.028**
14	n.s.	n.s.	n.s.	n.s.	**0.002**	0.085	**0.000**	**0.009**	n.s.	**0.028**	-

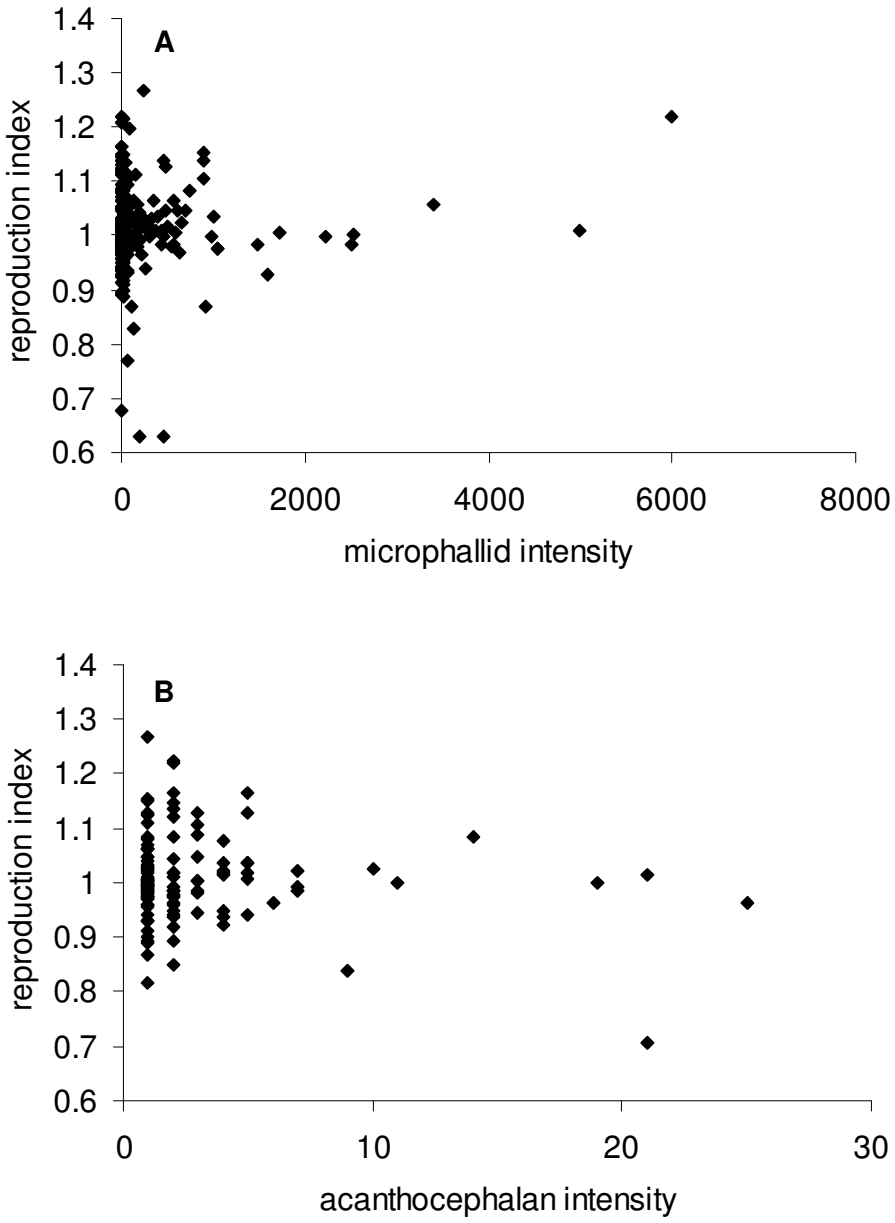

Figure 47: Influence of helminth intensity on the reproduction factor. **A** - microphallids: There is a significant correlation at intensities of over 1000 metacercariae (R_s=0.827, p=0.002). **B** - acanthocephalans (no significant correlation).

While *S. carcini* typically occurs as a single infection in European shore crabs, the density, dimensions and colour of the internal root system allow for a classification into different age classes of the parasite. The age of the parasite shows a significantly negative correlation with RI of the host (Figure 46) (R=-0.371, p=0.009).

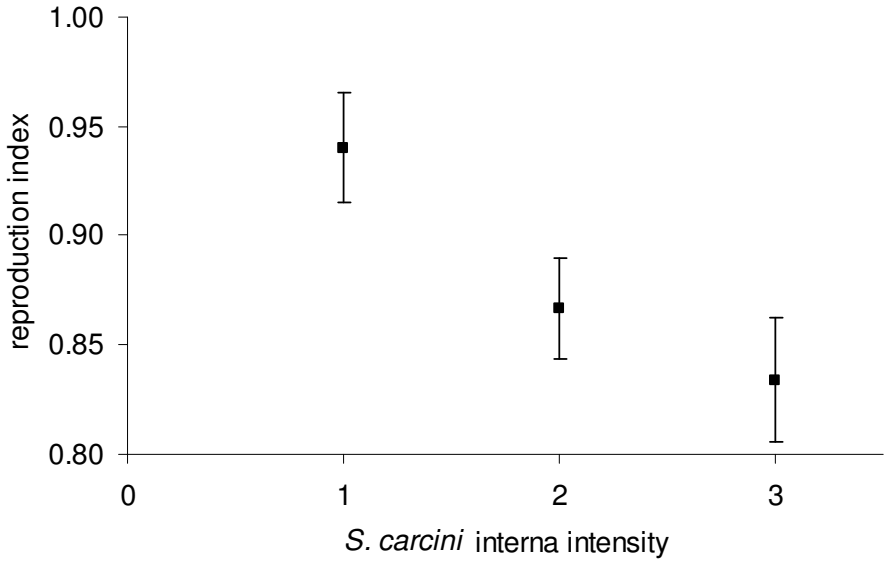

Figure 48: Influence of the age of the internal root system of *S. carcini* infection (stage 1 to 3) on mean reproduction index (+/- SE) (R=-0.371, p=0.009).

At the population level, neither parasite prevalence nor intensity were correlated with the mean reproduction index in the respective populations (Figure 50). Examining the invasive compared to the native crabs, I found that RI was significantly lower in South Africa (Mann-Whitney test: Z=-6.103, p<0.001). This, however, was not the case in the second invasive area in Australia, where testes size was comparable to European crabs (Figure 49).

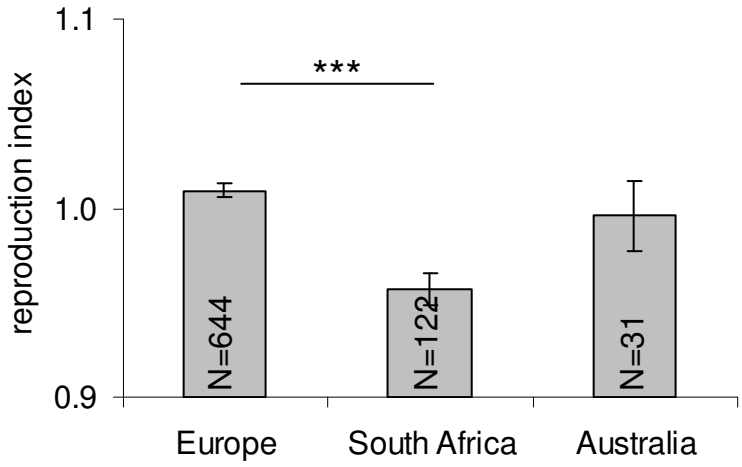

Figure 49: Mean reproduction index (+/- SE) in native populations of Europe, compared to invasive populations in South Africa and Australia. *** marks highly significant differences to the European population (Mann-Whitney test: Z=-6.103, p<0.001).

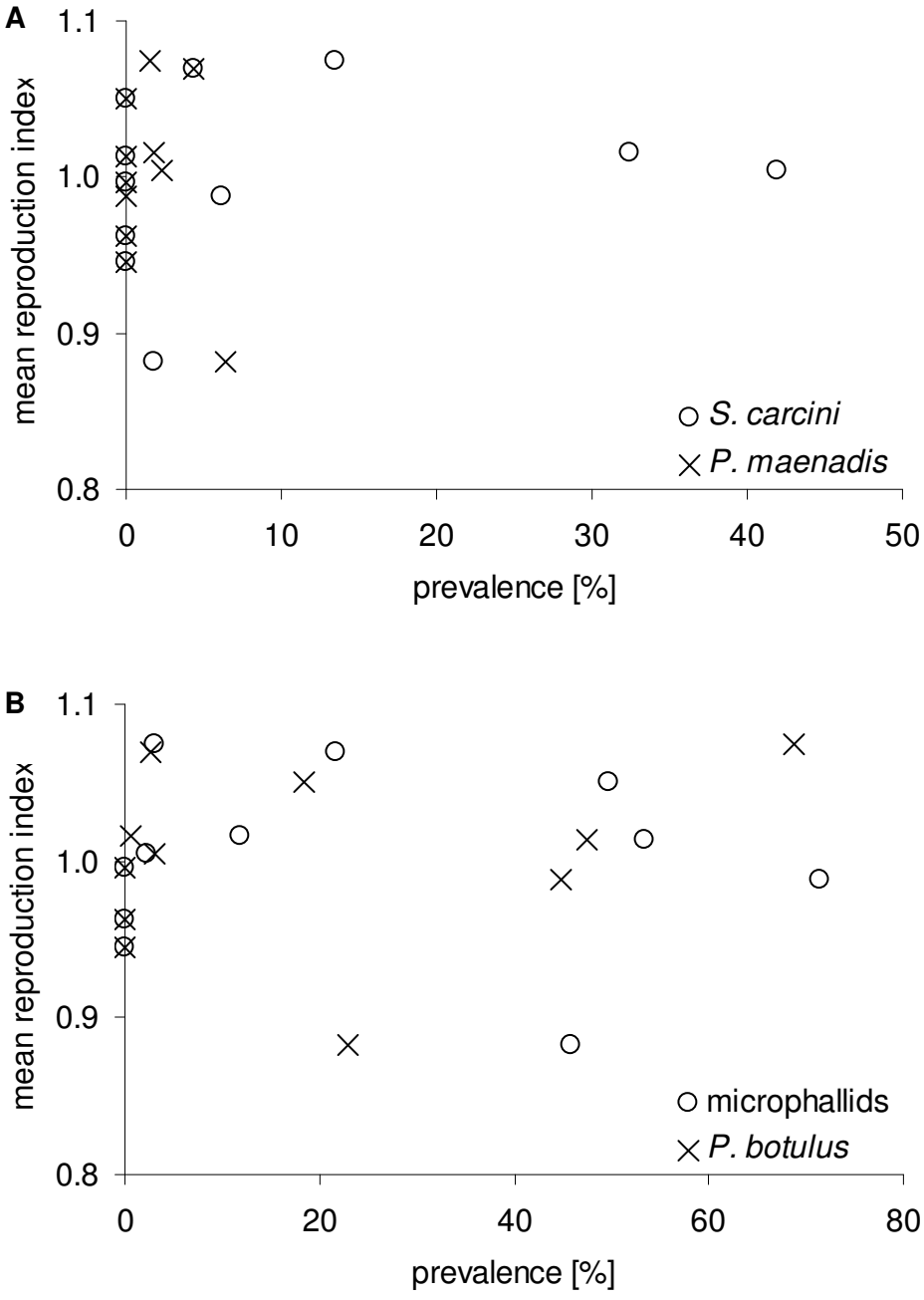

Figure 50: Mean reproduction index plotted against (**A**) prevalence of parasitic castrators and (**B**) prevalence of helminth larvae.

3.1.7.2 Dry weight of ovaries

In *C. maenas* females, ovaries pass through a number of stages during the reproductive cycle. For the analysis of ovary dry weight I could only dissect out mature ovaries, which reduced the sample size dramatically. Of 471 female crabs dissected, only 193 ovaries could be obtained. Approximately one third of these originated from Europe and thus provided a means to analyse possible parasite effects. Contrary to male crabs, gonad weight in females was not significantly different between European populations, which were therefore pooled for statistical analysis. However, as in male crabs colour morph had an impact on gonad weight in some areas (Figure 51). This was most pronounced in Europe (t-test: t=3.478, p=0.001), but it could also be observed to a lesser extent in South Africa (t-test: t=2.009, p=0.048). There were only very few red females among the crabs sampled in Australia (n=10), which might contribute to the finding that the observed size difference in gonads was not statistically significant there.

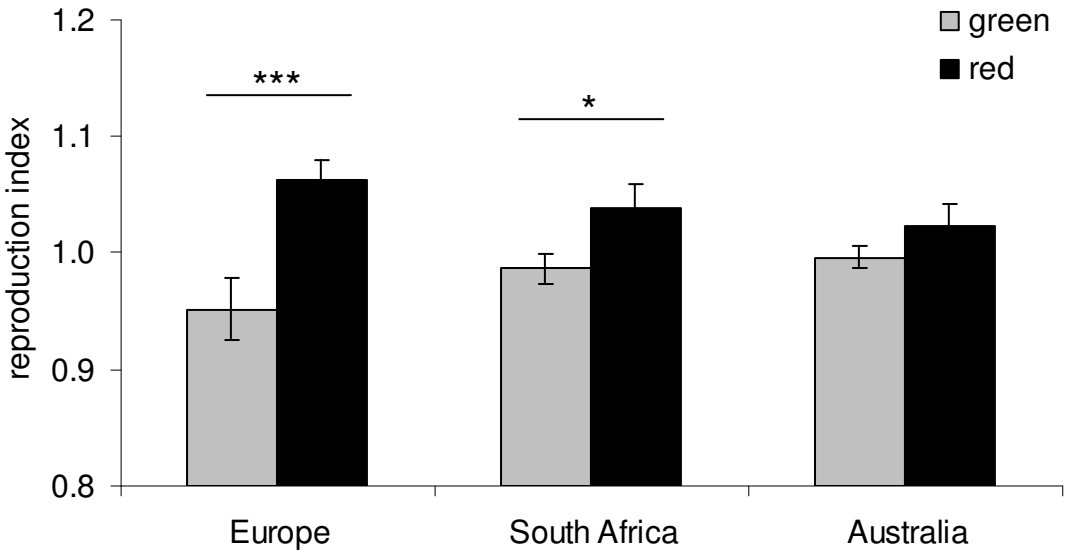

Figure 51: Mean reproduction index (+/-SE) in both morphs of female *C. maenas*. * marks significant differences between morphs (Europe: t-test: t=3.478, p=0.001, N=68; South Africa: t-test: t=2.009, p=0.048, N=88; Australia: N=74).

By controlling for the effect of colour morph on the reproduction index (RI), it was possible to compare invasive populations in South Africa and Australia with native populations in Europe, proving that they differ significantly in ovary weight (Kruskal-Wallis test: χ^2_2=41.078, p<0.001). Female crabs in both invasive populations have significantly larger ovaries

compared to females of the native range (Mann-Whitney test: South Africa: Z=-4.279, p<0.001; Australia: Z=-6.288, p<0.001). This effect was stronger in Australia, where females had larger ovaries than their South African counterparts (Mann-Whitney test: Z=-2.532, p=0.011).

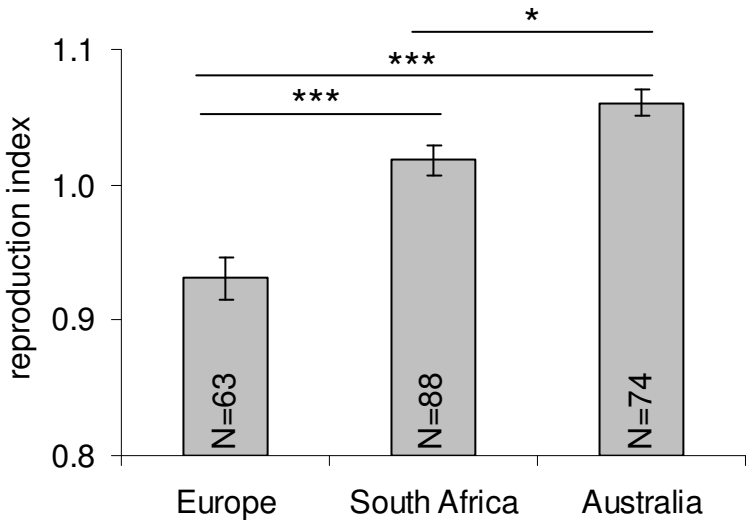

Figure 52: Mean reproduction index (+/- SE) of female *C. maenas* in native populations of Europe and in invasive populations of South Africa and Australia. * marks significant differences (South-Africa: Mann-Whitney test: Z=-4.279, p<0.001; Australia: Mann-Whitney test: Z=-6.288, p<0.001; between invasive populations: Mann-Whitney test: Z=-2.532, p=0.011).

Similarly to males, there was no significant effect of helminth parasite prevalence on the reproduction index of females as measured by ovary weight (Figure 53). In addition, there was no correlation of helminth intensity with RI, suggesting that there is also no effect at higher infection levels of these parasites (Figure 54). However, crabs infected with parasitic castrators had significantly smaller ovaries than unparasitized crabs (Mann-Whitney test: Z=-2.930, p=0.003).

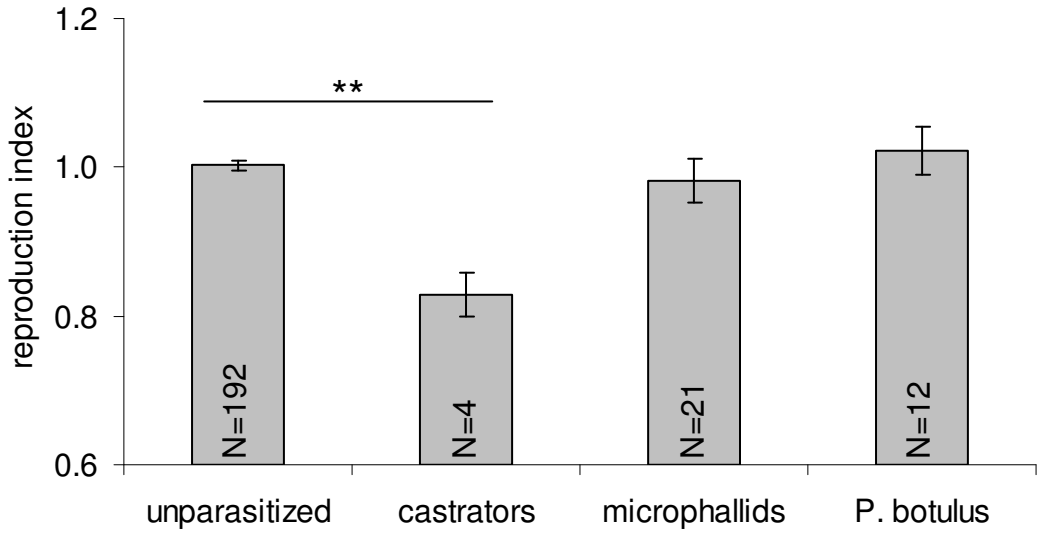

Figure 53: Mean reproduction index (+/-SE) in females infected with different parasites compared to unparasitized females. * marks significant differences (Mann-Whitney test: Z=-2.930, p=0.003).

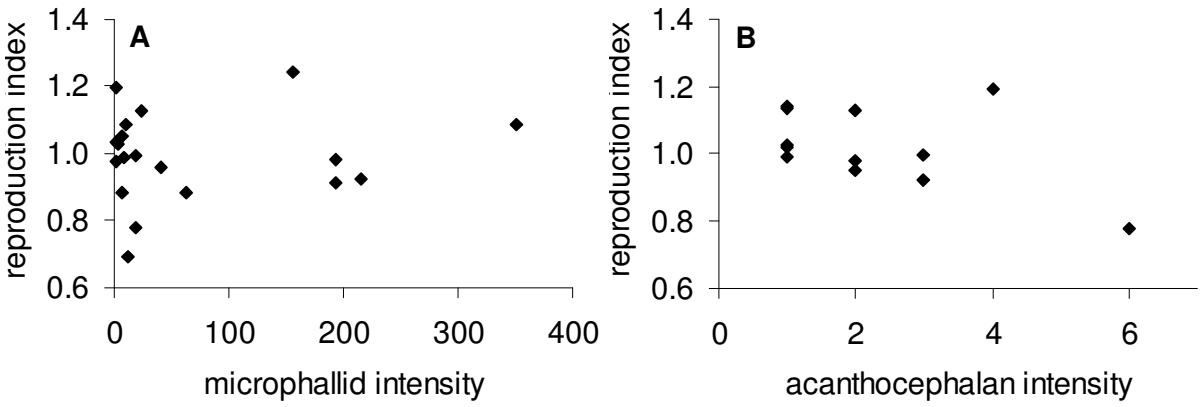

Figure 54: Reproduction index in females of *C. maenas* in relation to their infection intensity with larval (**A**) microphallids and (**B**) acanthocephalans (*P. botulus*).

3.1.7.3 Ovary stages

I distinguished between 4 different stages of ovarial development, all of which were commonly found in European females, although stage 0 was rarely encountered in crabs over 30 mm carapace width (Figure 55). The distribution of the ovarial stages appeared to vary between European sampling locations, but statistical analysis showed that there was no significant difference between the 5 locations that had a sufficient number of examined females for the analysis. This was the case despite differences in sampling time, as two of the locations were sampled in June and August, respectively, while all other locations were sampled between September and October (Table 17).

Similarly, among females from the three main invasive sites there was a very similar pattern in ovary stage distribution (Table 17). However, I found that they differ significantly from European females (χ^2_3=143.967, p<0.001). In all three invasive populations, I found almost exclusively females with deeply orange, mature stage 3 ovaries (Figure 55). These samples were taken between March and April in the Southern Hemisphere, and thus in the same season as the September samples from Europe.

Helminth infection did not have an impact on ovary stage. However, females infected with *S. carcini* were significantly more likely to be found in a lower ovarial stage (R=0.188, p=0.016).

Table 17: Sample size, location and month for mature female crabs in Europe, South Africa and Australia. Number of crabs with each ovary developmental stage is given. Location (loc) numbers refer to Figure 13.

sample location	sample time	ovary stage 0	1	2	3	N
loc 2	August	3	16	4	8	31
loc 3	June	0	19	14	10	43
loc 4	September	1	18	7	14	40
loc 6	September	0	5	3	7	15
loc 7	September	2	11	3	17	33
loc 12	March	0	2	1	31	34
loc 13	March	0	0	0	55	55
loc 14	March	0	1	0	73	74
total		30	77	32	220	359

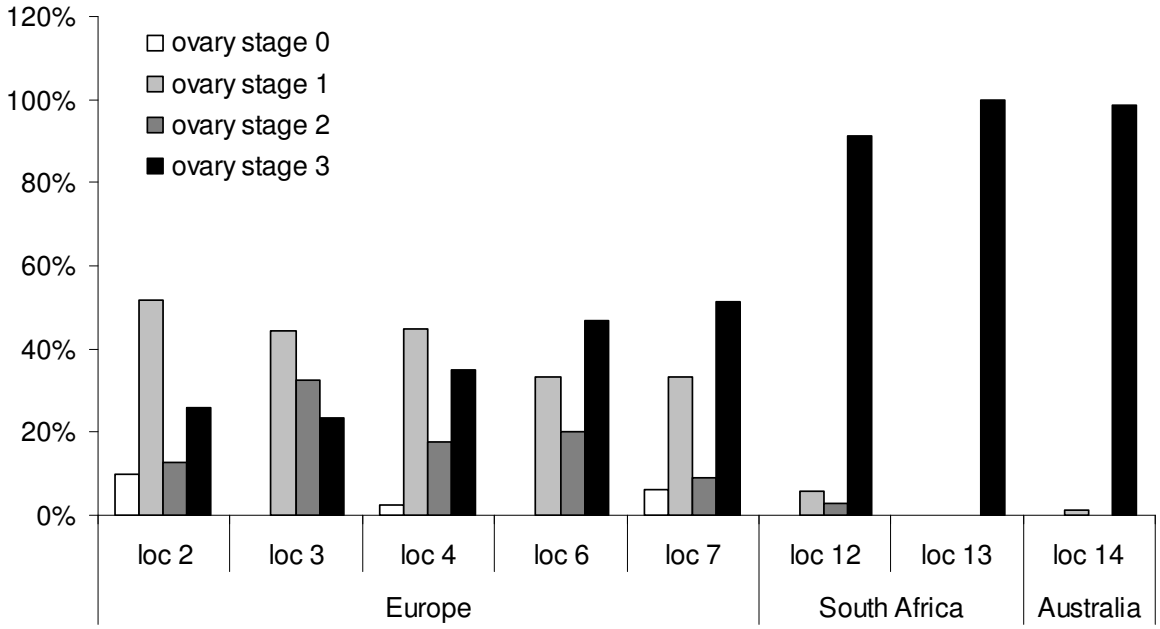

Figure 55: Distribution of ovary stages observed in mature female crabs in different locations. Location numbers refer to Figure 13.

3.1.8 Feminisation

3.1.8.1 Male crabs

Feminisation index (FI) in male crabs varied significantly between sample sites (ANOVA: $F=4.863$, $p<0.001$). A post-hoc test (Games-Howell test) showed that there are two distinct homogenous groups within Europe, seperating the western sampling sites of locations 2 to 6 from the eastern sampling sites of locations 7 to 10. FI at location 1 had an intermediate position, with no significant differences with either of the two European groups (Figure 56). Crabs from South Africa showed no significant differences among the three sampled sites and had an intermediate position between the two European extremes, with no significant differences to either. Australian crabs, however, had a significantly broader abdomen than crabs in locations 2 to 6 in Europe (Games-Howell test: $p<0.05$).

To analyse a possible impact of parasites on feminisation of host crabs, the homogenous locations 2-6 and 7-10 were pooled and analysed for differences between unparasitized and parasite infected crabs. Neither microphallid trematodes nor the acanthocephalan *P. botulus* showed any effect on FI.

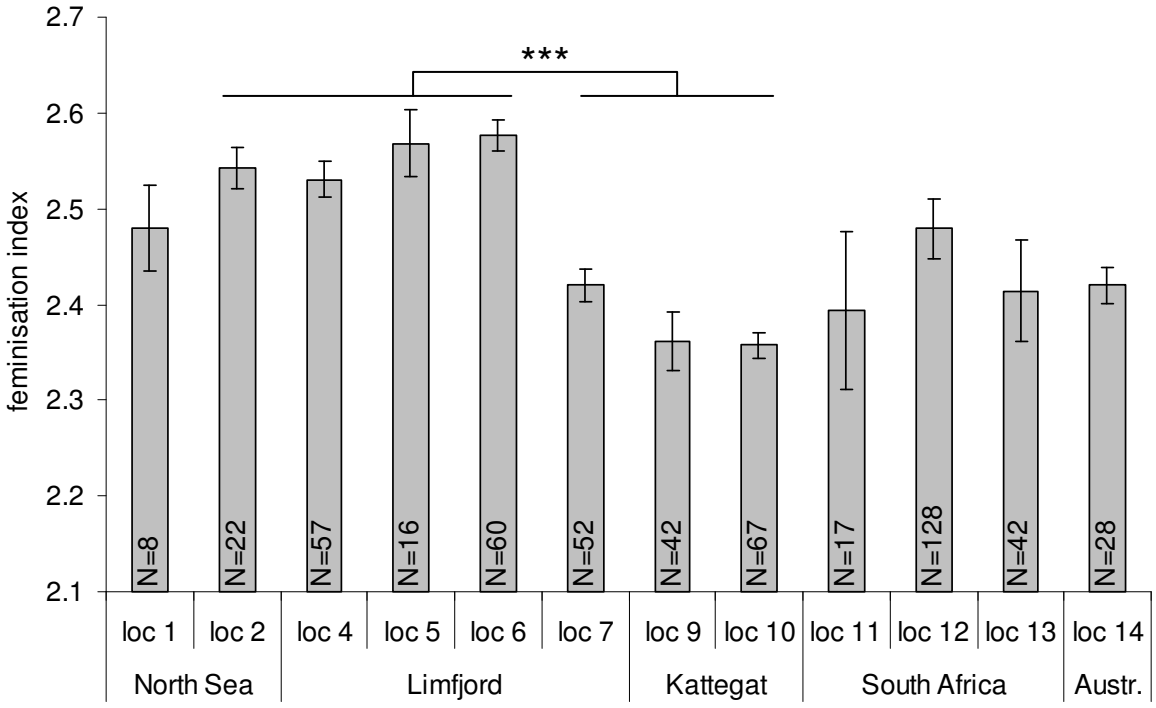

Figure 56: Mean feminisation index (+/- SE) in unparasitized crabs of different populations of *C. maenas* in Europe, South Africa and Australia. * = significant difference between crabs of locations 2 to 6 (western sample sites) and 7 to 10 (eastern sample sites) (Mann-Whitney test: Z=-9.800, p<0.001). Location (loc) numbers refer to Figure 13.

Sample sizes for *P. maenadis* were small (n<10) in both groups, yet crabs infected with this parasite showed the most pronounced reduction in FI, indicating a significantly broader abdomen in these crabs (location 2-6: t-test: t=4.136, p=0.008; location 7-10: Mann-Whitney test: Z=-4.781, p<0.001).

S. carcini was quite prevalent at locations 2-6 (n=47), also displaying a significantly reduced FI - indicating feminisation and broader abdomen - compared to unparasitized crabs (t-test: t=4.278, p<0.001). This was not the case at location 7-10, where only 6 crabs were infected with *S. carcini* and no difference to uninfected crabs was found. The age and strength of the *Sacculina* interna was not correlated to feminisation index.

However, where *S. carcini* had formed externa or where the scars of previous externa were present, crabs had a significantly reduced FI compared to crabs without these signs (t-test: t=2.499, p=0.016) (Figure 58). Indeed, abdomens of *Sacculina* infected crabs without externa were generally broader than those of entirely uninfected crabs, but not significantly so. The differences between crabs with and without *S. carcini* infection are therefore based mainly on those crabs with externa, which constituted 57% of the infected individuals in locations 2 to 6.

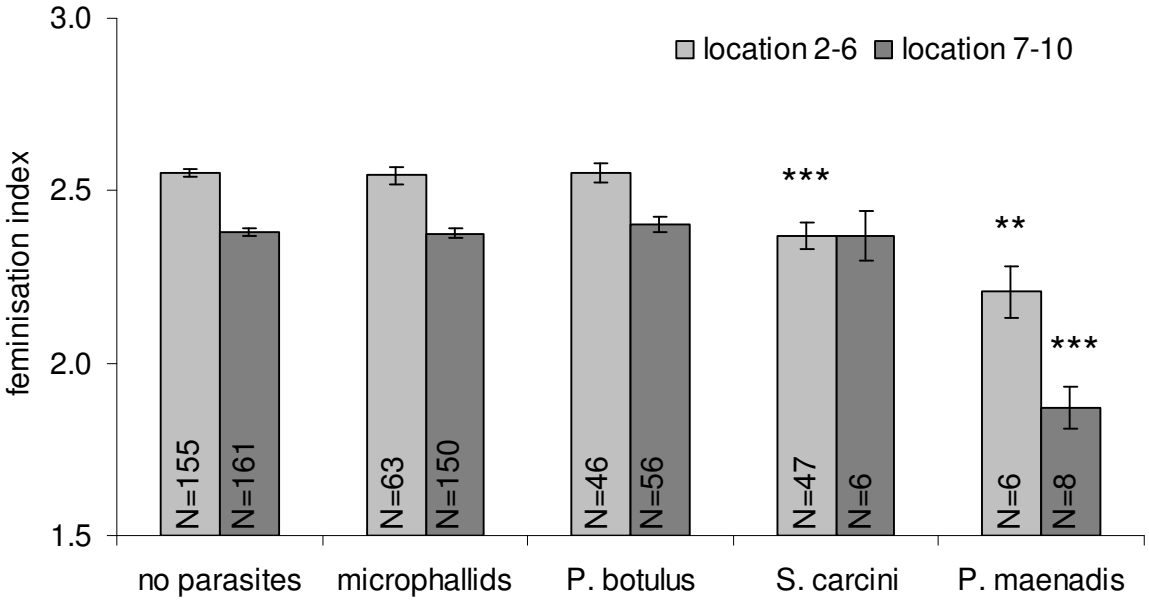

Figure 57: Mean feminisation index (+/-SE) of male *C. maenas* of western sample size (location 2-6) and eastern sample sites (location 7-10), in relation to infestation microphallids, *Profilicollis botulus*, *Sacculina carcini* and *Portunion maenadis*. * = significant differences compared to unparasitized crabs of the same area (see text for statistical results).

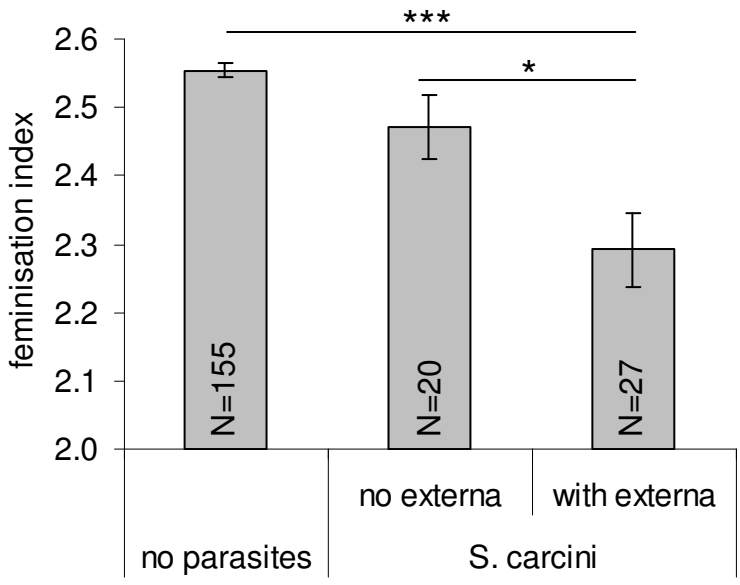

Figure 58: Mean feminisation index (+/-SE) of *S. carcini* infected crabs of locations 2-6: Differences between crabs without externa and crabs with either an externa or a scar as indication of an externa that has fallen off or was removed. Unparasitized crabs are included as comparison. * = significant differences (Between *S. carcini* infected crabs: t-test: t=2.499, p=0.016. Compared to unparasitized crabs: t-test: t=5.161, p<0.001).

3.1.8.2 Female crabs

Due to the low number of females at several of the European sample sites, only four of these sites were included in this analysis. These are location 2 in the North Sea, location 4 and 6 in the western Limfjord and location 7 in the eastern Limfjord. No significant differences in feminisation index were found among these sample sites. Similarly, there were no significant differences among the three South African sample sites. These sites were therefore pooled for further analysis. FI proved to be very similar on all three investigated continents (Figure 59).

Most parasites did not have a significant effect on feminisation index. However, crabs infected with the castrator *P. maenadis* had a significantly higher index (t-test: t=6.233, p<0.001), indicating a narrower than usual abdomen (Figure 60). However, only 3 crabs were infected with this parasite, sample size is therefore not high enough for a conclusive result.

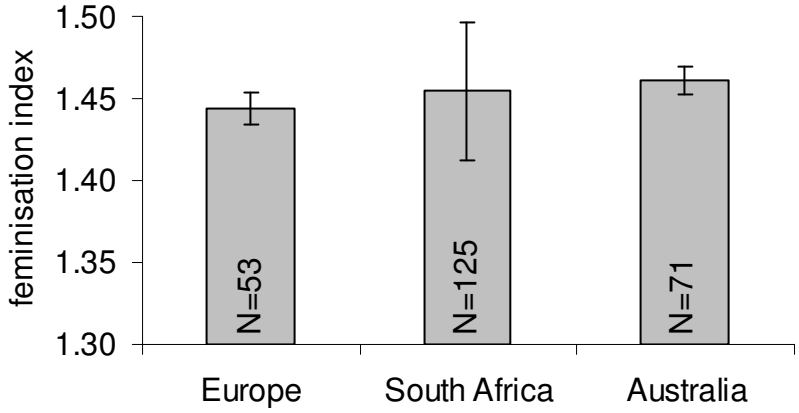

Figure 59: Mean feminisation index (+/-SE) in female, unparasitized *C. maenas* in Europe, South Africa and Australia.

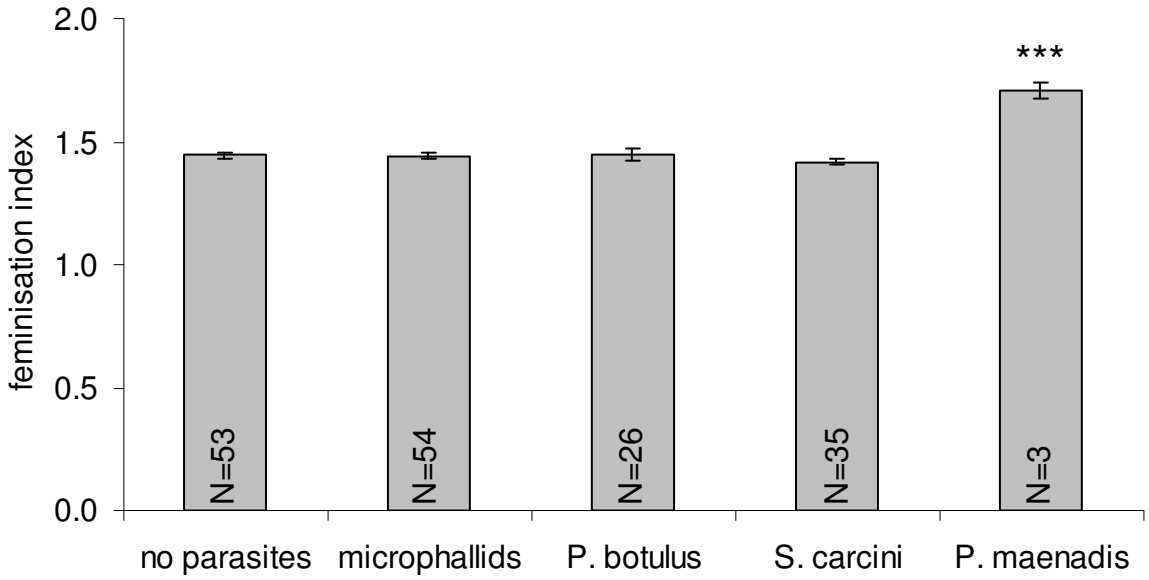

Figure 60: Mean feminisation index (+/-SE) in female *C. maenas* of Europe, with regards to their infestation with different parasites (microphallids, *P. botulus*, *S. carcini* and *P. maenadis*). * = significant differences compared to unparasitized crabs (t-test: t=6.233, p<0.001, see also text).

3.1.9 Haemolymph parameters

3.1.9.1 Total haemocyte counts

For analysis of haemocyte concentrations, *C. maenas* were generally collected in late summer and early autumn. The crabs from the North Sea (locations 1 and 2) were sampled in Sylt and Helgoland, with the first Helgoland sample being the only one that was gathered in summer at high water temperatures. Two more samples were collected in Denmark from the Limfjord (location 4-6, combined into location 5 for the purpose of THC abalysis) and from the Kattegat (location 9). In South Africa and Australia, *C. maenas* were collected in March and April of 2007 and 2008 (location 12-14). I found no difference in THC level between the investigated European populations (Figure 61), so these were pooled for the purpose of comparing invasive to native populations.

Crabs from Australia showed a significantly lower THC than European crabs (Mann-Whitney test, Z=-2.912, p=0.004) whereas the South African individuals showed a significantly higher THC (Mann-Whitney test, Z=2.501, p=0.012) (Figure 62). Consequently, there is also a significant difference between the two invasive areas in South Africa and Australia (t-test: t=6.088, p<0.001).

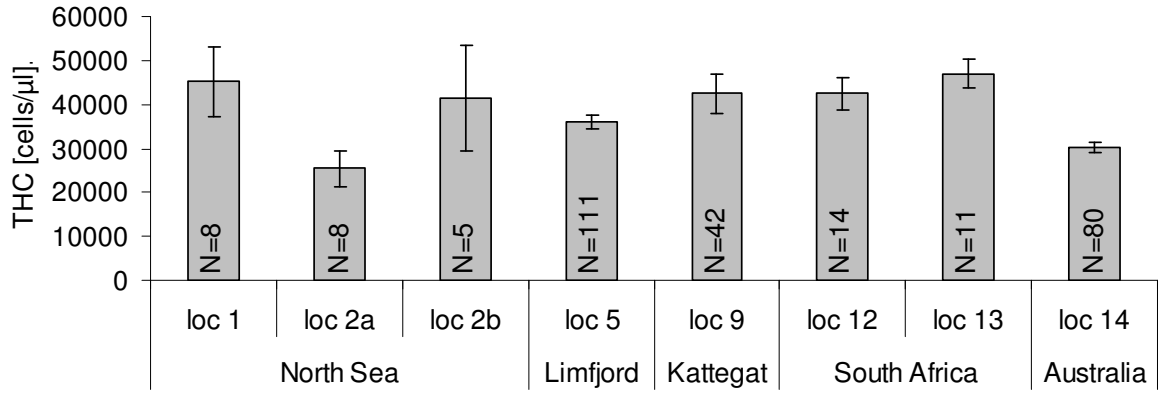

Figure 61: Mean THC values of unparasitized crabs in Europe (+/-SE). Location (loc) numbers refer to Figure 13. Crabs from location 2a were sampled in summer, as opposed to all other samples. There was no significant difference between sample sites.

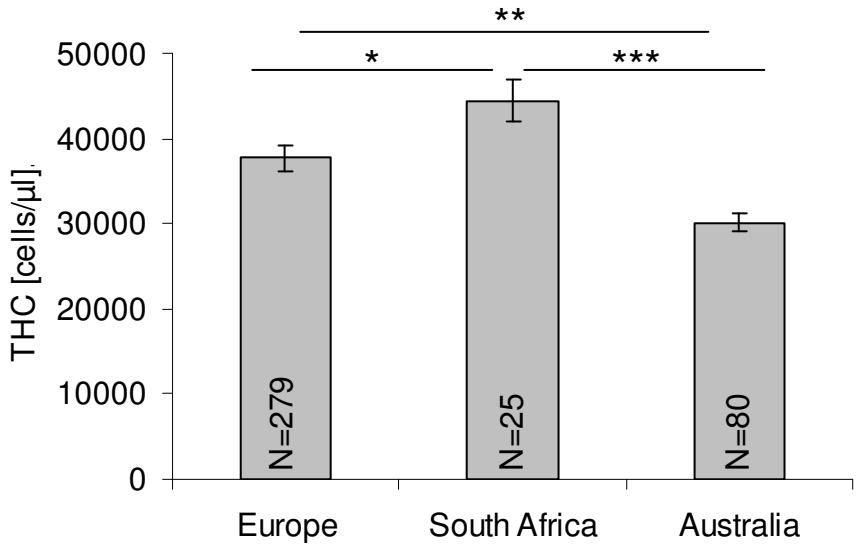

Figure 62: Mean THC values of unparasitized crabs in Europe, Australia and South Africa (+/-SE). * = significant differences (EU - AUS: Mann-Whitney test, Z=-2.912, p=0.004. EU - ZA: Mann-Whitney test, Z=2.501, p=0.012. ZA - AUS: t-test, t=6.088, p<0.001).

Colour morph had no significant effect on THC. Females showed a significantly lowered THC in Europe compared to males (Mann-Whitney-test: Z=-2.104, p=0.035), an increased THC in Australia (Mann-Whitney-test: Z=-2.074, p=0.038) and no difference to males in South Africa. For this reason, the analysis of parasite effects in Europe was limited to males, which

provided a sufficient sample size. However, parasite infection was not associated to altered THC (Figure 63).

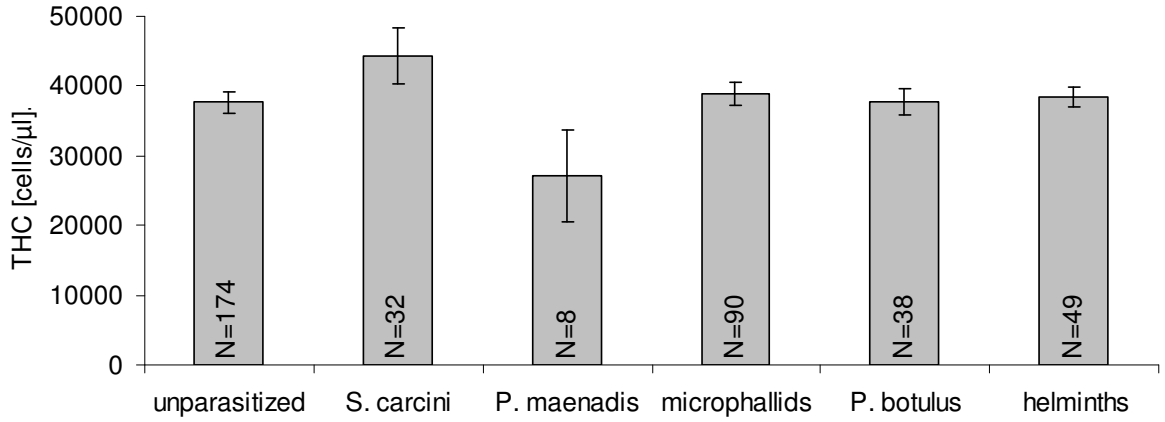

Figure 63: Mean THC (+/-SE) in all European male crabs, infected with different parasites: *Sacculina carcini*, microphallid trematodes, *Portunion maenadis*, *Profilicollis botulus* and "helminths", combining both microphallids and *P. botulus* infections.

Detrimental parasite effects often become obvious at high intensities only. Yet in our study there was no significant correlation between the intensity of microphallid and acantho-cephalan larvae and THC (Figure 64, Figure 65).

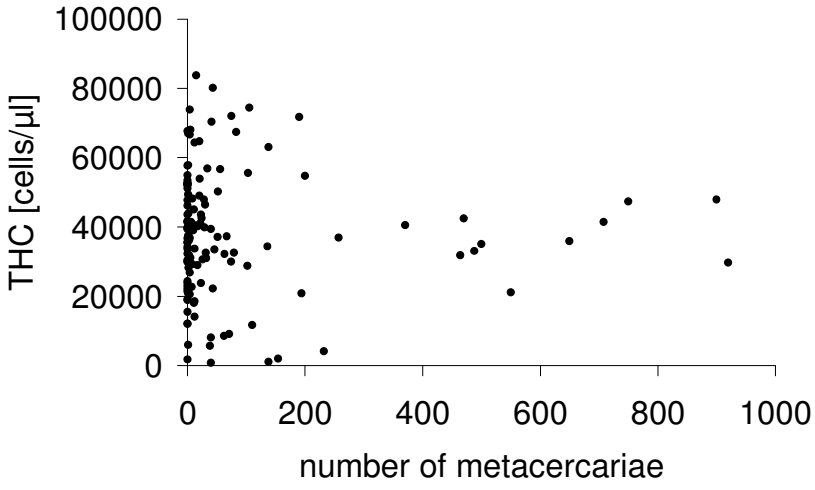

Figure 64: THC in relation to microphallid intensity. There was no significant correlation.

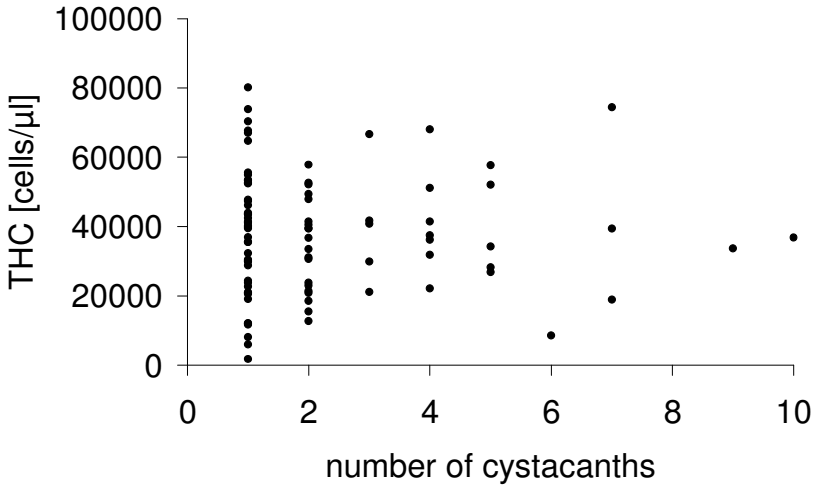

Figure 65: THC in relation to acanthocephalan (*Profilicollis botulus*) intensity. There was no significant correlation.

This was confirmed when looking at each population separately, with one exception. In the sample from Helgoland which was taken in August (location 2a), unparasitized crabs had a significantly lower THC than crabs infected with helminths in general (Figure 66), regardless of intensity (Mann-Whitney test: Z=-2.161, p=0.03). Separating the different helminth taxa proved difficult in this area, as 41% of all helminth infections were double infections with microphallids and acanthocephalans. Comparing all crabs that were infected with acanthocephalan cystacanths to completely unparasitized crabs, the difference was still significant (Mann-Whitney test: Z=-2.098, p=0.036). For microphallids, there was no such significance, but a clear trend (Mann-Whitney test: Z=-1.922, p=0.055). However, mean THC values show that it was indeed not the parasitized crabs that show an altered THC level. In fact, it is the unparasitized crabs that appear to have a significantly lowered THC level compared to both parasitized crabs of the same population and unparasitized crabs from other sampling locations.

To test for seasonal effects in this case, this area was sampled again in the following spring (location 2b). In this second set of data no effect of parasites was present (Figure 66).

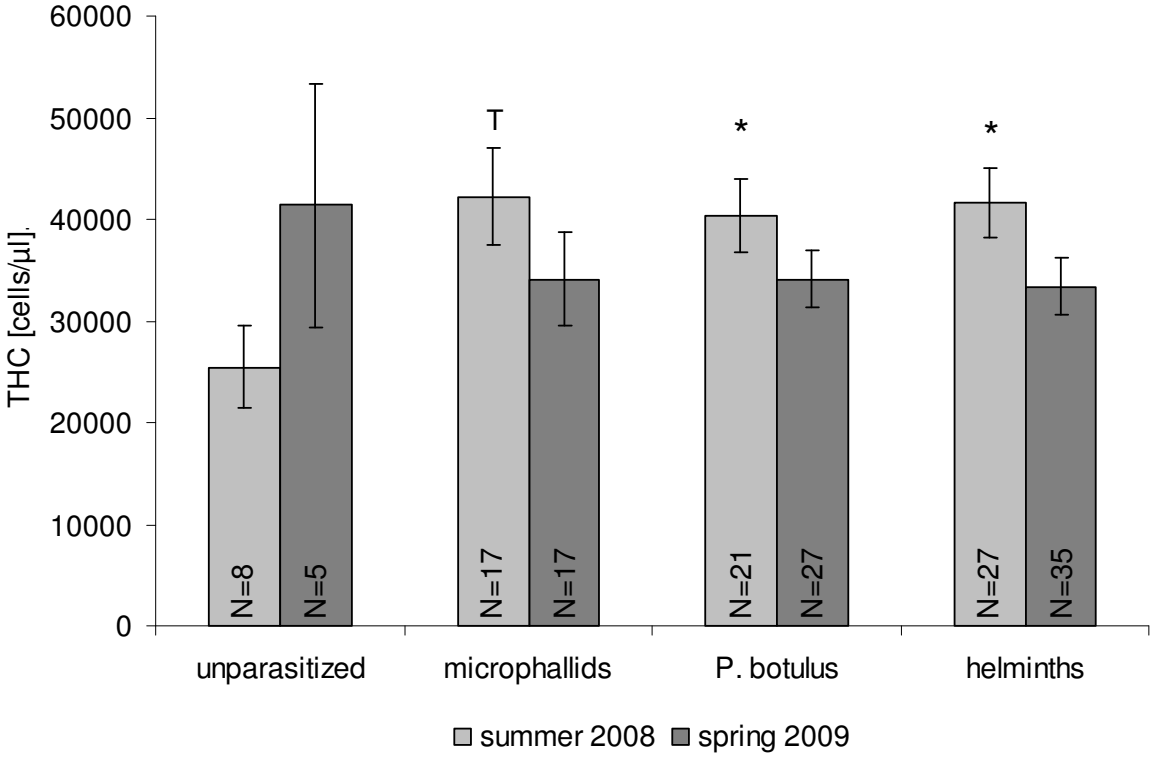

Figure 66: THC values (+/- SE) for crabs from Helgoland, sampled in summer 2008 and spring 2009. The helminth column includes crabs that were infected with either microphallids or acanthocephalans. * = significant differences compared to unparasitized crabs (helminths: Mann-Whitney test: Z=-2.161, p=0.03. Acanthocephalans: Mann-Whitney test: Z=-2.098, p=0.036). T = trend (Mann-Whitney test: Z=-1.922, p=0.055).

Haemocytes are also involved in the immune defence against other pathogens as well as in the melanization of injuries and foreign particles. Melanized structures within the crabs can be indicative of past infections, therefore I also analysed a possible connection between the presence of such structures and THC level. Due to the low sample size combined with differences between native and invasive populations I chose a slightly different method than a comparison of mean values. Instead, I calculated the 10% percentile for each population and determined if the THC of crabs with melanized structures lies within these percentiles more often than would be expected under random conditions. While this was not the case for the upper percentile (Figure 67) I confirmed that the lower percentile contains significantly more crabs with melanized structures than would be expected (χ^2_2=7.830, p<0.001). Crabs with melanized structures are therefore significantly more likely to have a low THC concentration.

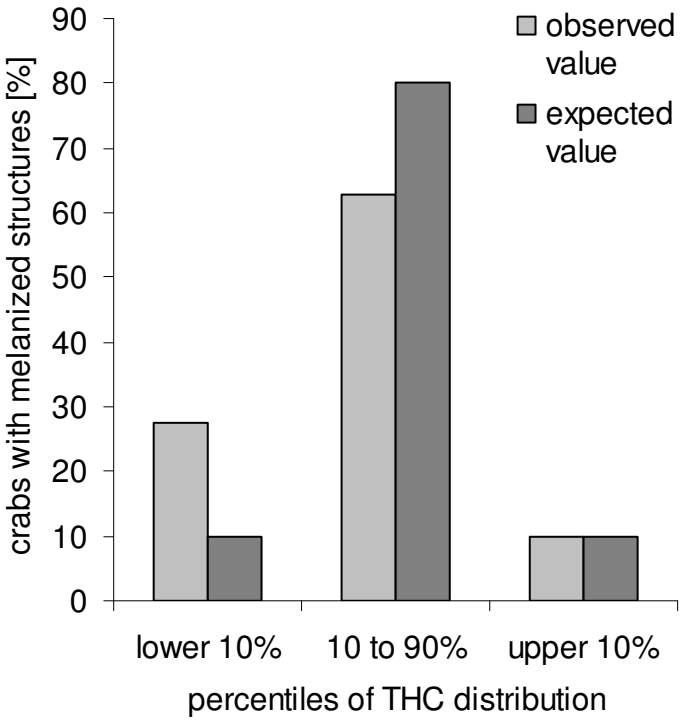

Figure 67: Distribution of crabs with melanized structures over THC percentiles: expected and actual situation are significantly different (Chi-square Test: χ^2_2=7.830, p<0.001, N=51).

3.1.9.2 Phenoloxidase activity

Phenoloxidase measurements on field samples were conducted on the population sampled in Sylt in 2008. I found that neither morph nor crab size has an influence on PO activity in these crabs (Figure 68). Differences between the sexes could not be detected as the sample consisted almost exclusively of male individuals. Therefore crabs could be pooled for statistical analysis of parasite impacts. These are limited to helminth parasites, as only microphallid trematodes and the acanthocephalan *Profilicollis botulus* occurred in this crab population. However, crabs infected with either of these parasites did not show a significantly different PO activity level from crabs that were not infected with the respective parasite (Figure 69). Similarly, metacercarial and cystacanth intensity was not significantly correlated with PO activity in infected crabs, indicating that there is no effect limited to high infection intensities (Figure 70).

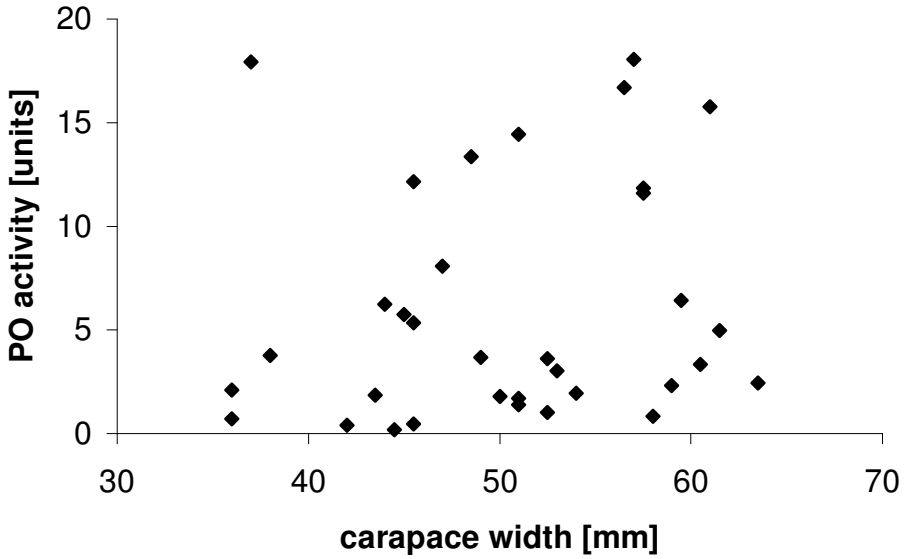

Figure 68: Phenoloxidase activity in relation to crab size. There was no significant correlation.

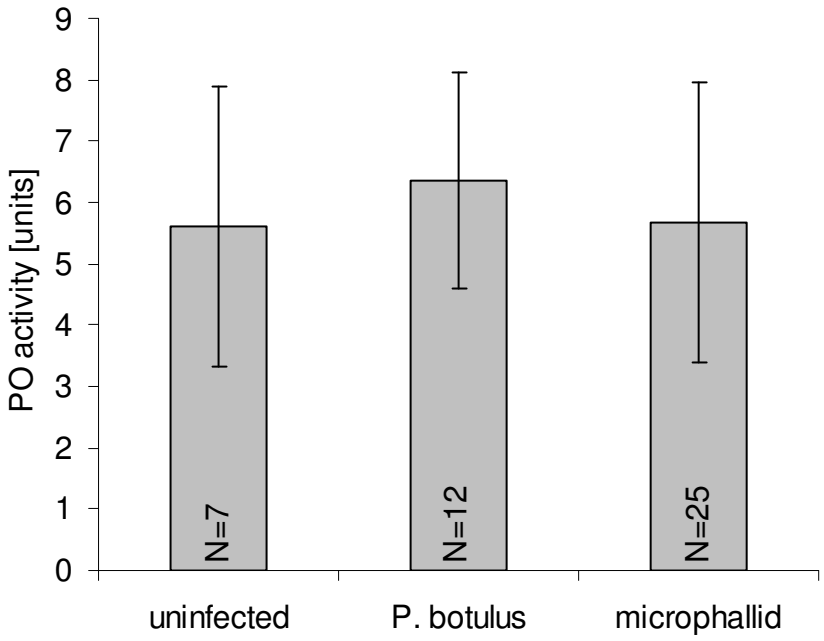

Figure 69: Mean phenoloxidase activity (+/-SE) in crabs infected with microphallids and acanthocephalans (*P. botulus*), compared to crabs that were free of parasites. There was no significant difference.

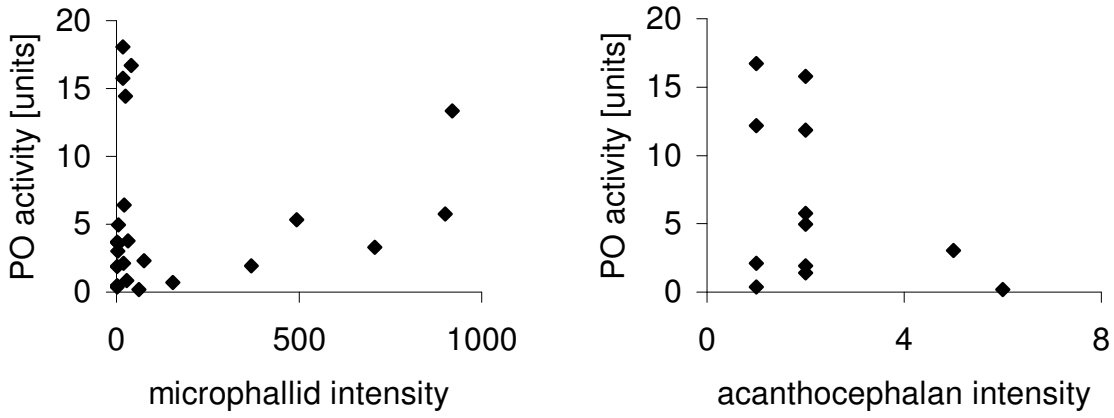

Figure 70: Relation between microphallid and acanthocephalan (*P. botulus*) intensity and phenoloxidase activity. There was no significant correlation.

3.1.9.3 Haemolymph protein

Haemolymph protein concentration was determined in crabs caught in Sylt in 2008. In contrast to the crabs used in the experimental infections (see below), these individuals did not show a correlation between size and haemolymph protein concentration (Figure 71). However, there was a significant difference in protein concentration between crabs of green and red colour morph. These were therefore analysed separately.

There was no difference in mean haemolymph concentration between red crabs infected with microphallids or acanthocephalans compared to crabs lacking the respective parasites. In green crabs, infected crabs showed a higher protein concentration which was nevertheless not statistically significant (Figure 72).

However, when turning to the effect of parasite intensity on protein concentration, there was a significant positive correlation for microphallids in green crabs (Figure 73), which may account for the above-mentioned differences in mean concentration ($R=0.771$, $p=0.005$). This effect was not present in red crabs, which also lacked individuals with exceptionally heavy microphallid infestation of over 500 metacercariae.

Similarly, no significant correlation between acanthocephalan intensity and protein concentration was found, but sample size for both colour morphs was very low ($N\leq7$).

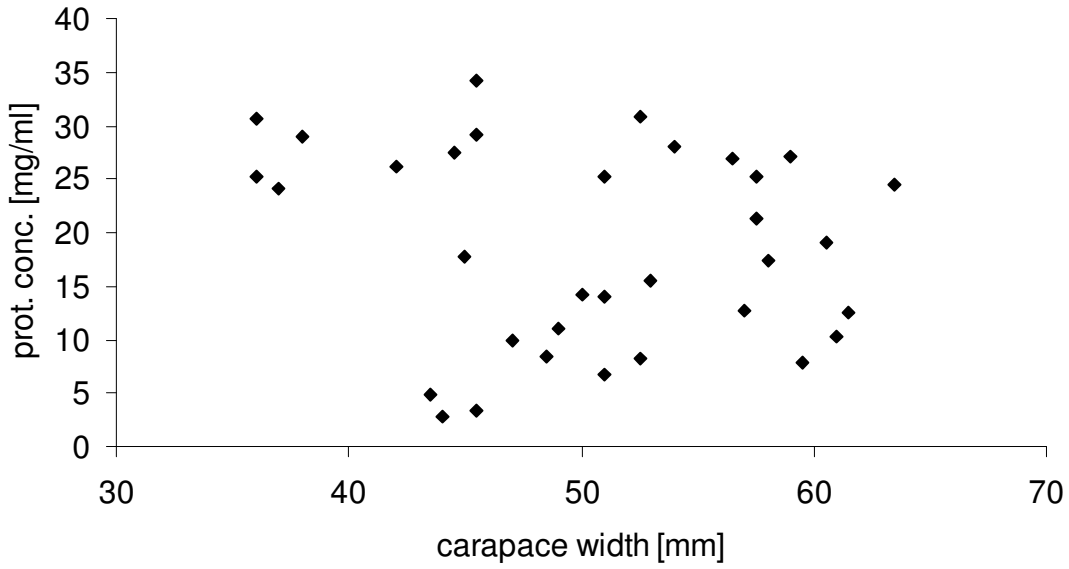

Figure 71: Relation between carapace width and haemolymph protein concentration. There was no significant correlation.

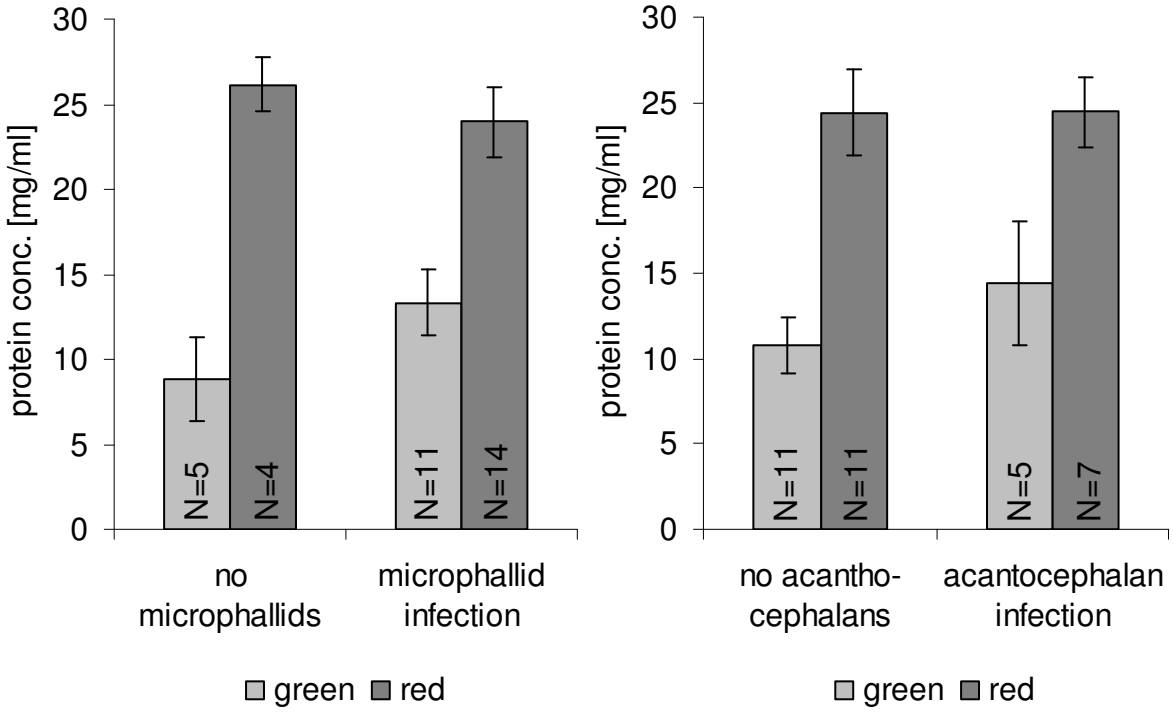

Figure 72: Mean haemolymph protein concentration (+/-SE) in uninfected crabs and crabs infected with *P. botulus* and microphallid trematodes.

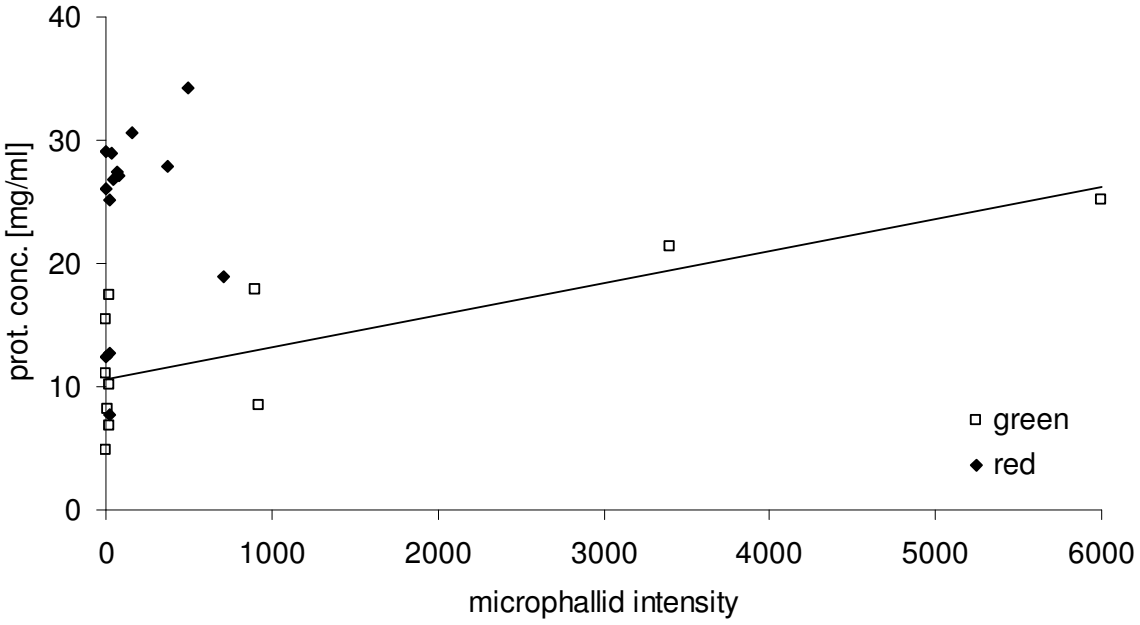

Figure 73: Relation between microphallid intensity and haemolymph protein concentration in green and red crabs. There is a positive correlation for green individuals, indicated by the trend line (R=0.771, p=0.005).

3.1.9.4 Interactions between gonad size and haemolymph parameters

There was a weak but significantly positive correlation between reproduction index and haemocyte concentration in European male crabs (Figure 74), indicating that these two fitness parameters may be connected (R_s=0.160, p=0.004). A similar but stronger correlation was found for RI and total haemolymph protein concentration (R_s=0.428, p=0.018) in male crabs from Sylt (Figure 76), but not in phenoloxidase activity (Figure 75).

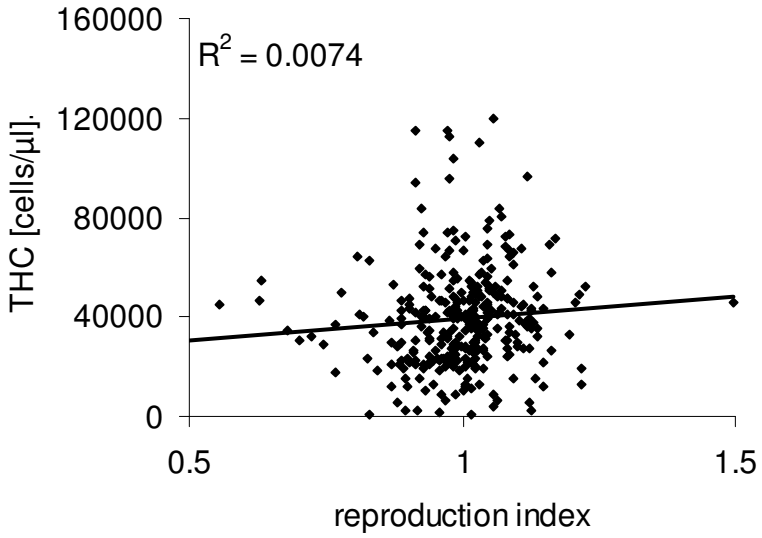

Figure 74: Correlation between THC and reproduction index in male crabs of Europe (R_s=0.160, p=0.004).

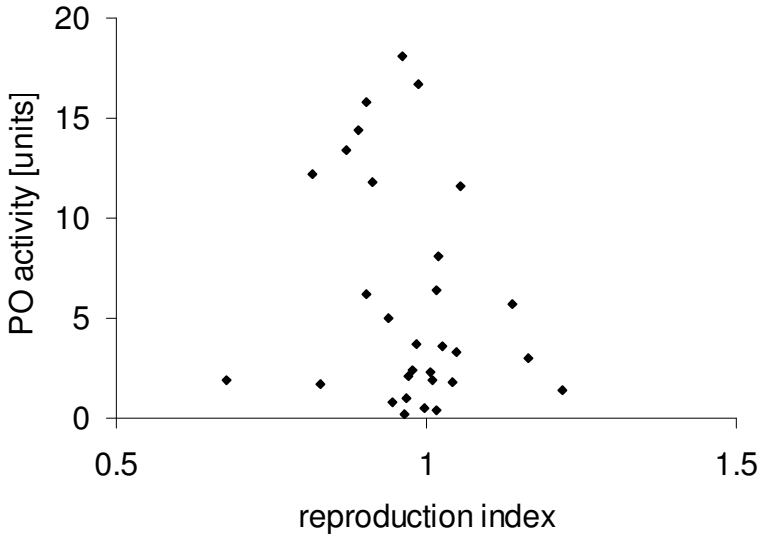

Figure 75: Relation between phenoloxidase activity (PO) units and reproduction index in male crabs of Sylt. There was no significant correlation.

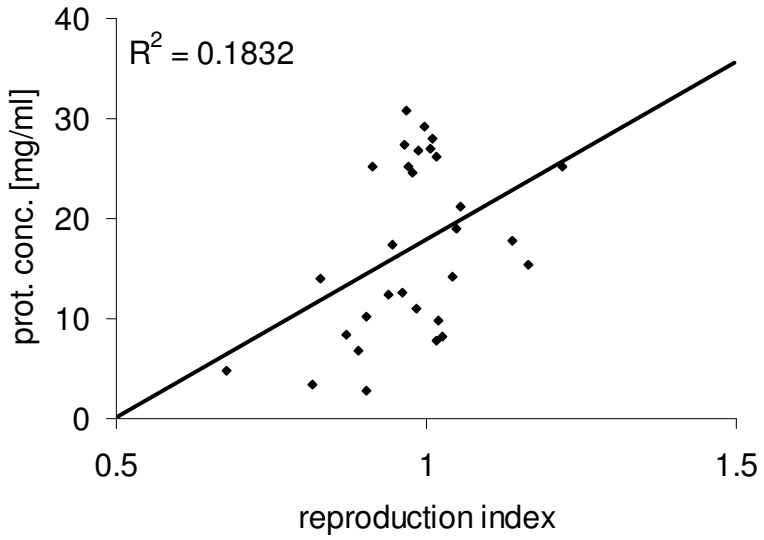

Figure 76: Correlation between total protein concentration in the haemolymph and reproduction index in male crabs of Sylt (R_s=0.428, p=0.018).

3.2 Results of the experimental infections

3.2.1 Infection success

Crabs that were treated with infectious eider duck faeces did not show mature or developing cystacanths on dissection. The experimental infection with *Profilicollis botulus* therefore failed.

The results of the experimental infections with microphallid trematodes are displayed in Table 18. Of the 14 crabs that were exposed to microphallid cercariae in week one of the sampling, only 4 turned out to be actually infected. This corresponds to an infection success of 28.5%. In these four crabs, intensity ranged from 24 to 213 metacercariae. Of the 15 crabs infected in week 3 of the experiment, only 12 could be dissected successfully as 3 crabs died prior to the infection or due to molting, which made the dissection of the remains impossible. Only 2 of the 12 remaining crabs were uninfected, resulting in an infection success of 83.3%. Intensity in these crabs varied widely, ranging from 5 to 1157 metacercariae.

Table 18: Crabs and metacercariae load of the two groups that were exposed to cercariae of microphallid trematodes in either week 1 or week 2 of the experiment. n.a.= not available due to early death or other circumstances.

week 1 infections		**week 3 infections**	
crab ID	no. of metacercariae	crab ID	no. of metacercariae
11	n.a.	41	41
12	0	42	1157
13	67	43	730
14	0	44	11
15	24	46	204
21	0	51	391
22	0	52	166
23	0	53	13
24	0	61	5
31	0	64	6
32	0	45	n.a.
33	213	54	n.a.
34	0	62	n.a.
35	54	63	0
		65	0

It is noticeable that among these week-3 crabs, the number of metacercariae was significantly positively correlated with crab size (R=0.732, p=0.007) (Figure 77). However, this result has to be considered with care, since it was not possible to ensure that the same number of infectious cercariae was presented to each crab.

During the course of the experiment a total of 14 crabs died prior to the final dissection. Approximately half of these deaths could be attributed to the haemolymph sampling, possibly caused by infections or stress, or to a molting event which is typically deadly in captivity. Mortality in infection groups was not significantly different from control groups.

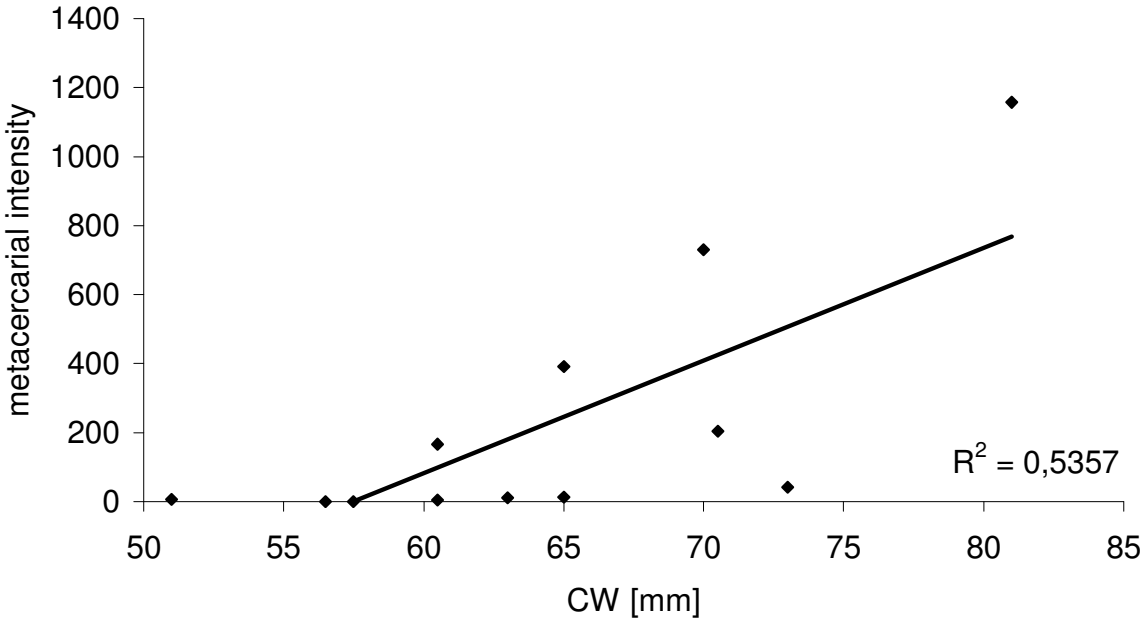

Figure 77: Correlation between crab size (carapace width - CW) and metacercarial load after the infection (R=0.732, p=0.007).

3.2.2 Total haemocyte concentration

Due to the unsuccessful infection with *Profilicollis botulus*, this section will only display results for the control group and the two groups infected with microphallids in weeks 1 and 3.

Within sampling events, there were no significant differences in THC between crabs of the second infection group and the control. The first infection group did not provide a sufficient sample size for conclusive results.

However, I did find differences between sampling events. Haemocyte concentration in the haemolymph of the experimental crabs showed a marked decrease between the first and the second sampling (Figure 78). However, this decrease proved significant only for the uninfected control group (t-test: $t=4.891$, $p<0.001$). Since the week 3 infection group was not exposed to parasites in this time frame, this effect cannot be attributed to the experimental infections themselves and might be caused by the high variation in THC combined with low sample sizes in the groups with microphallid infections. Haemocyte counts did not recover in any of the groups over the course of 70 days.

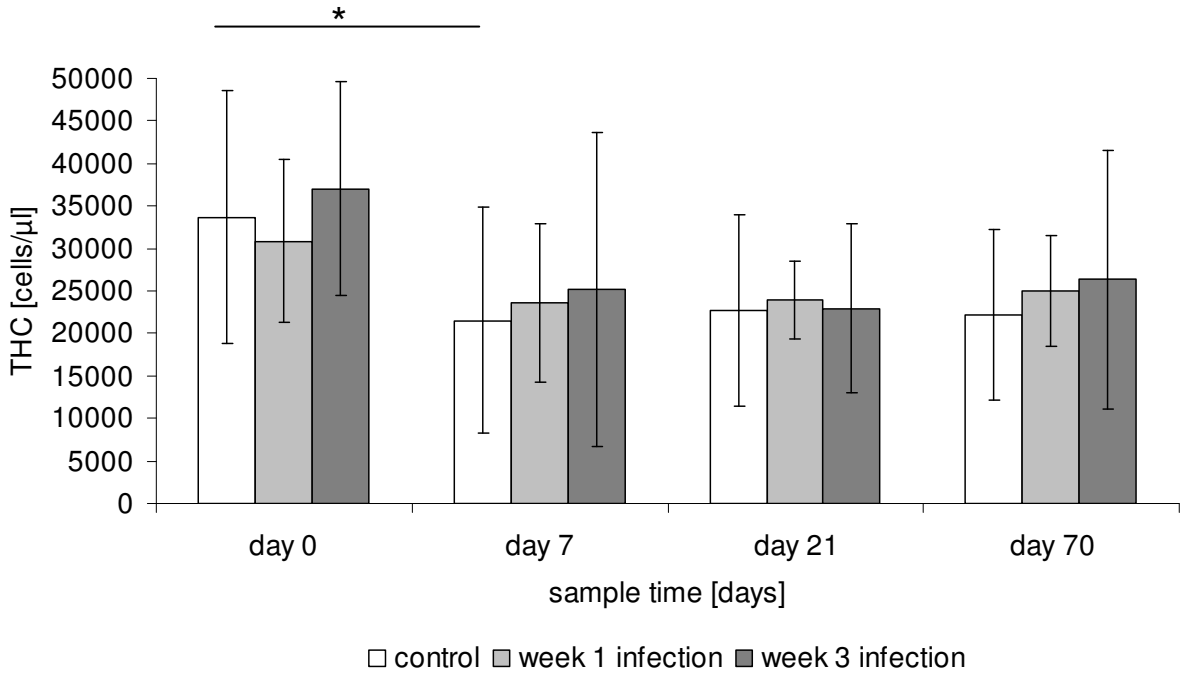

Figure 78: Mean THC levels (+/-SE) in uninfected crabs and crabs infected with microphallids before week 2 (week 1 infections) and after week 2 (week 3 infection). * = significant difference **(**t-test: t=4.891, p<0.001).

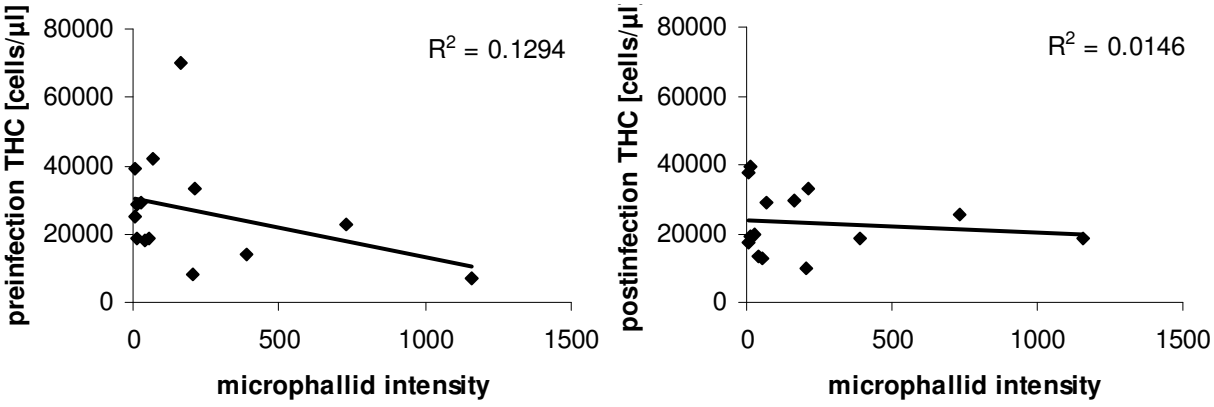

Figure 79: Infection success in relation to THC level before and after the infection event. In both cases, there is no significant correlation.

In group 3, where a sufficient sample size was available, there was no significant correlation between THC prior to the infection event and infection success, despite an apparent trend

(Figure 79). When dissecting the crabs, I found no melanized structures that would indicate that cercariae were stopped by the host immune system either while penetrating the crab or within the crab tissue. There was also no significant correlation between microphallid intensity and THC after the infection (Figure 79).

In uninfected crabs, THC was significantly negatively correlated with size at the beginning of the experiment (R=-0.45, p=0.003). However, this effect disappeared in later weeks and was not present in crabs infected with microphallids at week 3 (Figure 80). Lower sample size in the latter group may contribute to this result.

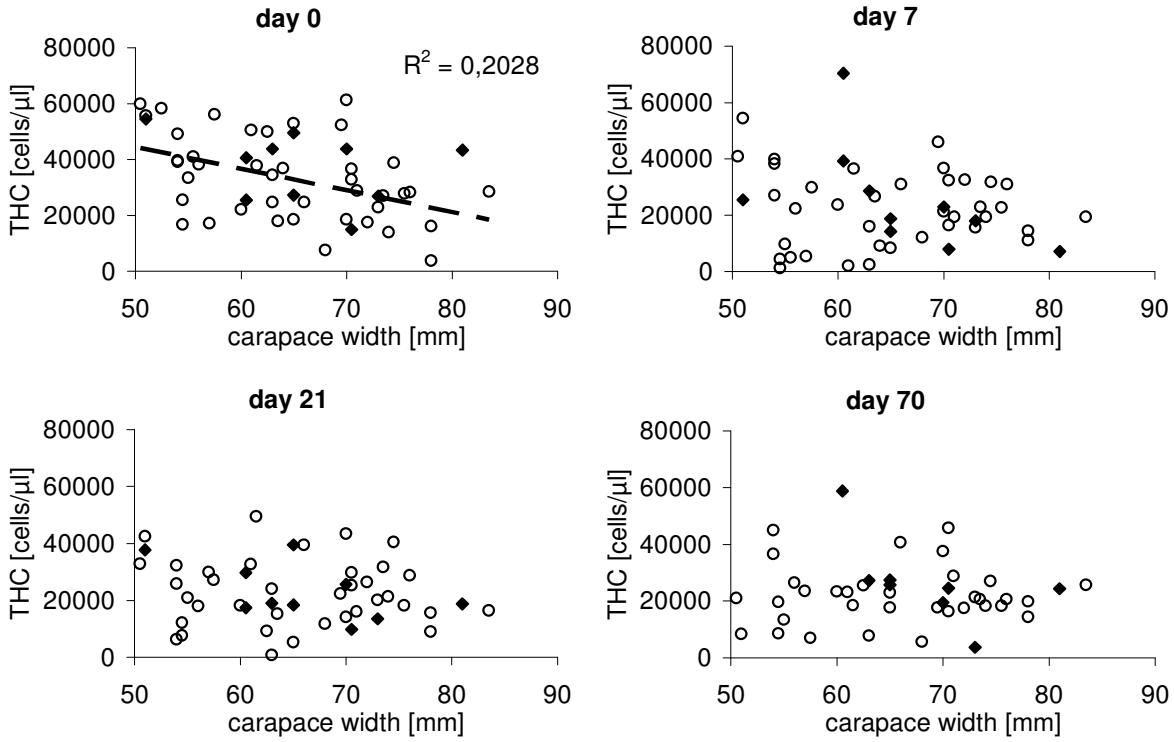

Figure 80: Relation between crab size (carapace width) and THC level in different phases of the experiment. Open circles represent uninfected crabs, filled diamonds represent crabs that were successfully infected with microphallids in week 3. A significant correlation was present for uninfected crabs on day 0 (R=-0.45, p=0.003).

3.2.3 Phenoloxidase activity

Due to the unsuccessful infection with *Profilicollis botulus*, this section will only cover results for the control group and the two groups infected with microphallids in week 1 and week 3. Overall, PO activities at the beginning of the experiment were comparable to those obtained in the field (see 3.1.9.2). There was no significant difference between the two groups.

Phenoloxidase (PO) activity showed very high variability in all three groups. No difference between control animals and the week 3 infection group was detected within sampling events. The week 1 infection group did not provide sufficient sample sizes for conclusive results.

In addition, there was no significant difference between the different sample times, confirming that neither the haemolymph sampling itself nor the parasite infection had an effect on PO activity (Figure 81).

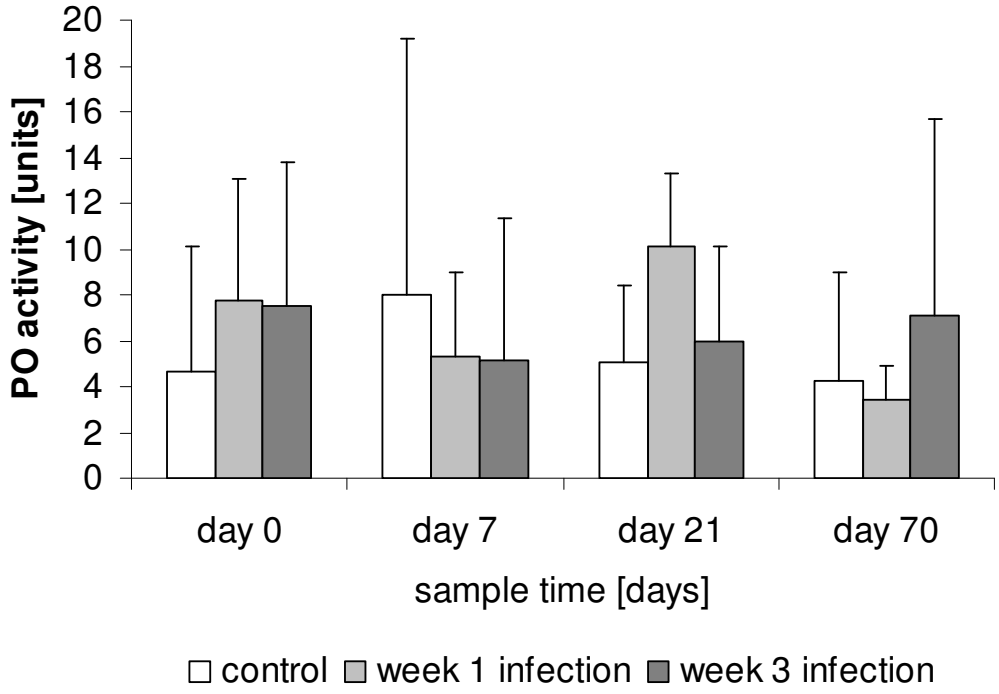

Figure 81: Mean phenoloxidase activity (+/-SE) in uninfected crabs and crabs infected with microphallids before week 2 (week 1 infections) and after week 2 (week 3 infection). No significant differences within or between groups were found.

Among the crabs infected with microphallids, there was no significant correlation between parasite intensity - and thus infection success - with the PO activity level prior to the infection event. Similarly, there was no significant correlation between infection intensity and PO activity one week after the infection occurred (Figure 82).

In uninfected crabs, PO activity was significantly negative correlated with size in the second week of the experiment (R_s=-0.361, p=0.026). This effect was not reflected in other sampling events during the experiment and it was not present in crabs infected with microphallids in week 3 (Figure 83). This can possibly be contributed to lower sample size in this group.

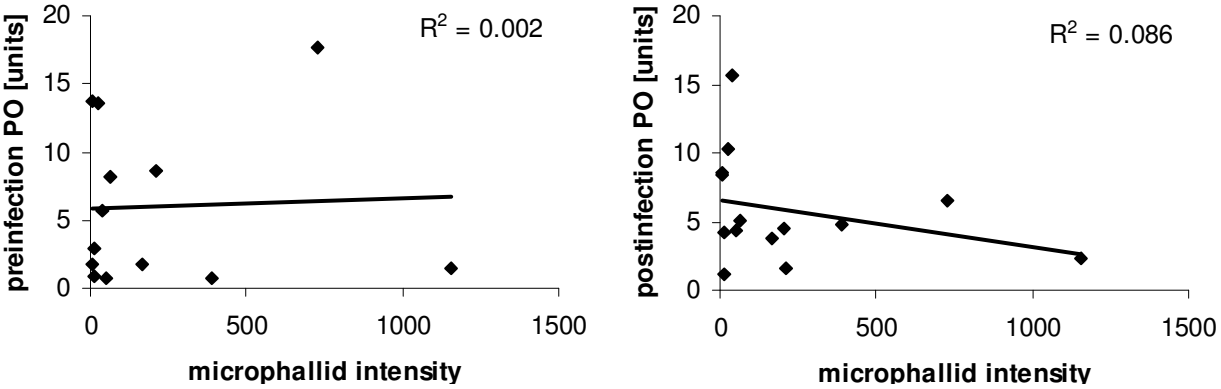

Figure 82: Infection success in relation to phenoloxidase activity before and after the infection event. In both cases, there is no significant correlation.

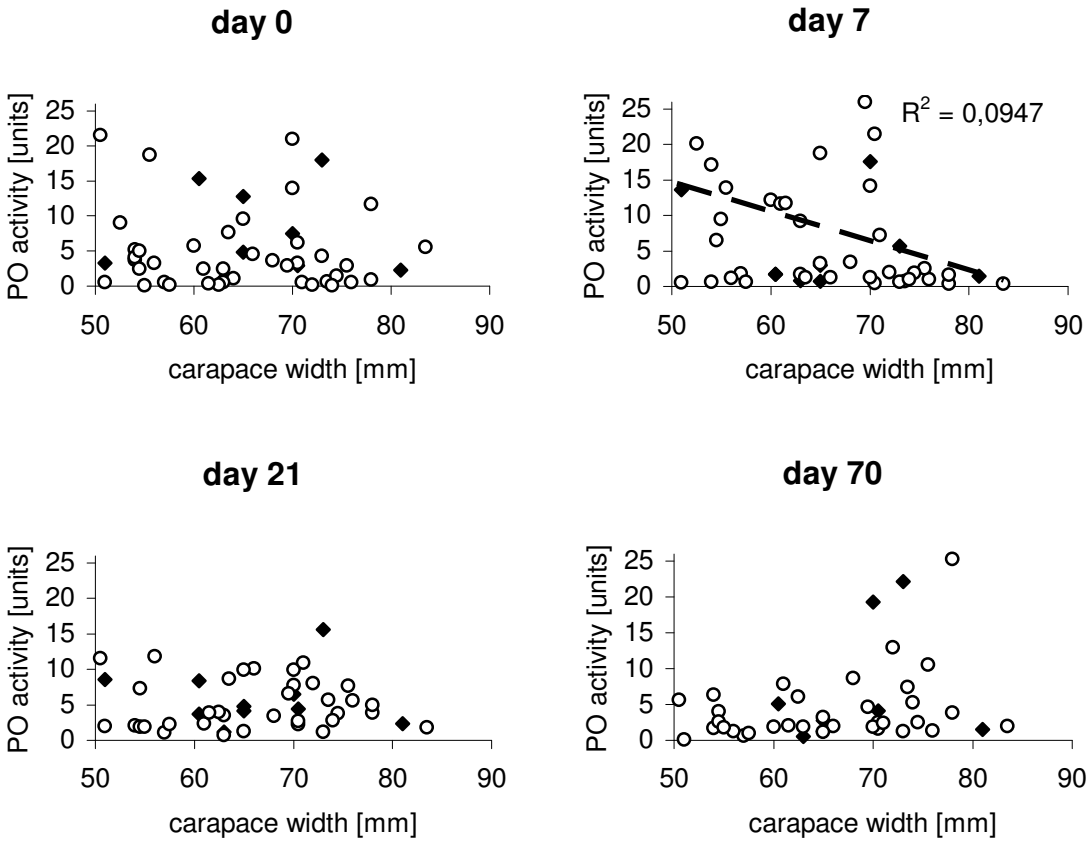

Figure 83: Relation between crab size (carapace width) and phenoloxidase activity in different phases of the experiment. Open circles represent uninfected crabs, filled diamonds represent crabs that were successfully infected with microphallids in week 3. A significant correlation was present for uninfected crabs on day 7 (R_s=-0.361, p=0.026).

3.2.4 Haemolymph protein

Due to the unsuccessful infection with *Profilicollis botulus*, this section will only cover results for the control group and the two groups infected with microphallids in week 1 and week 3. The week 1 infection group was analysed statistically, but due to the very low sample size for this group results have to be interpreted with caution. Morph did not have an influence on haemolymph protein in the crabs used for these experiments. Both crab morphs were therefore pooled.

I found that on any given sampling session, there was no significant difference in total protein concentration in the haemolymph comparing infected and uninfected crab groups.

However, there were differences between sampling events at different times during the experiment. The control group showed a significant drop in protein concentration after the initial sampling (t-test: t=2.623, p=0.012), but no subsequent changes occurred. In contrast to that, the week 3 infection group showed significant changes in later stages of the experiment (see Figure 84). It displayed a significantly reduced protein concentration on day 70, compared to both day 7 (t-test: t=3.742, p=0.01) and day 21 (t-test: t=3.390, p=0.012) (see also Table 19).

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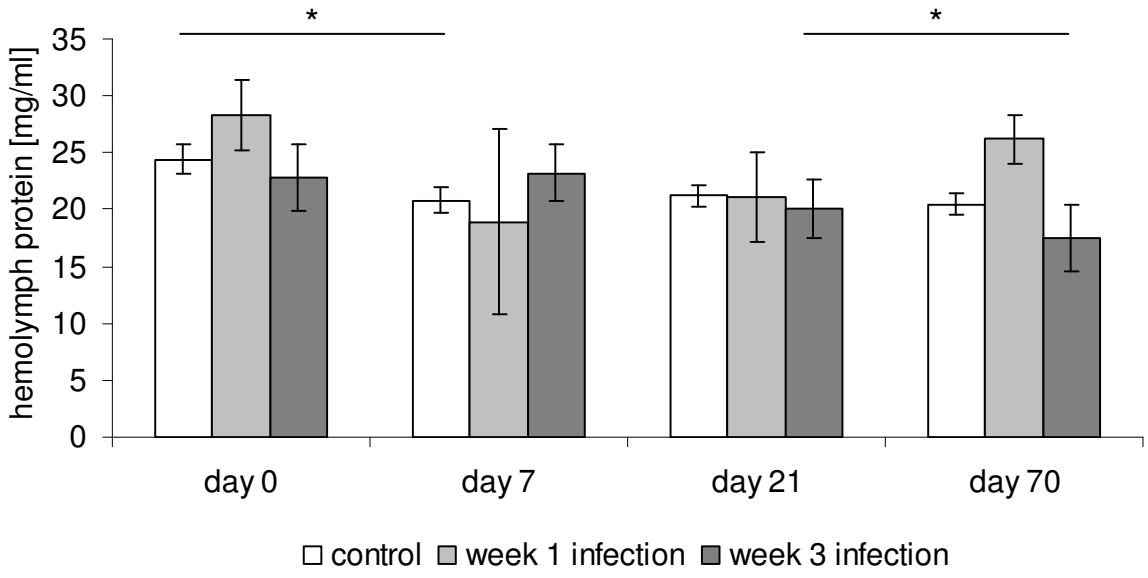

Figure 84: Mean haemolymph protein concentration (+/- SE) in uninfected crabs (control) and in groups infected with microphallids in week 1 and week 3 of the experiment. * = significant differences (see Table 19 for statistical analysis).

Table 19: Results of paired sample t-tests for haemolymph protein concentration in uninfected (control) and microphallid infected (group 1 and 2) groups at different times during the experiment. Group 1: infection in week 1. Group 2: infection in week 3. Significant correlations indicate that crabs with high protein concentration in the first measurement also display high concentration inthe second measurement. Significant effects are printed in bold.

paired samples	infection group	paired t-test		correlation		
		T	p	R	p	N
day 0 ↓ day 7	control	**2.623**	**0.012**	-0.133	0.420	39
	group 1	1.554	0.218	0.471	0.529	4
	group 2	0.795	0.453	0.315	0.447	8
day 7 ↓ day 21	control	-0.113	0.911	-0.090	0.612	34
	group 1	-1.005	0.389	**0.994**	**0.006**	4
	group 2	0.605	0.562	0.456	0.218	9
day 21 ↓ day 70	control	1.601	0.119	**0.834**	**0.000**	33
	group 1	-0.984	0.429	-0.359	0.767	3
	group 2	**3.390**	**0.012**	**0.947**	**0.000**	8

There was no significant correlation between infection success and protein concentration before or after the infection events. However, there was a correlation between crab size and protein concentration in most haemolymph samplings. Therefore, differences between week 3 infections and control animals were also analysed by ANCOVA using carapace width as a covariate (Table 20). The test showed no significant differences between the two groups over all of the sample days. It is notable, however, that the significant correlation between size and protein concentration was not present during the second sampling event (Figure 85, Table 20). This second sampling occurred only one week after the first, indicating that this time is not sufficient for the crabs to recover from the removal of haemolymph.

Table 20: Results of the ANCOVA conducted on control crabs as well as crabs infected with microphallids in week 3 of the experiment. The influence of carapace width and infection group on haemolymph protein concentration is displayed, as well as results for the corrected model incorporating both factors. Significant relations are marked in bold.

sample day		F	p	R^2	$N_{control}$	$N_{infection}$
day 0	corrected model	**7.035**	**0.002**	0.201	40	9
	carapace width	**13.497**	**0.001**			
	infection group	0.183	0.671			
day 7	corrected model	2.045	0.142	0.043	38	9
	carapace width	2.620	0.113			
	infection group	1.438	0.237			
day 21	corrected model	**10.824**	**0.000**	0.309	35	10
	carapace width	**21.278**	**0.000**			
	infection group	0.372	0.545			
day 70	corrected model	**13.754**	**0.000**	0.384	34	8
	carapace width	**24.944**	**0.000**			
	infection group	0.774	0.384			

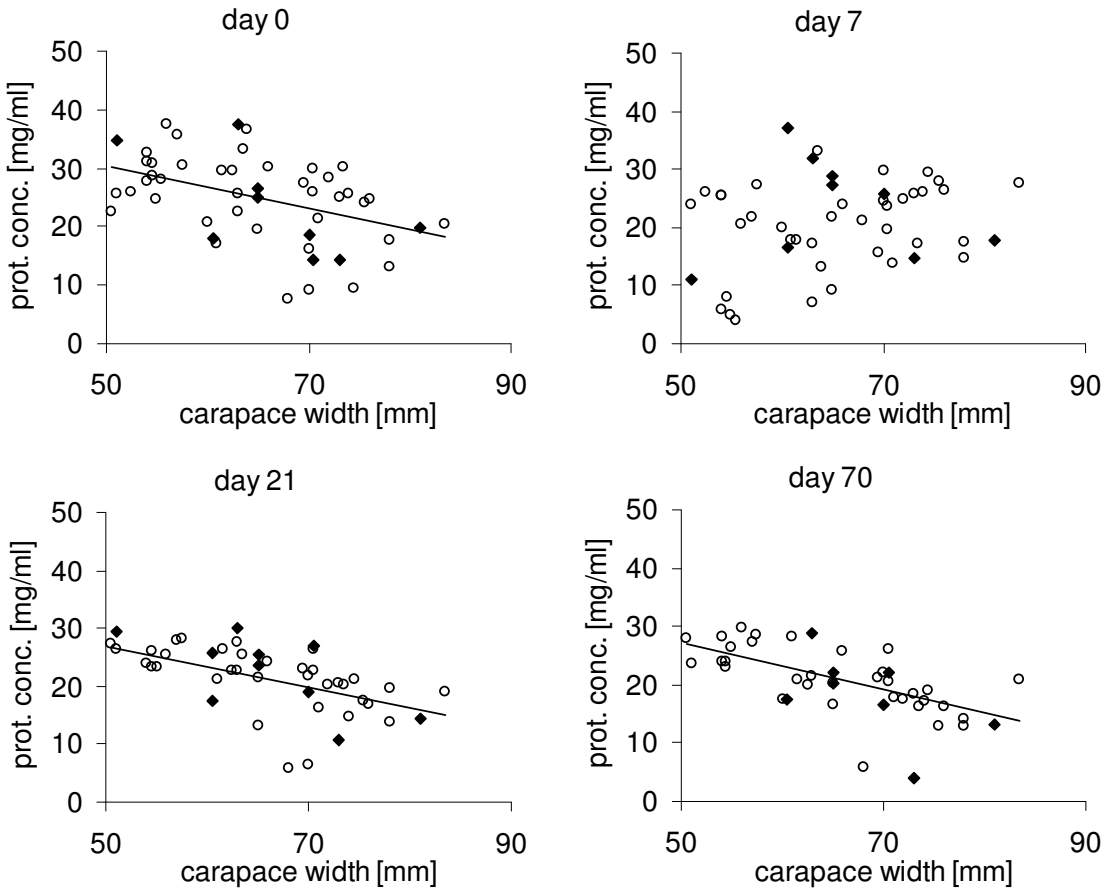

Figure 85: Correlation between crab size measured in carapace width with haemolymph protein concentration. Open circles represent unparasitized control crabs, filled diamonds represent crabs infected with microphallids in week 3 of the experiment. See Table 20 for statistical results of the ANCOVA.

4 Discussion

4.1 Population characteristics of *Carcinus maenas* in Europe and in invasive populations

4.1.1 Size distribution

In invasive populations of European shore crabs, the individuals often grow to significantly larger sizes compared to the native populations (TORCHIN et al. 2001; GROSHOLZ & RUIZ 2003), suggesting that they are actually more successful in these areas.

I found that crabs in populations from Denmark and Germany are very similar in size, showing rather little variance. The situation within South Africa was comparable. Only two large populations were investigated here, but the mean size was very similar in both. As in the previous studies, crabs in South Africa were much larger than their European counterparts. However, my findings from Australia are in stark contrast to this. In fact, mean crab sizes in Australia were well in line with what I found for Europe. The only difference I found was a slight skew towards higher size classes in female crabs from Australia, but not the astonishing size increase observed in South African individuals.

It is notable that the only difference between European and Australian *C. maenas* was found in females. Similarly, size differences between European and South African crabs were also distinctly more pronounced in females than in males, with a 37% and a 21% increase, respectively. This indicates that females may benefit more from the altered environment in the invaded areas. In *C. maenas*, females invest a large amount of energy in clutch production and brood care. It is therefore possible that limited resources force females in native populations to trade-off further growth against reproduction. If South African females are less limited by resources, they are not necessarily forced into such a trade off. An effect like this was described in female hermit crabs that are limited in their growth by the availability of appropriate shells. Here, growth is traded off against reproductive effort, resulting in higher clutch frequency when no shell of adequate size is available (BERTNESS 1981). Once a larger shell is acquired, the crabs start to allocate resources into growth and reduce clutch frequency.

Whatever the reasons, size is a crucial factor determining reproductive success in females, as it is closely connected to clutch size (THESSALOU-LEGAKI 1992; D'UDEKEM-D'AKOZ 1994; LATHAM & POULIN 2002a). Additionally, more continuous growth may be an indicator for a better nutritional status. These factors are likely to lead to higher fecundity for females in invasive areas and may well have a significant impact on the success and continuous

flourishing of this species - at least in South Africa and possibly, but to a much lower degree, in Australia.

The size increase of invasive *C. maenas* populations is one of the main anchor points that the supposed invasion success of this species rests on (TORCHIN et al. 2001). That it was not observed in Australia is therefore an interesting result. Unfortunately, only one population could be sampled and a generalisation to other invaded areas in Australia is not possible. The crabs caught from this single population lived in an estuarine ecosystem and were thus subject to salinity changes, as opposed to all other sampling locations in Europe and South Africa. MCGAW & NAYLOR (1992) reported that red colour morphs are more rarely found in such an environment, because of a reduced salinity tolerance over longer periods. Since red crabs are more common in higher size classes, this could explain the comparative lack of very large crabs in this estuary. Unfortunately, sample sizes for males were too small to compare the relative abundance of red colour morphs to that of other areas, so I was unable to test this hypothesis.

The situation in South Africa is much clearer, with a very distinct size difference to European shore crabs. Obviously, numerous factors can contribute to a size increase in invasive populations. A genetic component has often been hypothesised. It is possible that a bottle neck effect or selection pressure during and after the invasion process led to the evolutionary development of larger sizes. However, this could not be confirmed in a study on invasive plants (WILLIS et al. 2000), rather, the size differences could be attributed to a plastic reaction towards a benign environment. This is quite likely also the case in *C. maenas*.

One of these environmental factors to be considered in this respect is latitude and the resulting differences in water temperature. Indeed, GROSHOLZ & RUIZ (2003) found a correlation between size and latitude in *C. maenas*. Most invasive populations inhabit coasts at lower latitudes. However, in ectotherms it is typically expected to find smaller body sizes at higher temperatures (ATKINSON & SIBLY 1997); the size increase of *C. maenas* is therefore very unusual (GROSHOLZ & RUIZ 2003).

C. maenas do not moult and grow indefinitely. Rather, they enter a hormonally controlled state of terminal anecdysis at a certain point in their life (CARLISLE 1957). However, how many moults an individual crab can undergo is variable, with estimates ranging from 18 to 23 (CARLISLE 1957; CROTHERS 1967; MOHAMEDEEN & HARTNOLL 1989b). In South Africa, the larger size classes still contain a comparably large number of green colour morphs, which indicates a recent moult. There are two possible explanations for this which are not mutually exclusive. Crabs may either moult more often until they reach terminal anecdysis, or the

moult increments may be significantly increased and thus the maximum number of moults is reached at a bigger size.

It is well established that both of these growth parameters are affected by temperature and nutritional status (see HARTNOLL 2001 for an overview). Interestingly, it could be shown that increased temperatures lead to higher moulting frequency, but also to moult increment decreases, possibly due to insufficient time to accumulate the necessary energy reserves (MOHAMEDEEN & HARTNOLL 1989b). However, the net effect of higher temperatures is an increased growth rate because changes in moult frequency have a stronger impact.

Not unexpectedly, starvation has considerable negative effects on growth, reducing both moult frequency and moult increment (ADELUNG 1971; KLEIN-BRETELER 1975; MOHAMEDEEN & HARTNOLL 1989a). And again, the duration of the inter-moult period is the decisive factor governing growth rate.

There are no studies that explore available food resources and nutritional status in South African shore crabs compared to European populations. However, there are obvious differences in temperature regime between the two locations. The mean temperature is quite similar at around 13°C along the western side of the Cape and approximately 12°C in different areas of the Limfjord. But while the temperature in South Africa is relatively stable, the water in the Limfjord is subject to seasonal variations that range from under 0°C in winter to over 20°C in the summer.

In Europe, the crabs are forced to withdraw to deeper water during the winter months; they cease to moult and reduce feeding and other activities, typically resulting in one moult per year in adult crabs (CROTHERS 1967). In South Africa, similar restraints do not apply, possibly offering the crabs a longer time window, both for moulting and for gathering the necessary resources.

4.1.2 Ovary development and reproductive cycle

Since size differences were more pronounced or even limited to the female proportion of invasive populations, it is interesting to explore other attributes of female crabs. CHEUNG (1966) described four different ovarian stages in the European shore crab, all of which are part of the female reproductive cycle. Directly after the deposition of the fertilized eggs under the abdomen, the ovaries are empty and in a translucent, nearly invisible state. They will take on a white colour when they start to redevelop, pass through a yellow stage and finally turn deeply orange on reaching maturity. In this phase, the eggs are ready again for deposition under the abdomen.

It was observed early that the reproductive cycles vary between European crabs and invasive crabs of the US east coast (Table 21). In Europe, the mating season in summer is followed by egg deposition in early winter and hatching of the eggs in the following spring (CROTHERS 1967). This cycle is delayed in Maine in the northeastern USA. Despite a mating season in summer, egg deposition does not occur until the following spring, and the larvae hatch in summer (BERRIL 1982).

Table 21: Reproductive cycle of female *C. maenas* in Europe (Crothers 1967) and Maine, USA (Berril 1982). Observed fragment of the cycle in Australia and South Africa based on the present study.

	Europe	**North east USA (Maine)**	**Australia & South Africa**
mating season	summer & autumn	summer & autumn	?
egg deposition	early winter	early spring	autumn
hatching	spring	summer	?
megalopae settlement	early summer	late summer	?

In my study I could only observe a short segment of the reproductive cycle of female *C. maenas* in late summer and early autumn. However, the differences between Europe and both invasive locations were striking. In Europe, the crabs showed a mixture of ovarian colours ranging from white to deep orange, with only a few specimens having recently spent ovaries. This is the expected outcome for populations that spawned last year's clutch in spring and are preparing for a new egg deposition in winter. Both invasive areas examined are located on the southern hemisphere, but they were investigated during the same season. Here, however, females were almost exclusively in the final ovarian stage directly before egg deposition. This suggests an earlier egg deposition time compared to Europe, possibly in autumn (Table 21).

It is notable that both South Africa and Australia showed a very similar result with regard to ovarian stages. While not unexpected, it is an interesting result when compared to the two areas of the northern hemisphere, Europe and US east coast, which showed such marked differences despite similar conditions. The differences are probably mainly caused by temperature effects, as water temperatures in Maine are lower than in Europe. While mean temperatures in South Africa are not necessarily higher than in Europe, they are more stable throughout the year, which may lead to the earlier breeding.

4.1.3 Predation pressure

The European shore crab is prey for numerous animals in its native habitat. Bird and fish predators typically focus on young adult crabs, while marine mammals as well as invertebrates like octopi and other crab species will also prey on larger specimens (CROTHERS 1968).

C. maenas individuals can escape attack by a predator by autotomising limbs (MCVEAN 1976), and occurrence of autotomy has been used as an indicator for the predation pressure that acts on any given population of shore crabs (e.g. TORCHIN et al. 2001).

The populations investigated for this part of the study showed considerable variation in the frequency of autotomy, with predation in invasive areas well in line with similar European locations. Previous studies yielded similar results of highly variable autotomy incidences in Europe (MCVEAN 1976; MCVEAN & FINDLAY 1979; ABELLÓ et al. 1994; MATHEWS et al. 1999), ranging from under 10% to over 50% of the examined crabs.

In my samples, autotomy was typically independent of sex, but in several areas larger crabs were more likely to have missing limbs. Similarly, red colour morph crabs appeared to be more vulnerable to limb loss, mostly in the same populations where size also plays a role. It is likely that these two factors interact, due to the higher percentage of red crabs in larger size classes. Adult shore crabs have the ability to regenerate limbs over the course of two to three moults (MCVEAN 1976), resulting in a possible bias as younger crabs moult more often. However, after the pre-adult moult, crabs are only thought to moult once a year regardless of age (CROTHERS 1968). A bias caused by regenerated limbs is therefore not expected for this study, as only adult crabs were examined for autotomy.

In previous studies on autotomy in *C. maenas*, reports on limb loss in male and female crabs were conflicting. MCVEAN (1976) found that male crabs in a sublittoral population were more prone to autotomy than females, whereas there was no difference between the sexes in intertidal populations. In contrast, MATHEWS (1999) observed an increased occurrence of autotomy in female crabs in four different populations from sublittoral and intertidal zones. My findings add to this confusing situation as sex differences in autotomy were only found in a single population. In *C. maenas* from Australia, male crabs were significantly more likely to have missing limbs, which is akin to the situation in MATHEWS et al. (1999), with the difference that the Australian population was exclusively intertidal.

In their paper, MATHEWS et al. (1999) suggested that higher autotomy in some crab groups could be caused by a lower autotomy threshold. This could evolve because limb loss in males results in lower mating success (ABELLÓ et al. 1994), therefore a higher threshold might be favourable for them. However, in the light of the conflicting findings on autotomy in

both sexes, a genetically fixed difference seems unlikely. Rather, females - or males in the case of MCVEAN (1976) - can be more vulnerable to predation due to differences in behaviour and size. Females have a tendency to stay in deeper water (ATKINSON & PARSONS 1973). This is even more pronounced in ovigerous females, which also feed less (ROPES 1968) and are in general less active. Due to higher energy expenditure for the production of eggs, they may be forced to be more active during other phases of their life cycle.

In combination with varying dominant predators in different habitats, for example birds rather than fish, these differences in behaviour may account for the conflicting reports on autotomy in both sexes.

A similar mechanism may also explain another observation made in my study: The incidence of autotomy was correlated with the depth in which the populations were sampled. Crabs from deeper water had consistently more missing legs than crabs from shallow water. There are few other data available on the effect of water depth on autotomy, but MCVEAN (1976) provided raw data in his study that shows a similar trend. However, this may be biased by the fact that smaller size classes have lower levels of autotomy and are typically found in intertidal areas.

The correlation of predation with water depth is a somewhat surprising result as birds, mainly the herring gull, are often thought to be the main predators of *C. maenas* (CROTHERS 1967). These birds typically forage in intertidal areas and cannot dive deeper than 1-2 meters. This suggests that other predators that are more active in deeper water are potentially more efficient in attacking and damaging *C. maenas*.

4.1.4 Epibionts of *C. maenas*

Carcinus maenas is often colonized by a great variety of epibionts, including barnacles, hydrozoa, bryozoa, mussels, algae and the calcified tubes of polychaete worms (ABELLÓ et al. 1997). In my study, the most common colonizing organisms were barnacles, bryozoa and polychaetes. While isolated cases of algal and mussel growth were observed, these were generally rare and the amount of fouling on a single crab by these organisms was negligible.

In the populations examined for this project, the most common type of epibionts were barnacles. Barnacles were also the only epibionts which were found in all European populations, whereas bryozoans and polychaetes were more patchily distributed.

In some areas, epibiont prevalence was higher in males than in females. ABELLÓ & CORBERA (1996) explain this phenomenon with different moulting patterns, because females only have a short time window for their moult during the mating season. However, similar to predation

pressure, the risk of being colonized by epibionts may also vary by habitat and sex specific behaviour (ROPES 1968; ATKINSON & PARSONS 1973).

Prevalence differences between the two colour morphs, on the other hand, were much more distinct and showed that red crabs are more often colonized by epibionts. Moulting is an extremely effective defence against fouling of all kinds (WAHL 1989) and the prolonged inter-moult stage in red individuals results in an expected increase in fouling.

Following the same reasoning, it is also expected that the intensity of epibiont colonization - along with prevalence - is larger in red *C. maenas,* and indeed this is what previous examinations have found (MCGAW et al. 1992; WOLF 1998). However, this was not observed in my study, where no significant intensity differences between colour morphs were present.

The invasive populations in South Africa show a similar pattern. It is notable that bryozoans were absent from all invasive populations, but also from several European populations. In addition, bryozoans may be limited by host specificity, which was observed by ABELLÓ & CORBERA (1996) for bryozoans of the crab *Goneplax rhomboides*.

More interesting is the complete lack of epibionts in Australia, which is also the only location where this was the case. The reason for this may lie in the exceptional sampling location, which was in the estuary of a small river. As a consequence, variations in water salinity and possibly also in other abiotic factors may be the reason for this phenomenon, as epibionts are not necessarily adapted to such an environment (KEY et al. 1996). Additional evidence for this hypothesis can be found in the epibiont prevalences of the Kattegat. The sample sites in this area were located in the Great and Little Belt, which separate the Danish islands Fyn and Sjaelland from each other and the mainland. Depending on wind direction, the belts are subject to influx of water with higher salinity and oxygen concentration from the North Sea (DÖÖS et al. 2004). I would therefore expect a lower amount of epibionts in these areas, and this was confirmed by my data from the Kattegat locations.

A similar observation was made in a survey by ABELLÓ et al. (1997), who examined a crab population along the environmental gradient of a Danish fjord. While they typically found other epibionts (mainly hydrozoa, algae, mussels and barnacles) than the present study, all of them were conspicuously absent from parts of the fjord with high salinity and temperature fluctuations. It could therefore be concluded that, contrary to *C. maenas*, most of its epibionts are indeed not adapted to unstable environments.

Mean epibiont coverage on the carapace of crabs was correlated strongly with the water depth at which the crabs were sampled. This can have two non-mutually exclusive explanations. On the one hand, behavioural differences lead to more juvenile and young crabs in intertidal habitats (ATKINSON & PARSONS 1973; HUNTER & NAYLOR 1993). Due to the

comparably fast moulting of these crabs epibionts are expected to be less common among them. At the same time, the intertidal habitat also has highly changing environmental conditions, resulting in the same problems for epibionts as in sampling locations with strong salinity changes (ABELLÓ et al. 1997, see above).

Colonization by epibionts can have distinct consequences for the host (WAHL 1989; WAHL et al. 1997). The basibiont faces an altered interface between its surface and the surrounding water, possibly hampering movement and flexibility in crab hosts. The epibionts also increase the weight of the crabs, and can possibly increase carapace brittleness. In addition, epibionts covering the carapace of crabs may interfere with both recognition and handling by predators. Indeed, crabs which are covered with epibionts offer a better grip for human experimenters and it can be inferred that the same is true for other predators. On the other hand, epibionts can offer camouflage and make the crabs harder to locate in many habitats (WAHL et al. 1997). None of these factors have been investigated for crabs, so far. Epibiont effects on predation were observed in mussels, where the presence of barnacles increased predation by crabs, while hydrozoan colonization decreased it (WAHL et al. 1997).

In my study, only a single population from South Africa showed increased autotomy in crabs colonized by barnacles. This may suggest a possible effect of epibionts on predation, but without further research a causal connection between the two factors cannot be established. It is entirely possible that both epibionts and autotomy are covariates of environmental parameters.

4.1.5 Parasites in *C. maenas* populations of Europe

My study shows that the distribution and abundance of parasites differs highly between the sampled populations of *C. maenas* in Europe. This is not unexpected as both abiotic and biotic habitat characteristics can influence the distribution of parasites. For example, transmission and development rate of many parasites is linked to temperature (e.g. JENSEN et al. 1998; HARVELL et al. 1999). The list of other abiotic parameters that can influence parasite transmission is long and includes salinity, pH, water hardness, UV radiation and pollutants (reviewed in PIETROCK & MARCOGLIESE 2003). At the biotic end of the scale, the presence of alternative hosts, predation and competition can lead to variation in parasite distribution (reviewed in THIELTGES et al. 2008b).

P. botulus was mainly found in the North and Baltic Sea where the final host, the eider duck *Somateria molissima*, is common (PETERSEN et al. 2006). Parasite loads were typically low, which is not unusual for this helminth (LIAT & PIKE 1980; THOMPSON 1985a). The only area

where both prevalence and mean parasite load were high was location 8 in the Kattegat, although no satisfying explanation can be provided for this observation.

Microphallid trematodes were more patchily distributed. Their abundance appeared not to be solely connected to the occurrence of their final seabird hosts. Instead, it is likely that the distribution of the first intermediate host, the mud snail *Hydrobia ulvae* is another crucial factor (MOURITSEN *et al.* 1997). *H. ulvae* is known to have a scattered distribution with highly fluctuating populations, depending partly on the floating migration of juveniles (ARMONIES & HARTKE 1995), as well as habitat characteristics and competition (FENCHEL 1975).

Parasitic castrators, despite being attributed an important role in parasite release (TORCHIN et al. 2001), were not highly prevalent in most of the populations I investigated, and were completely absent in two locations. Where *P. maendadis* was present, it was typically found at prevalences of less than 5%.

The more common *S. carcini* is monecious with planktonic larvae, allowing for possibly high distribution rates. Yet the highest prevalences of between 40% and 65% only occurred in samples from 2006 in the Limfjord, while all other samples showed considerably lower prevalences ranging between 0% and 27%. In any case, these values are at the lower end of the prevalence range observed in previous studies in France, the British Isles, Skandinavia and Spain (reviewed in TORCHIN et al. 2001), which report prevalences of up to 80%, especially considering that these studies often refer to crabs with externa only, and are therefore underestimates.

Within the Limfjord, *S. carcini* prevalence appeared to be connected to salinity, with the lowest prevalences in the eastern part, close to the brackish Kattegat (BLEIL 2006) and continuing with even lower prevalences into the Kattegat itself. Prevalence increased towards the western part of the Fjord, where salinity is higher in proximity to the North Sea (DMU 2006). Larval development in rhizocephalans may be limited by salinity (TOLLEY et al. 2006), resulting in this varying distribution of *S. carcini*. However, in the North Sea locations themselves the rhizocephalan was almost completely absent. This indicates that, while salinity may be a factor, other parameters must be equally important (KASHENKO & KORN 2002). One of them may be physical isolation, for example in fjords with comparatively little water fluctuation. This has been shown for a deep water cirriped (SLOAN 1984) and may promote *S. carcini* prevalence in the Limfjord as opposed to the open sea areas of the North Sea and the Kattegat where parasite larvae are more likely to be carried away by currents.

In addition, RAINBOW et al. (1979) showed that *S. carcini* prevalence can also vary with water depth, with the highest levels found in the older crabs of deeper subtidal waters. Water depth may not be the deciding factor for this; the distribution can also be caused by preferences of

the parasite for certain size classes of crabs. Older and larger crabs are typically found more commonly in subtidal areas (ATKINSON & PARSONS 1973; HUNTER & NAYLOR 1993).

These differences in parasite communities between the European populations are not limited to large scale trends, however. Similar variability is also found on smaller geological scales.

For example, there are major differences in helminth prevalence between the two North Sea islands Sylt and Helgoland. Three sampling locations on Helgoland itself provided the opportunity to observe parasite communities in different habitats, which also showed considerable variation. In all three sublocalities, helminth parasites were predominant and parasitic castrators were absent. But crabs sampled on the dune area of Helgoland showed a trend to higher helminth prevalence in the subtidal area, compared to the intertidal crabs. The third sample area on the main island was also an intertidal one, but here again distinct differences could be observed with substantially higher numbers of digenean trematodes on the one hand, and lower acanthocephalan prevalence on the other, compared to both dune populations.

Higher prevalences in the sublittoral population of the dune island of Helgoland may in part be explained by age differences in these two areas. Young crabs are typically found more often in the intertidal area, whereas older crabs demonstrate both tidal and seasonal migration (ATKINSON & PARSONS 1973; HUNTER & NAYLOR 1993). Metacercariae and cystacanths are dormant larval stages, with neither reproduction nor vertical transmission taking place, and they remain alive and infective until the death of the host. Hosts accumulate them over time and both prevalence and intensity are expected to increase with host age (HUDSON & DOBSON 1995). At least for microphallids, this was indeed the case in our study and may explain some of the differences between these two subpopulations.

However, it cannot easily explain the observations made on the third sampling location, which was also an intertidal area containing many young and even juvenile crabs. However, contrary to the other locations, this sampling site was situated in close proximity to the rocky cliff line. These so-called "bird rocks" are popular nesting grounds for rock breeding seabirds like kittiwakes (*Rissa tridactyla*) and the common murre (*Uria aalge*). These can act as final hosts for trematode parasites of *C. maenas* and their presence is likely to benefit microphallid prevalence in the other hosts. Eider ducks, on the other hand, are the only final host for the more specific acanthocephalan *P. botulus*, and these birds are probably less common in this rocky habitat, which can explain the reduced number of acanthocephalans.

Crabs examined in the Limfjord, close to the island of Mors, offer a second opportunity to examine prevalence differences between populations in relatively close proximity. Differences between the three sampling sites around Mors were only found for the parasitic

castrator *S. carcini*, which was considerably more common at the southernmost site. However, it is not clear how these differences can be explained. The distribution of the free swimming larvae of *S. carcini* may be influenced by currents (SLOAN 1984), since other parameters between these sites appear to be very similar.

Marked differences between parasite prevalences were also observed in the three Kattegat populations. However, this is not unexpected as the distance between these sampling sites was substantial.

In addition to geographical differences, my data also allowed the evaluation of temporal variation within sampling sites. One location in Helgoland and two locations in the Limfjord were examined in consecutive years, with distinct differences in parasite prevalence between the two sampling sessions.

From these examples it is obvious that the parasite community is indeed highly variable even within Europe and between populations in close proximity. This is especially important in the light of the invasion success of *C. maenas* and the suggested mechanism of parasite release for this species. It is clear that the parasite community of the specific source population needs to be considered, as opposed to the much higher overall parasite diversity across the whole native range in Europe (TORCHIN et al. 2001).

In two cases, parasite prevalence was connected to crab colour morph. Among crabs infected with *S. carcini* or with microphallids, red colour morphs were more common then green ones. In the case of *S. carcini*, this can be explained by the moulting inhibition this parasite causes once its externa emerges (HØEG 1995). Microphallids accumulate in crabs over time, and older crabs are more likely to be infected (present study). Older and larger crabs are also more likely to have reached terminal anecdysis and cease moulting; as a result the fraction of red crabs increases.

4.1.6 Parasites in invasive *C. maenas* populations

In the South African and Australian populations investigated, none of the European parasites were present. In fact, crabs in South Africa were completely free of parasites. This was previously observed by TORCHIN et al. (2001), but my study provides a much more substantial sample size. This lack of macroparasites in South Africa can therefore be assumed with greater certainty.

In Australia, very low prevalences of two native parasites were found. These parasites, the trypanorhynch cestode *Thrimacracanthus aetobatidis* and a nematode of the genus *Proleptus*, were previously described in Australian and Tasmanian *C. maenas* by GURNEY

(2006). Both parasites utilize *C. maenas* as an intermediate host, very likely with elasmobranch final hosts (IAN BEVERIDGE, pers. comm.). This is in marked contrast to European helminths of the shore crab, whose typical final hosts are different types of sea birds.

A second trypanorhynch described in GURNEY (2006), *Dolfusiella martini*, was not found in the population examined for my work. Additionally, both the prevalence and intensity observed by me correspond to the very lowest range of the values recorded in the previous study.

There are a number of reasons for the lack of parasites in invasive populations, particularly in marine invaders. One is the possibility of the introduction of larval forms, which are typically not parasitized (LAFFERTY & KURIS 1996). In addition, the introduction process acts as a bottleneck, in which only part of the natural parasite fauna of an invader is introduced along with the host (DOBSON 1988). Low host densities at the onset of invasions may be below the persistence threshold of the parasite (SWINTON et al. 2002), and the lack of other obligatory hosts may completely prevent parasite transmission (TOMPKINS & POULIN 2006).

For these reasons parasite diversity is typically reduced in invasive populations (TORCHIN et al. 2003), including *C. maenas*.

4.2 Host-parasite interaction in *C. maenas*

Parasites can influence host fitness in two major ways, either by reducing fecundity or by increasing mortality (TOMPKINS et al. 2002). In two theoretical papers, Roy Anderson and Robert May marked a turning point in parasite ecology when they demonstrated that macroparasites can potentially regulate host populations (ANDERSON & MAY 1978; MAY & ANDERSON 1978).

The true effect of parasites on wild populations is very hard to demonstrate, as it often requires long-term studies involving the addition or removal of parasites from whole populations, as well as appropriate control populations. One of the rare examples where this has been conducted is the red grouse in Scotland infected with the nematode *Trichostrongylus tenuis*. HUDSON et al. (1998) treated the birds with an anthelmintic, and as a result the population crashes which regularly occurred all but disappeared.

A comparable approach is not possible in the shore crab *C. maenas* and its parasites. However, it is possible to determine parasite effects on fitness and thus the general potential of these parasites to influence the host population. Impacts on mortality and reproduction are

the most direct measures of fitness. However, parasites may also indirectly influence their hosts, for example by redirecting energy resources to defence mechanisms.

4.2.1 Parasite-induced mortality

In the field, one of the methods to determine parasite induced mortality is the analysis of parasite prevalence and intensity in different age groups. This method can be used successfully for the helminth parasites which use *C. maenas* as an intermediate host for larval stages. Both cystacanths of acanthocephala and metacercariae of digenean trematodes do not reproduce within the crab and survive until the death of the host. Consequently, hosts acquire and accumulate parasites over time and both prevalence and mean intensity are expected to increase with host age (HUDSON & DOBSON 1995).

If high numbers of parasites cause mortality in the host, the age-intensity function will have a convex shape, as older crabs which have accumulated high amounts of parasites are removed from the population (ANDERSON & GORDON 1982). In addition, if the rate of parasite acquisition is variable, higher degrees of aggregation are expected for older crabs. Mortality in highly infected individuals would shift the range of highest aggregation to the intermediate size classes.

A second line of possible evidence for mortality comes from autotomy data. The incidence of autotomy is considered an indicator for the predation risk of *C. maenas*. Parasite-induced changes in behaviour that increase predation risk are expected to be reflected in higher autotomy rates in affected crabs. This is an effect common for helminths with complex life cycles, which depend on transmission to a predatory definitive host (see CÉZILLY et al. 2010 for a review).

The parasitic castrator *S. carcini*, however, depends on the survival of its crab host and as a consequence this parasite should not promote crab mortality. And indeed my data confirms that crabs infected with this cirriped are not subject to more predatory attacks than uninfected crabs.

The situation in the two types of helminths found in my study is more complex.

4.2.1.1 Mortality caused by microphallid trematodes

Similar to previously discussed population parameters, evaluation of the mean intensity and aggregation of microphallid trematodes in different age classes revealed substantial differences between samples sites. In one population where mean intensities were generally

extremely low, these did not increase with age at all. This suggests that trematodes are either too rare to accumulate in a meaningful way in older crabs or that the parasites moved into the area only recently and all infections are comparatively fresh.

In all other sampling sites with a sufficient abundance of trematodes, however, the mean intensity did indeed increase with age. In two of these, both situated in the Kattegat, there was no evidence for parasite-induced mortality, whereas such an effect was observed in both populations from the North Sea and one from the Limfjord. This suggests that a possible effect of microphallids on crab survival may be tied to secondary environmental factors (LAFFERTY & KURIS 1999; LAFFERTY & HOLT 2003).This is not unusual for macroparasites, which are typically expected to cause morbidity rather than mortality (HUDSON et al. 2002).

Variations in the effect of parasites on different, even neighbouring host populations are not unheard of. In studies on the New Zealand amphipod *Paracalliope novizealandiae*, mortality caused by the trematode *Maritrema novaezealandensis* in the high-shore population was distinctly higher than in the low-shore population from the same coastal area (BATES et al. 2010). The numerous factors that contribute to parasite transmission dynamics can also facilitate such differences (PIETROCK & MARCOGLIESE 2003; THIELTGES et al. 2008b).

The second parameter for mortality is aggregation in relation to size. Peak dispersion of parasites in intermediate size classes would confirm an effect of metacercariae on mortality or morbidity. However, in most populations the degree of aggregation, measured as the variance to mean ratio, was not correlated with age at all. A similar finding has been interpreted by LATHAM & POULIN (2002a) as a confirmation of mortality, since it indicates that individuals with heavy parasite loads are removed from the population. Further support is provided by two populations in which the variance to mean ratio is indeed correlated with crab age, but with a peak at an intermediate size class.

A second possible explanation for the lack of correlation in the 4 populations is that extreme levels of infection, in the range of several thousand metacercariae per crab, are not related to crab age and are thus not accumulated slowly. Rather, it seems plausible that such high levels of metacercarial infestation are acquired in single infection events, for example by preying on a highly infected *Hydrobia* individual and releasing cercariae in the process. These events may be sufficiently rare to be distributed randomly over age classes. The frequency distribution of metacercariae within infected hosts support this to a degree, showing that a majority of affected crabs carries less than 100 cysts and only rare cases harbour more than 1000.

However, the partly ambiguous findings on the variance to mean ratio question the conclusions that can be drawn from the convex relationship between mean intensity and host

age. Is it indeed caused by parasite-induced mortality or is it rather an artefact of sampling bias or behavioural differences between the age classes (WILSON et al. 2002)?

That trematode infections can have deadly consequences on their crustacean second intermediate host is now well established. In fact, the very species that infect *C. maenas* are known to cause mass mortalities in an alternative intermediate host, the amphipod *Corophium volutator*. In 1990, a *C. volutator* population was eradicated completely, which was attributed to the massive occurrence of microphallid trematodes caused by exceptionally high temperatures (JENSEN & MOURITSEN 1992). A trematode-induced effect on mortality in this host species was also confirmed in laboratory experiments (JENSEN et al. 1998). Similarly, trematodes were also found to cause mortality in other crustacean taxa, like gammarids (THOMAS 1995) and isopods (JENSEN et al. 2004), as well as other invertebrates (e.g. THIELTGES et al. 2006).

However, when discussing parasite-induced mortality it is important to note the different mechanisms that may be at work to cause this effect and how they are connected to the results of my study.

Parasites can mediate behavioural changes that increase predation risk for the host, either as an adaptive process or as a consequence of pathology (CÉZILLY et al. 2010). With regards to trematodes, the textbook example for this phenomenon is the lancet liver fluke *Dicrocoelium dendriticum*, which causes its ant intermediate host to climb vegetation to increase transmission to grazing final hosts (CARNEY 1969). Similar examples can be found in marine environments. One is the previously mentioned *C. volutator*, which spends more time outside of its burrow when infected with metacercariae of microphallid trematodes, exposing it to seabird predation (JENSEN & MOURITSEN 1992). The microphallid *Microphallus papillorobustus* even specfically targets the brain of its gammarid intermediate host to facilitate behavioural changes which benefit transmission to final hosts (HELLUY 1983, 1984).

However, an increase in predator attacks on infected *C. maenas* individuals was not observed. Trematode infection did not coincide with a higher occurrence of autotomy, which is generally used as an indirect indicator for predation risk. This suggests that infected crabs are not attacked more often than healthy individuals. Rather than a gradual increase in predation there may also be a threshold intensity over which predator attacks are mostly successful, removing highly infected crabs from the population. In this case autotomy would not show up more often in such crabs, because they cannot escape predators. This could indeed be the case in populations with a convex intensity-age relationship, but such a threshold seems counterintuitive and does not comply with past studies. Where no

specialised metacercariae directly affect the host brain, trematode effects on the host are usually correlated with intensity (e.g. JENSEN et al. 1998).

Besides behavioural alterations, parasites can of course cause host pathology directly in numerous ways. They can bind host energy resources by nutrient competition or by inducing costly immune responses. Penetration of the host's cuticle can facilitate secondary infections and movement through host tissue has potentially negative consequences as well.

In the amphipod *C. volutator* mortality was not related to the total amount of metacercariae carried, but rather to the amount of cercariae invading the host within a short time frame (JENSEN et al. 1998). The authors suggested that the penetration itself causes mortality, possibly due to haemolymph loss or secondary infections. The subsequent movement of the cercariae through host tissue is also likely to have negative consequences, caused the production of lytic enzymes or similar mechanisms (MEIßNER & BICK 1999). Finally, the cercariae undergo a growth phase in which they absorb nutrients from surrounding tissue (GALAKTIONOV et al. 1996). During these first three stages of penetration, migration and growth, the effect on the host is probably most pronounced (FREDENSBORG et al. 2004). Once the cyst stage is reached, no more nutrients are absorbed and there is no interaction with the host, except for the possible problem of tissue and organ displacement.

If fully formed cysts are relatively benign, then the intensity-age relationship is only of limited use in determining mortality, and it would certainly account for my ambiguous results in *C. maenas*. However, the experimental infections of shore crabs with microphallids, despite their largely preliminary nature, allow a tentative conclusion of the effect of the first three stages of the infection process to be made. If the invasion of a large number of cercariae within a short time causes mortality, I would expect this to be reflected in the results from these experiments. However, no mortality was observed in crabs exposed to several hundred cercariae. While infection success was very varied, not even the single crab which was later found to carry more than 1,000 cysts showed any signs of pathology. While these experiments suffer from a low sample size and a yet to be perfected methodology, they do suggest that microphallid infections are not as detrimental to adult shore crabs as they are in the formerly investigated amphipods and isopods.

Isolated observations of peak intensities seem to support this. Crabs can carry several thousand metacercariae and survive. In an isolated case, a single crab of medium size was observed to be so heavily infected that the body cavity was filled with metacercariae which displaced the hepatopancreas and the gonads almost completely (personal observation). The cysts were so numerous that they were impossible to count, but the intensity was

estimated at well over 10,000 metacercariae. Yet this specimen was alive and even survived a stressful transport and subsequent change of environmental conditions.

It remains to be seen whether host size is a relevant factor for the impact of parasites. Previous examples of trematode induced mortality typically stem from amphipods and isopods, which are significantly smaller than European shore crabs. Indeed, the only reports of crab mortality caused by microphallid trematodes are centred on juvenile crabs, whose size is much closer to that of gammarids (LAUCKNER 1986; LAUCKNER & SÖHL 1990). Unfortunately, these reports are only found in so-called grey literature and a connection between host size and trematode induced mortality cannot be concluded with certainty.

However, this is not the first time that host-parasite size ratio is suggested to be relevant for the parasite's effect on its host. LATHAM & POULIN (2001, 2002b) formulated a similar hypothesis in the case of an acanthocephalan infecting the crab *Macrophthalmus hirtipes*, which did not result in the expected behavioural alterations. This explanation seems to be particularly likely in cases where host behaviour changes as a consequence of pathology rather than an active manipulation by the parasite.

In general, it is possible that the same parasite has very different effects on different hosts. The trematode *M. papillorobustus*, for example, has a considerable impact on the survival of its gammarid host *G. insensibilis*, but the closely related host species *G. aequicauda* appears to be barely affected (THOMAS et al. 1995). Experiments with *M. claviforims*, one of the microphallids which also infect *C. maenas*, showed a similar situation. The parasite causes mortality in *C. volutator*, but not in the congeneric species *C. arenarium* (JENSEN et al. 1998). These differences in influence on hosts may well extend to the taxonomically much more distant shore crab.

In summary, there is some interesting evidence that infestation by digenean trematodes leads to mortality in adult *C. maenas*, and that this effect is a consequence of the presence of fully formed metacercariae, despite their apparently dormant state. The absence of mortality in several populations indicates that either biotic or abiotic stressors are necessary for this effect, resulting in parasite-induced morbidity rather than mortality.

4.2.1.2 Mortality caused by *P. botulus*

The principles applied to mortality caused by microphallid trematodes in the shore crab apply for the most part to the acanthocephalan *P. botulus* as well. Mean intensity is expected to be correlated with host age, and if mortality is a factor the function should have a convex shape.

However, contrary to my findings on microphallids, there was no correlation at all between mean cystacanth number and crab age. Similarly, the variance to mean ratio did not increase with crab age either. In most of the populations this may be explainable by the generally very low intensity and thus the comparative rarity of the parasite. Typically, an average of 3 cystacanths was found per infected host, it is therefore possible that the infection risk was simply too low for a strong accumulation within older hosts.

However, in a single location (location 8) both high prevalence and high intensity were combined and yet there was no observable accumulation effect. It could be argued that this type of age-mean intensity distribution is caused by a threshold intensity, above which crabs are removed from the population by mortality. Such an effect would be reflected in decreasing aggregation within older crabs as more hosts approach this threshold. This is not supported by my data, therefore it cannot serve as evidence that *P. botulus* causes significant mortality in adults of the European shore crab.

Crabs acquire cystacanths by ingesting eggs accidentally with their food. This leads to a "trickle infection" and consequently accumulation with age is the intuitive conclusion. This is exactly what was found in previous research, for example on crab populations in the Ythan Estuary in Scotland (LIAT & PIKE 1980; THOMPSON 1985a). The overall intensity and prevalence of cystacanths in these studies was comparable to my findings, but here both mean intensity and aggregation increased with crab age in several populations. It should also be noted that neither study found a decrease of parasite intensity in older crabs, which could be indicative of mortality. The results of these studies also show very clearly that the lack of accumulation in my samples cannot be attributed to mortality, as cystacanth levels were too low.

It is not clear how the lack of such data can be explained. The frequency distribution of the parasite over the whole crab population, however, suggests a general lack of aggregation. In fact, in almost all locations the variance to mean ratio was below 3, indicating that the cystacanths are close to randomly distributed within their hosts. The single exception is again location 8, which shows considerable aggregation, albeit still to a much lower degree than what was observed for microphallids.

It is obvious that neither the distribution of mean intensity nor the aggregation over size classes allows any conclusions regarding mortality caused by *P. botulus*. I can only rely on previous studies which report no levelling of cystacanth load in older crabs (LIAT & PIKE 1980; THOMPSON 1985a), therefore questioning an intensity dependant effect on mortality.

Data on autotomy could potentially provide additional evidence for mortality, if it is caused by behavioural alterations leading to increased predator vulnerability. However, crabs bearing

cystacanths were not found to be more susceptible to attacks, regardless of parasite load. This suggests that *P. Botulus* does not change the behaviour of the host to increase transmission probability to the final host.

The distinct lack of evidence regarding crab mortality is in stark contrast to what is typically expected of acanthocephalans in their intermediate host. A number of studies spanning several decades has confirmed that acanthocephalans usually alter host behaviour, making them more susceptible to predators (e.g. BETHEL & HOLMES 1973; BAKKER et al. 1997; BAUER et al. 2005; BENESH et al. 2005). Most of these studies were carried out on small crustaceans like amphipods or isopods, which are the most common intermediate hosts.

Nevertheless, there is also some information available on brachyuran crabs from New Zealand. These crabs are typically found more exposed when they are infected with *Profilicollis* spp. cystacanths (LATHAM & POULIN 2001, 2002b). This may be in parts caused by a parasite-induced increase in metabolic rates, as well as activity (HAYE & OJEDA 1998). Only recently it was found that the parasite manipulates dopamine levels, which can lead to these behavioural changes (ROJAS & OJEDA 2005). Infected crabs also show clear signs of intensity-dependant mortality, with a levelling of mean intensity in larger size classes (LATHAM & POULIN 2002a).

This parasite occurs in very similar prevalences and intensities as *P. botulus* in *C. maenas* (BROCKERHOFF & SMALES 2002; POULIN et al. 2003); a similar behaviour altering effect would therefore be not unexpected for this closely related acanthocephalan. And indeed, there are reports of negative impacts of *P. botulus*, particularly on young female crabs (LAUCKNER & SÖHL 1990). As in the case of microphallid trematodes, these reports are not backed up by peer-reviewed papers and can therefore not be used as a reliable source.

Nevertheless, the results from these studies are interesting. On the one hand the authors found cystacanth distributions and other indicators suggesting that young infected female crabs are prevented from further moulting. This results in a longer time in which they are appropriate prey for the definite host, since Eider ducks prefer the small size classes (LIAT & PIKE 1980; THOMPSON 1985b). On the other hand, older crabs in this study also displayed a sharp drop in mean cystacanth intensity, which could indicate mortality. However, parasite load in intermediate size classes reached a peak at over 300 cystacanths, which is an extreme value compared to intensities observed both in my own study and in comparable studies in Europe (see above). This suggests quite clearly that possible pathogenic effects are limited to intensities that are not at all typical in most populations of *C. maenas*. It also shows why neither previous studies, nor my own research revealed signs of mortality.

The analysis of parameters for parasite-induced mortality therefore does not provide any supporting evidence for a possible effect of the acanthocephalan *P. botulus* on crab population dynamics under normal circumstances.

4.2.2 Parasites and haemolymph parameters

4.2.2.1 Immune parameters

The immune system of invertebrates is a complex mechanism involving both cellular and humoral components (YOSHINO & VASTA 1996). Studies on immune competence therefore often focus on some aspects of this system by necessity. I chose two parameters that are considered crucial for the immune defence in arthropods.

Haemocytes are central to many aspects of the immune system. They encapsulate pathogens or perform phagocytosis, but they also release antimicrobial peptides and store the ingredients of the phenoloxidase system (SCHNAPP et al. 1996; CERENIUS & SÖDERHÄLL 2004). Total haemocyte counts (THC) can therefore provide a good estimate on the immune status of an individual (ESLIN & PREVOST 1996). The phenoloxidase (PO) system itself is the second aspect that was investigated in my study. This is also a commonly used parameter for immune competence (HAUTON et al. 1997; VASS & NAPPI 2000; LIU et al. 2007).

I found that both PO activity and THC vary strongly between individual crabs. There was no difference between red and green colour morph crabs. This suggests that there is no trade-off regarding immune competence involved in the two different life-history strategies. It is notable that female THC levels sometimes deviate from the values observed for male crabs, but not in a consistent way. In Europe I observed higher cell concentrations in females, but in Australia they were lower. The cause for this is not clear, but it may be connected to different migration patterns in females, which can expose them to varying environmental conditions. Unfortunately, the same analysis could not be conducted for PO activity, as the number of available females at the time of the sampling was too low. In addition, the test groups for the experimental infections consisted entirely of male individuals. As a consequence, the following evaluation of parasite influence on immune parameters is focused on male *C. maenas*.

The population examined for PO activity was mainly affected by helminth parasites, and the only successful experimental infections were conducted with microphallid trematodes. The activity of PO, despite being highly variable between individual crabs, was not influenced by the presence of these parasites. This was the case in both field samples and in the experimental infections, and regardless of parasite load in the host crabs. It appears,

therefore, that neither the presence of mature helminth cysts nor the movement and growth of cercariae within host tissue have any effect on PO activity.

This is quite contrary to previous observations on bacterial infections, which elicit a significant response in *C. maenas* (HAUTON et al. 1997). Crabs that were challenged with a bacterial dose showed an immediate drop but subsequently a long-term increase in PO activity, probably as a result of the previous stimulation of the immune system. This is clearly not the case for infections with microphallids, which can have three possible explanations. On the one hand, the parasites may be able to avoid the crab immune system actively. This idea is weakly supported by my observation of isolated cases of dead and melanised metacercariae (pers. observation). But active avoidance is typically accompanied by a drop in immunocompetence which was not observed in my study (e.g. SHELBY et al. 2000). On the other hand, parasites can avoid the immune system passively, for example by using molecular mimicry (SALZET et al. 2000; ZAMBRANO-VILLA et al. 2002). Lastly, PO is only one aspect of the immune defence and while it is clearly involved in bacterial infections (HAUTON et al. 1997), the same is not necessarily true for macroparasites.

My results also show that crabs with high PO activity were not significantly less infected in the wild population. It follows that this specific immune parameter apparently does not confer resistance to helminth parasites. Despite the wide-spread use of PO activity in invertebrate immunology, my results are not unique. There are obviously cases where higher PO levels protect their hosts from pathogens. SHIAO et al. (2001) demonstrated this for mosquitoes infected with another helminth parasite, the microfilara *Dirofilaria immitis*. But more examples were found for resistance against microparasites, bacterial and even viral pathogens (NIGAM et al. 1997; REESON et al. 1998). However, my study adds to the cases where PO activity was not correlated to either an infection event or defence against a particular pathogen. MUCKLOW et al. (2004) found that high PO activity does not confer higher resistance of *Daphnia* against bacterial and microsporidian pathogens and parasites. ADAMO (2004) made similar observations in an insect host and reported that other immune parameters rather than PO predicted host survival.

Such a variation in immune response is not unexpected. Vertebrates are well known to adopt different immune defence mechanisms depending on the identity and the quantity of the pathogen they are exposed to (SHUDO & IWASA 2001). Our knowledge of the immune system of invertebrates is much less complete, but it is likely that they have similar mechanisms.

For this reason total haemocyte counts as a second immune parameter were investigated throughout my study. However, THC analysis provided a very similar picture to the results found for PO activity. Typically, crabs that were infected with parasites in natural populations

had neither a significantly higher nor a lower THC than uninfected crabs. Similar to PO activity, I can therefore conclude that THC does not confer resistance to parasitic castrators or helminth parasites. Nor does it appear to be involved in immune defence against them, which would be indicated by reduced values (HAUTON et al. 1997). Similar results were obtained in the experimental infections, which showed no connection between parasite infection and THC.

Contrary to PO data, THC measurements are available for a number of European populations, which would allow me to detect any population level effects. Such an adaptive increase in immunocompetence in response to high parasite pressure was observed in amphipods infected with a microphallid, *Maritema novaezealandensis* (BRYAN-WALKER et al. 2007; BATES et al. 2010). However, there were no significant differences in mean THC between European populations and, consequently, no correlation to parasite prevalence.

There was only one case where the presence - or rather the absence - of parasites was associated with an altered THC. In the first sample of crabs from Helgoland, THC in uninfected crabs was significantly lower than that of crabs infected with metacercariae of cystacanths, which were the predominant parasites in the area. Incidentally, this was also the only population sampled in August, while most other European samples were taken between late September and October. A second sampling of the same site in the following March confirmed a suspicion that seasonal effects may be involved, as the THC difference between crab groups disappeared in this second sample.

Due to the lack of supporting data from other populations, explanations for this phenomenon can only be speculative. One possible explanation is an external stressor that acts on this host-parasite system. In general, the impact of stressors is hard to estimate, as they can act on both host and parasite. A typical consequence, however, is a reduction in immunocompetence (LAFFERTY & HOLT 2003).

August is the time of highest water temperatures around Helgoland, adding temperature stress to crabs that may already be under stress due to the mating season that lasts from summer into early autumn (CROTHERS 1967). This may lead to an overall lower THC over summer compared to the cooler seasons. Heat stress can also amplify the risk of helminth infections (JENSEN & MOURITSEN 1992). For already infected crabs, it could therefore be adaptive to allocate more resources into immunocompetence to avoid further infections.

The lack of any supporting evidence for a role of THC in the immune reaction against helminths questions this hypothesis. Unfortunately it cannot be excluded that this isolated finding is an artefact caused by the low sample size for uninfected crabs in this area. But final

conclusions have to be withheld until further research on the influence of season and temperature on immune parameters provide us with more evidence.

Despite this possible exception, the overall trend clearly points to an at best marginal role of THC for resistance against parasites, under normal circumstances. Once again this is at odds with what we know about the immune reaction to bacterial pathogens, which have quite distinct consequences on THC in *C. maenas* and other crustaceans (HAUTON et al. 1997; LORENZON et al. 1999).

My own work supports this as reduced THC levels were observed in crabs with melanised structures in their body cavity, which were interpreted as a consequence of small scale infections that were deterred by the immune system. In addition, the bleeding of crabs in the course of the experimental infections also lead to a reduced THC level, which did not recover even after more than 7 weeks of regeneration time. These crabs were later found to exhibit melanised structures in proximity to the bleeding site as a consequence of wound healing, but probably also caused by contaminants and pathogens entering through the open wound. These findings stress the role of THC in different aspects of immune defence and also wound healing.

In other invertebrate hosts, THC is also clearly involved in the defence against parasites and parasitoids (YOSHINO & VASTA 1996). In noctuid Lepidoptera, infection with an insect parasitoid is accompanied by a significant drop in THC (PREVOST et al. 1990) while in drosophila, elevated THC levels provide resistance against another parasitoid (ESLIN & PREVOST 1996).

It is therefore surprising that parasitic infections in *C. maenas* do not elicit the degranulation and clotting of haemocytes which leads to the sudden THC decrease after bacterial infections (JOHANSSON et al. 2000). In addition, I very rarely encountered melanised parasites in the course of examining hundreds of infected crabs, even though melanised structures were not rare by themselves. These findings along with the results on PO activity suggest that the chosen immune parameters are not simply inappropriate for metazoan parasites in *C. maenas*, but that helminth parasites in the shore crab avoid the host immune system almost completely.

Of course, immune evasion is a major premise for the success of every endoparasite (LOKER 1994). Parasites can actively influence host defence, for example by releasing immunomodulatory substances (SHELBY et al. 2000), or by directly attacking cellular components of the immune system (UBELAKER et al. 1970). However, active immunosuppression results in a reduction in immune competence, which should be measurable. An example for this is the interaction between the crustacean *Gammarus pulex*

and acanthocephalan parasites of the genus *Pomphorhynchus*, where cystacanth infections result both in lowered PO activity and lowered THC values (RIGAUD & MORET 2003; CORNET et al. 2009). My results show that neither of those criteria is met in *C. maenas* infected with helminth parasites, making passive evasion more likely. Due to a lack of data regarding parasitic castrators, a similar conclusion cannot be drawn for this parasite group. However, both castrators, and *S. carcini* in particular, rely heavily on the health and well-being of their host. Immunosuppression increases the host's risk for secondary infections, resulting in great risk for these parasites (LOKER 1994). For these reasons passive evasion of the host immune system is also more likely for parasitic castrators.

Passive evasion can be achieved by molecular mimicry, either by mimicking host epitopes on the parasite surface or by integrating host proteins (Damian 1964). Unfortunately, the bulk of research on molecular mimicry has been conducted on vertebrates, most notably humans (e.g. ZAMBRANO-VILA et al. 2002). It is well known that helminths are capable of this evasion method, and MAIZELS & YAZDANBAKHSH (2003) reviewed the mechanisms they apply in the process. However, it is not clear if similar mechanisms are at work in invertebrate intermediate hosts and further research is very much needed to investigate this question.

Due to size and position of the invading stages of *S. carcini*, it is not possible to estimate the chance of a successful infection with this parasite once the cypris larva has entered the host. Melanised invasion stages would be very hard to spot during a typical dissection. The larval stages of microphallids and acanthocephalans, on the other hand, should be easily spotted in thorough examinations of the hepatopancreas and the digestive tract. The almost complete lack of melanised structures that could be identified as metacercariae or cystacanths (found in only one crab, pers. observation) points to a very high success rate and, in turn, to a high susceptibility of *C. maenas*.

The Red Queen hypothesis suggests that parasites and hosts are caught in a constant arms race, in which the parasite constantly optimizes its infection process to avoid the host immune system (DAWKINS & KREBS 1979). This leads to selection pressure on hosts resulting in turn in better host defences. The high susceptibility of *C. maenas* to microphallids and acanthocephalans may easily lead to the conclusion that these parasites result in little pathogenicity and consequently do not exert substantial selection pressure on this host. This is indeed a valid possibility, and the lack of evidence for fitness impacts in my study lends some support to it. Yet the development of resistance against a specific parasite depends on more factors than just pathogenicity, such as parasite density, trade-offs with other fitness parameters and genetic drift (FREEMAN & HERRON 2004).

It can, however, be said with considerable confidence that the parasites involved in my study do not cause indirect energy constraints on the host by eliciting a costly immune defence, or by reducing immunocompetence which would imply the risk of secondary infections.

4.2.2.2 Total protein concentration

Contrary to the total haemocyte counts and PO activity, total haemolymph protein is typically not associated with immune competence. Rather, haemocyanin as the largest protein component is supposed to act as a nutrient reserve and as a parameter for general physical fitness (HAGERMAN 1983; DEPLEDGE & BJERREGAARD 1989). However, total haemolymph protein is also closely associated with the survival of crickets infected with bacteria (ADAMO 2004). In *C. maenas* total haemolymph protein is typically a very variable parameter (UGLOW 1969a), an observation which was confirmed in my analyses, and which can potentially mask changes in protein level. However, despite this problem I observed interesting albeit on the first glance contradictory results, both in field samples and in experimental infections.

In the course of the experimental infections, crabs infected with metacercariae showed a significant decrease in total protein concentration over time, up to the last haemolymph sample 7 weeks post infection. In previous research, a diminished protein concentration in *C. Maenas* was a typical consequence of starvation (UGLOW 1969b). In *B. glabrata* snails, a reduced protein level was associated with both starvation and infections with the trematode *S. mansoni* (ONN LEE & CHENG 1972). It is therefore possible that parasite growth leads to nutritional stress for the host, which causes it to exploit the reservoir function of haemolymph proteins, mainly haemocyanin (DEPLEDGE & BJERREGAARD 1989).

A quite different result was obtained for the field samples. It was noticeable that red crabs had significantly higher values than green crabs in all cases. This adds to the already noticeable differences in life-history traits between the two colour morphs and may be caused by a better nutritional status. Two factors may add to this. On the one hand, red individuals have stronger chelae and can crush mussels and other prey more easily (KAISER et al. 1990). On the other hand, the reduction or complete cessation of growth in red crabs may free up resources otherwise bound for costly moult events (STYRISHAVE et al. 2004).

Additionally, there was also a difference between colour morphs in their reaction to helminth infection. While red crabs showed no alteration in total protein, green individuals infected with either microphallids or *P. botulus* had an increased protein concentration compared to uninfected crabs. This difference was not significant, but further analysis showed a significantly positive correlation between total protein and microphallid intensity. Sample size for cystacanths was too low to determine whether a similar effect occurs for this parasite.

This appears to be in direct opposition to the results for the experimental infections. However, it has to be noted that the two investigations examine different phases in a microphallid infection: the invasion and growth phase immediately following the infection event as opposed to the dormant stage of mature metacercariae present in the crabs which were sampled in the wild. It has already been established that the invasion of the host and the subsequent considerable growth are likely to be the most detrimental parts of parasitisation (GALAKTIONOV et al. 1996; JENSEN et al. 1998; FREDENSBORG et al. 2004). It is therefore conceivable that this phase leads to nutritional stress, explaining the reduced total protein levels in this phase.

Once the cercariae are fully encysted, they do not absorb nutrients from host tissue anymore and do not cause any tissue damage (GALAKTIONOV et al. 1996); nutritional stress should therefore be alleviated at this point. What then causes the increase in total protein with increasing numbers of metacercariae? Several authors have argued that antimicrobial peptides may play a role (ADAMO 2004; ANGELO et al. 2010).

Antimicrobial peptides are ubiquitous in vertebrates and invertebrates alike. As parts of the innate immune system they act against different types of pathogens, including parasites like *Plasmodium* and *Trypanosoma* (see ZAIOU 2007 for a review). In decapod crustaceans, including *C. maenas*, several antibacterial and antifungal peptides have been characterised (SCHNAPP et al. 1996; DESTOUMIEUX et al. 1997; KHOO et al. 1999). These peptides are typically stored in haemocytes, emphasizing the relevance of these cells for immunocompetence, although there are also examples of antimicrobial peptides in the haemolymph plasma of shrimp (DESTOUMIEUX-GARZÓN et al. 2001). Peptides may be released into the plasma upon contact with a pathogen. Unidentified proteins appearing in the haemolymph of bacterial infected *C. maenas* may lend some support to this hypothesis (HENKE 1985). In addition, it was shown that haemocytes of resistant snail strains produce significantly more peptides and protein when maintained in cell culture (YOSHINO & LODES 1988). Thus, an adaptive increase of immunocompetence in response to the presence of microphallid metacercariae may go along with an increase in total protein in *C. maenas*.

However, HENKE (1985) found no change in total protein in infected crabs, while another study found reduced concentrations in crabs with an active bacterial infection (SPINDLER-BARTH 1976). This is in line with my own observations in the experimental infections. DEPLEDGE & BJERREGAARD (1989) suggest that immunological reactions are generally unlikely to significantly lead to an increase in total protein concentration. PO release to the plasma could also contribute to an increased protein concentration. But I did not find increased PO levels in infected crabs from the field samples, so this explanation is unlikely. Antimicrobial peptides have not been shown to have any influence on metazoan parasites.

Overall, the increase of protein concentration in the haemolymph of infected *C. maenas* cannot conclusively be traced back to immunological reactions for these reasons.

It is therefore likely that we have to look into other possible explanations for the increase in total protein with metacercarial infection level. For example, it would be conceivable that the parasites themselves are the source of proteins that elevate the total haemolymph protein level. However, if this is the case we would expect to find the same elevated levels in crabs with the red colour morph.

On the other hand, crabs with high amounts of mature metacercariae could indeed have a better nutritional status, mirrored in their haemolymph protein concentration. This may be the case if infected crabs have longer activity times than uninfected ones (HAYE & OJEDA 1998). Even though such behaviour probably leads to higher predator susceptibility it could also result in more time spent foraging. This could be masked by the overall better nutritional status of red crabs, explaining the lack of any effect in this group. However, as previously discussed there is no indication for behavioural alterations in *C. maenas*.

4.2.3 Parasites and reproductive potential

There are a number of ways in which parasites can influence the reproduction of their host. Some parasites, for example schistosomes in their snail intermediate hosts, grow and replicate within the gonads and also feed on them (MINCHELLA & LOVERDE 1981). Other parasitic castrators use a more indirect approach, but the result on host reproduction is similar (BAUDOIN 1975). Nutrient competition by the parasite, pathological effects enforcing tissue regeneration, and costly defence mechanisms can cause resource constraints in the hosts, indirectly leading to reduced reproductive effort (POINAR & KURIS 1975; VIVARÈS & CUQ 1981; HENKE 1985; LAUCKNER 1986, 1987; HAYE & OJEDA 1998; HURD 2001; ZUK & STOEHR 2002; SCHMID-HEMPEL 2005).

Reduced reproductive effort is the most obvious and intuitively negative effect of parasites on host fitness, but not the only one. In fact, short-term increases in reproductive effort can also indicate fitness impacts of parasites. For some parasites, the impact on the host increases with time or is otherwise delayed. In such cases, life-history theory predicts that hosts are selected to minimize the fitness impacts of the parasite by increasing reproductive effort (FORBES 1993; AGNEW et al. 2000). An example for this is the snail *Biomphalaria glabrata*, which increases egg output upon infection with *Schistosoma mansoni*. In uninfected snails, this strategy leads to an overall fitness loss, but in infected snails it is a relative gain due to

the longer-term castrating effect of the parasites (MINCHELLA & LOVERDE 1981; THORNHILL et al. 1986).

As a consequence, alterations in reproductive effort in both directions can be indicators of a negative impact of parasites on the general fitness of their host. In my study, gonad weight was used as a parameter for reproductive effort, but parasite effects on this attribute were mainly limited to parasitic castrators.

4.2.3.1 Parasitic castrators and *C. maenas*

Host castration is a strategy employed by a number of parasites (BAUDOIN 1975), with several benefits for the castrating parasite. For example, host castration in *Daphnia magna* releases available resources for parasite reproduction and minimizes the risk of host mortality caused by the two-fold stress of parasitisation and reproduction (EBERT et al. 2004).

The most common parasitic castrators in crustaceans are the rhizocephalan cirripeds, which reap additional benefits from effectively castrating their host (HØEG et al. 2005): infected crabs are manipulated into cleaning and caring for the parasite instead of their own brood. Male hosts are feminized both in behaviour and in morphology, caring for the parasite in the same way the infected females do.

In *C. maenas*, *Sacculina carcini* is the predominant parasitic castrator, and it is known that this species causes degeneration of the androgenic gland, as well as inhibition of spermatogenesis (RUBILIANI-DUROZOI et al. 1980; RUBILIANI 1983). General morphological and behavioural feminisation of male crabs also occurs (HØEG 1995).

The effect of *S. carcini* on testes weight has not previously been studied. It is often assumed that infections lead to smaller or even completely reduced testes; however this has never been quantified. Our results show that testes weight is only reduced by an average of 12% in infested crabs, demonstrating that “parasitic sterilization” is a more appropriate term than the more commonly used “parasitic castration” (HØEG 1995). The reduction in testes weight also depends on how far the infection has proceeded, with mature parasites causing a stronger degeneration of the testes. Similar results were obtained for female crabs, which show substantially reduced ovary weight when infected with *S. carcini*.

However, feminisation of male crabs was not as pronounced as is usually described in the literature (WERNER 2001). In fact, only infected crabs from the eastern Limfjord and the Kattegat had a significantly broader abdomen than uninfected males, while no such difference could be observed in the other European areas. Most studies on *S. carcini* infections in the shore crab focus on crabs with externa, and consequently only deal with

mature parasites. In contrast, my study evaluated all crabs infected with this parasite which may explain the discrepancy in feminisation. Yet overall, strong feminisation was a much better predictor for a *P. maenadis* infestation than for a *S. carcini* parasite.

P. maenadis does not gain the same benefits from host castration and feminisation as *S. carcini*, as it has no external brood sac that requires care by the host. Yet it has an even more profound influence on host gonad weight. Both testes and ovaries are significantly reduced by about 20% in infected shore crabs. This is mirrored by my results regarding the feminisation of male crabs, which is decidedly more pronounced in individuals infected with *P. maenadis* compared to *S. carcini*.

Are there changes in life history strategy as a response to infection with a castrator, considering the severe impact of these parasites, most notably the more common *S. carcini*? Reports of such an effect on crustaceans exist, for example, in *D. magna* infected with a bacterial castrator, indicating that such adaptations are possible in crustaceans (EBERT et al. 2004). However, there appears to be no adaptive increase in reproductive effort at the onset of a *Sacculina* infection in *C. maenas*. Even crabs with young interna in the first infective stage show a reduced testes weight, indicating that the parasite begins to change host behaviour and physiology very early in the infection.

In *Biomphalaria*, even the mere contact with the castrating parasite is enough to trigger the switch to increased reproductive effort, which shows that a successful infection is not necessary (MINCHELLA & LOVERDE 1981). If a similar mechanism is at work in *C. maenas* a higher mean reproductive effort would be expected in uninfected crabs from populations with a high *Sacculina* prevalence. But this is not supported by my data, suggesting that *C. maenas* does not increase reproductive effort in an attempt to mitigate the consequences of *S. carcini* infection.

This is not without precedence. Obviously, organisms are limited in their ability to adapt both by environmental and genetic factors. An example for this can be found in crickets of the genus *Acheta*. These insects increases egg laying rate on contact with a deadly bacterium in order to minimize the fitness loss incurred by its imminent death (ADAMO 1999). However, the very same species does not react in the same way to infection with a parasitoid fly, even though it is also deadly.

The fact that no population level effects regarding the prevalence of *S. carcini* could be detected at all has a second implication. It suggests that there is no trade-off between reproduction and potentially costly defence mechanisms in high prevalence areas. Three different models can explain this. First, there is investment in defence mechanisms, but a resource trade-off has an impact on other life-history traits like survival or growth, rather than

reproduction (SCHWARZENBACH & WARD 2006). Second, the cost of defence may be negligible, which is indeed not uncommon (COUSTEAU et al. 2000; RIGBY et al. 2002; BROWN 2003). Third, there may be no investment in defence mechanisms and no subsequent resource trade-off, which seems to be the case in *C. maenas*, based on haemolymph data gained in my study.

4.2.3.2 Helminth parasites and *C. maenas*

While helminths, and trematodes in particular, can castrate their hosts, this is not typical for crustaceans and has never been reported in decapods. Indirect effects on reproductive effort are therefore what I expected to find in *C. maenas*.

While much of the research on the costs of parasitism and resource trade-offs involving defence mechanisms has been conducted on vertebrates, there are also several examples for invertebrates which confirm the theoretical model. In *Lymnea stagnalis*, higher resistance is coupled with a reduced breeding probability and lower egg numbers under certain circumstances (RIGBY & JOKELA 2000). Indian meal moths with a higher immune competence suffer from lower egg viability (BOOTS & BEGON 1993), and yellow dung flies that are subject to selection due to polyandry develop larger gonads but have to trade off immunocompetence for this benefit (HOSKEN 2001).

A cost to parasite resistance may not be universal or only detectable under very specific circumstances (COUSTEAU et al. 2000). Nevertheless, parasite infections have the potential to affect the reproductive success of hosts, although I found no support for this in *C. maenas*. Neither male nor female gonads were significantly smaller in crabs infected with either microphallids or the acanthocephalan *P. botulus*.

This extends to population level effects. In general, the reproduction index is highly variable over different sampling areas, indicating that it is modulated by environmental factors. However, it appears that the threat of parasite infection is not one of these. Unfortunately, the analysis was limited to male crabs, as the sample size for females over several populations was not sufficient. Mean testes weight was in no way significantly correlated with the prevalence of either microphallids or acanthocephalans.

Contrary to the parasitic castrators, the fitness impacts of the helminth parasites on *C. maenas* are largely unknown. However, as discussed earlier, increased gonad weight could be an indicator for adaptive life-history strategy changes (FORBES 1993; AGNEW et al. 2000). It follows that if helminths cause a future loss of reproductive potential, for example by incurring mortality at higher intensities, endangered shore crabs should increase their

reproductive effort in an attempt to offset this future loss. This could only be investigated for male crabs, but my data does indeed provide weak evidence for such an effect caused by microphallid trematodes. In crabs infected with very high doses of these parasites, there is a weak significant correlation between testes weight and intensity.

It has already been shown that invertebrates can modulate the adaptive response to future fitness losses based on parasite intensity. Male *Drosophila* invest more time and energy in courtship behaviour when infected with ectoparasitic mites which have a negative impact on longevity (POLAK & STARMER 1998). This response was shown to be dose-dependent and males scaled their mating behaviour according to the intensity of their mite infestation.

A similar mechanism may be at work in *C. maenas* infected with microphallids at high intensities. Unfortunately, sample size for this intensity range is comparatively small and it is therefore not possible to draw definitive conclusions. However, it is an interesting result and warrants more research.

4.2.3.3 Overall influence of parasites on *C. maenas* reproductive fitness

Overall, parasitic castrators and helminth parasites appear to lie on opposing ends of the spectrum with regards to their potential influence on host reproductive potential. *S. carcini*, which is the more common of the two castrators, reduces reproductive fitness of the host to zero. Prevalences of over 50% in some populations indicate that this parasite may indeed be crucial for *C. maenas* population dynamics. Additionally, in a similar crab-rhizocephalan system, both infected females and feminised males are chosen as mating partners by males, resulting in sterile mating (SHIELDS & WOOD 1993). It was therefore suggested that the true effect of this parasite on host populations is greater than would be expected based on prevalence alone (HØEG et al. 2005).

Clearly, the same cannot be inferred from my results on helminth impact on crab reproduction. But is the chosen parameter of gonad weight even appropriate to estimate reproductive effort and potential in *C. maenas*? This is an especially important question regarding males, which represent the majority of the examined crabs in this study. While ovary size is without doubt important for clutch size and thus reproductive success in females (D'UDEKEM-D'AKOZ 1994), it is not obvious that testes size is a similarly relevant factor for male reproductive effort.

However, studies on insects show that testes size can indeed be a measure of resource allocation to reproduction in invertebrates (GAGE 1994, 1995; HOSKEN 2001). A look at the life-history of *C. maenas* itself helps to further evaluate this question. European shore crabs

occur in two different colour morphs, green and red. These are intimately connected to the moult cycle. Freshly moulted crabs have a light green colour, which gradually turns to a deep red. This transformation is accompanied by a number of physiological and morphological changes in male crabs, and usually coincides with the mating season (WOLF 1998). This leads to the conclusion that the two morphs represent two different strategies, with red crabs forgoing further growth for an increased investment in reproduction (STYRISHAVE et al. 2004).

Red crabs benefit from stronger chelae and a thicker carapace (KAISER et al. 1990; MCGAW et al. 1992), which are advantageous in mate competition for females. Observational and experimental evidence has confirmed that red crabs do have a significant advantage over green individuals in gaining access to females (REID et al. 1997). STYRISHAVE et al. (2004) could show that these benefits are accompanied by significantly larger testes in red shore crabs, indicating that testes weight is an appropriate measure of reproductive effort.

Additionally, research on the two colour morphs also provides insight regarding resource trade-offs in *C. maenas*, because the benefits of red crabs in reproduction come at a price. They suffer from increased susceptibility to hypoxic stress and salinity changes (REID AND ALDRICH 1989; REID et al. 1989), which limits their ability to survive in diverse habitats. This supports strongly the hypothesis that reproduction is indeed subject to resource trade-offs in the shore crab.

As a consequence, the lack of robust evidence for an impact of non-castrating parasites on *C. maenas* could show that neither microphallids nor acanthocephalans have a noticeable influence on reproductive fitness of this host. This questions once again the potential of these ubiquitous parasites as a factor for host population dynamics. However, it is quite possible that a reduction in reproductive potential is not mirrored in gonad weight.

In males, success in mating competitions may be similarly or even more relevant for overall reproductive fitness (HOWARD & MINCHELLA 1990). For example, gregarine gut parasites significantly reduce the competitiveness of male *Libellula* dragonflies in a study by MARDEN & COBB (2004). Nematodes were found to have a similar effect in male *Drosophila testacea* flies (JAENIKE 1988). Male shore crabs do fight for access to females and have to guard receptive females for long periods of time. Any negative effect of parasites on physical fitness could therefore result in reduced mating success. This was confirmed when male *C. maenas* which suffered from chela loss were observed to have greatly diminished mating success (ABELLÓ et al. 1994; VAN DER MEEREN 1994). However, I have already established that limb loss - including chela loss - is not connected to parasite infection. I can therefore exclude this particular way in which parasites could potentially reduce crab reproductive fitness, but other behavioural alterations were beyond the scope of the present study.

Similar to this variety of factors influencing male reproductive success, mating success as well as egg and larval survival could be influenced by parasite infection in female crabs. Grass shrimp infected with microphallid trematodes provide an example in which egg mass was not affected by parasites, but other factors apparently were: ovigerous females were significantly less infected than non-ovigerous individuals (PUNG et al. 2002). It is therefore possible that other reproductive parameters are reduced, rather than gonad weight. However, my data agree well with what little we know about the effects of helminth on female reproduction in crabs. LATHAM & POULIN (2002a) studied fecundity of two decapod crab species, and also found no effect of acanthocephalan infestation on egg mass.

Life-history trade-offs do not necessarily result in reduced reproductive effort. SCHWARZENBACH & WARD (2006) showed that selection for high resistance in their study animal, the yellow dung fly, did not lead to decreased reproduction, but instead to reduced longevity under starvation. In *Drosophila*, increased THC results in better parasitoid resistance, but is accompanied by fitness loss in larval food competitions (KRAAIJEVELD et al. 2001). Such effects are almost impossible to detect in wild populations, but my data does provide evidence that there is no general trade off between reproductive effort, immunocompetence and general physiological constitution in male *C. maenas*. I found a weak but significantly positive correlation between testes weight, THC and total haemolymph protein. Protein concentration is a measure for the nutritional status of the crab individual, and it is not surprising that crabs with more available resources invest more in both immunocompetence and reproduction. But this also clearly indicates that immunocompetence is not traded off for reproduction. Further experiments on *C. maenas* may provide more insight in the interaction of parasites, host defences and the consequences for host fitness.

4.2.4 Other consequences of parasite infection for *C. maenas*

The parasitic castrator *S. carcini* is known to inhibit moulting in infected shore crabs. It is therefore expected that these crabs accumulate more epibionts compared to uninfected crabs (ABELLÓ & CORBERA 1996). This was confirmed in a study by MOURITSEN & JENSEN (2006), who found that *C. maenas* individuals with *Sacculina* externa were colonized over twice as often as uninfected ones. This could be partly confirmed in my study, with only a single sample site showing a discrepancy in epibiont prevalence between infected and uninfected crabs. Similarly, the moult inhibition results in a higher percentage of red colour morphs among infected crabs, which was also only observed in a single population. However, pooling populations showed that overall, red crabs are indeed more common

among *Sacculina* infested crabs, showing that the lack of findings in isolated populations was caused by low sample sizes.

The authors of the previous study attributed part of the difference in epibionts not only to the moulting inhibition, but also to behavioural alterations in infected crabs. These are less likely to burrow and are therefore more exposed to the larval stages of epibionts. If this is the case it may explain the lack of increased epibiont growth on infected crabs in many of my sample sites, as environmental conditions may not permit burrowing behaviour in all locations.

On the other hand, there was a clear correlation between *Sacculina* intensity (or age class) and epibiont abundance in all populations with a considerable castrator prevalence, indicating that the lack of statistically significant results on prevalence may be caused by insufficient sample sizes. MOURITSEN & JENSEN (2006) based their work solely on crabs with external cues indicating *Sacculina* infection, and thus mature parasites. They hypothesized that higher epibiont prevalences would also be found in crabs with *Sacculina* interna only.

My data show, for the first time, how epibiont infestation relates to different stages of the *Sacculina* infection. The increase in epibiont growth with the age of the parasite infection shows that moulting inhibition is mainly a characteristic of the mature parasite. While this goes against the suggestions of MOURITSEN & JENSEN (2006), it is not an unexpected result. *S. carcini* is, after all, known to feminize male host crabs. This includes morphological changes to the abdomen which require at least one moult, possibly more, to happen (HØEG 1995).

The degree of feminisation was not only investigated for male crabs and parasitic castrators, however, my data confirm that there is no hyperfeminisation in female crabs which are infected either by *S. carcini* or *P. maenadis*. As expected, the two types of helminth parasite have no influence on female abdomen width whatsoever.

4.3 The role of parasites for population dynamics and invasion success of *C. maenas*

As one of the most common invertebrates along the coasts of northern and western Europe, the European shore crab has always been the subject of extensive research. An increasing awareness of the dangers of invasive species has shifted much of the attention towards *C. maenas* as an important and exceptionally successful invader. Over the course of several centuries, the crab has colonized five continents and can now be found along both North American coasts, in South Africa, Australia and Japan (CARLTON & COHEN 2003), and most recently also in South America (HIDALGO et al. 2005).

The significantly negative impact of this invader on native invertebrate fauna, including bivalves and crustaceans of relevance for the fishery industry (ROPES 1968; MORGAN et al. 1980; MENGE 1983, 1985; TETTLEBACH 1986; GROSHOLZ 2000; GROSHOLZ & RUIZ 2002), has sparked an interest in the reasons for the overwhelming success of *C. maenas* as an invader (THRESHER 1997).

One of the most commonly invoked reasons for invasion success is the enemy release hypothesis (ERH, MACK et al. 2000), suggesting that a loss of natural enemies during the invasion process promotes invasion success. The ERH can be extended to parasites and pathogens, and indeed many studies have found evidence that invasive populations usually suffer less from these species than do native populations (TORCHIN et al. 2002; MITCHELL & POWER 2003; TORCHIN et al. 2003). Similarly, invaders with a greater release from pathogens are more likely to have harmful effects on the newly colonized environment, at least in plants (MITCHELL & POWER 2003).

ANDERSON & MAY (1978) were the first to show that macroparasites have the potential to regulate host populations, even if their impact on host fitness is usually not as pronounced as is the case for parasitic castrators. Experimental evidence both from wild and laboratory populations support these models (e.g. SCOTT 1987; HUDSON et al. 1998). Some parasites are also known to reduce host population density without the density-dependent effect that is required for true regulation (LAFFERTY 1993). At a community level, parasites can influence interactions between competitors as well as predators and prey (HATCHER et al. 2006). Where invasive hosts are affected by parasites with such effects, a release from them in the invasive habitats may facilitate increased invasion success.

4.3.1 Size increase in invasive *C. maenas* - a case of release from parasitic castrators?

TORCHIN et al. (2001) reported that *C. maenas* does indeed experience a loss of parasite diversity, prevalence and intensity in the newly colonized areas, and suggested that this parasite release is a major factor for the invasion success of this species. They supported this hypothesis with their observation of a negative correlation between mean size and catch per unit effort (CPUE) with the prevalence of parasitic castrators in the respective populations.

While *S. carcini* does prevent host crabs from moulting once the externa emerges, the mean size differences observed by TORCHIN et al. (2001) could not be explained solely by this effect. The authors could not convincingly exclude environmental factors influencing both

parasite prevalence and crab growth, but they suggested that the smaller crab size in populations with high *S. carcini* prevalence are likely to be caused by a cost of resistance that is proportional to parasite exposure, or by changes in life-history in uninfected crabs. Such a change in life-history could be earlier maturation in response to the potentially higher risk of later castration (JOKELA & LIVELY 1995), which could result in crabs entering terminal anecdysis at a smaller size.

My study now permits me to put these two hypotheses to the test. They predict higher investment in immunocompetence in populations threatened by parasitic castrators, or alternatively a higher reproductive effort in the same areas. However, neither of these predictions is supported by my data. Castrator prevalence has no effect on either mean THC level or mean reproduction index in the respective populations. This indicates that, while *S. carcini* absence may lead to regulatory release in invasive areas, compensatory release by a reduction in defence mechanisms is probably not happening in *C. maenas* (COLAUTTI et al. 2004). As previously discussed, the two parameters investigated in my study are not an exhaustive representation of all possible ways in which reproductive effort or immunocompetence may be influenced, and yet my results are a first indicator that the explanations offered by TORCHIN et al. (2001) may not be sufficient, and that the possible role of an environmental covariate should be considered with more seriousness.

In addition, even if the role of a parasitic castrator's prevalence on mean crab size is confirmed, it can only explain part of the size discrepancy between European and invasive crab populations. This discrepancy is present even for native *C. maenas* populations that are not affected by parasitic castrators (TORCHIN et al. 2001; GROSHOLZ & RUIZ 2003), so we have to look for other explanations. One such explanation can be found in latitude, which is typically lower in invasive populations and which is correlated with crab mean size (GROSHOLZ & RUIZ 2003). I also expected to find a possible further explanation in the presence of helminth parasites which are, in fact, more common than parasitic castrators in populations investigated for this study.

4.3.2 Can helminth parasites contribute to a parasite release effect?

Helminth parasites are often highly aggregated. In addition, they have complex life-cycles involving very mobile definitive hosts as well as first intermediate hosts. Due to these two characteristics, it is unlikely that the trophically transmitted helminths have a density-dependent effect resulting in regulation (LAFFERTY 1993; WILSON et al. 2002). However, negative impacts on fitness that could lead to a reduction in population density - either

directly or as a consequence of community interactions - could still be a factor that leads to parasite release in the invasive populations of *C. maenas*.

I found some supporting evidence for the negative impact of helminths on crab fitness. I could show that infestation with microphallid trematodes can in some cases lead to mortality in larger size classes. Yet there is little in the way of supplemental data backing this up. A compensatory increase in reproductive effort in very heavily infected crabs can be interpreted as an additional hint that mortality is caused by heavy infections, but sample sizes were too small for a definitive conclusion. A reallocation of resources into defence mechanisms in response to helminth parasites was limited to a single sample site and is likely to be a consequence of environmental stress. The experimental infections showed that the initial phase of microphallid invasion, movement and growth within the crab tissue leads to diminished haemolymph protein levels, indicating reduced physical fitness.

Overall, these results suggest that there are fitness impacts of helminth parasites in the weeks following the infection event. The long-term negative impacts of the encysted parasites, on the other hand, appear to be only relevant where environmental factors cause additional stress for the crabs. Also, these results appear to be mainly limited to microphallids, while *P. botulus* acanthocephalan infections did not lead to decreased fitness in the crabs.

This positive evidence, however, stands in contrast to my other results. Apart from the exceptions mentioned above, my data shows that there is typically no relationship between helminth infection and either reproductive effort or immunocompetence. This was found in chronic infections in the field samples as well as in the time immediately following experimental infection with microphallid trematodes. On the population level, increased helminth prevalence resulted neither in adaptive changes in immunocompetence, nor in a subsequent trade-off for reproductive effort.

As a result, my study provides limited support for negative impacts on host fitness caused by two types of helminth parasites in Europe. Mortality in *C. maenas* infected with high numbers of microphallids may contribute to reduced population density when parasitism coincides with secondary stressors. As larger and older crabs accumulate metacercariae and are removed from the population, this effect can also result in smaller mean crab size in populations where microphallids are very common. This shows that under certain circumstances, microphallid infection can contribute to the size differences observed between native and invasive populations of the European shore crab (TORCHIN et al. 2001; GROSHOLZ & RUIZ 2003). The loss of these parasites can therefore lead to regulatory release similar to the parasitic castrators (COLAUTTI et al. 2004), although the extent of this release remains unknown.

4.3.3 Immunocompetence and reproductive effort in invasive populations of *C. maenas*

My research also provides more insight into the differences between crabs in European and in invasive populations in relation to mean gonad weight as well as THC. However, only the reproductive index in females showed a consistent pattern in both invasive locations. It was significantly increased, indicating that females invest more in reproductive effort than in their native populations. This was not mirrored in male reproductive index. I observed no difference between Australian and European males, while testes weight in South Africa was even diminished. Results for THC analyses were also quite contradictory, with an increased value in South Africa and a decreased value in Australia, both compared to European crabs.

Along with the previously observed size increase in South Africa (TORCHIN et al. 2001), the changes in female gonads could also be a sign for compensatory release (COLAUTTI et al. 2004). This form of parasite release is a consequence of costly defence mechanisms employed by hosts in the native range. Due to the low sample size I cannot confirm that mean ovary weight in a given European population is influenced by parasite prevalence. However, the analysis of THC values suggests that crabs do not in fact invest in costly defence mechanisms. As a consequence, the changes in both size and relative ovary weight may be more readily explained by environmental benefits such as better resource availability or different temperature regimes that are a consequence of the latitude differences in invasive populations (GROSHOLZ & RUIZ 2003).

Nevertheless, the changes to female gonads are particularly interesting with regards to the invasion success of *C. maenas*. It adds to the already increased reproductive potential that is a consequence of the larger mean size of crabs in the invasive areas (THESSALOU-LEGAKI 1992; D'UDEKEM-D'AKOZ 1994; LATHAM & POULIN 2002a). Although recruitment mechanisms of *C. maenas* are not fully understood, female fecundity could be a key factor for the population dynamics of the crab. Together with the findings of TORCHIN et al. (2001), this provides more evidence that the European shore crab is more successful in invasive areas compared to its native habitat in Europe, even though this cannot conclusively be traced to the loss of parasites.

4.3.4 Parasite release depends on the origin of the invading individuals

There is an abundance of research, including the present study and the previously discussed paper by TORCHIN et al. (2001), showing that parasite prevalences are highly variable within the European native range of *C. maenas*. This does have serious implications for the

applicability of the parasite release hypothesis, because it indicates that the source of the invasive populations has to be taken into account. Depending on the parasite infestation at this source population, parasite release may be much less of a factor than what is suggested by impressive lists of the numerous parasite species found over the whole European home range. Yet until recently, very little was known about the invasion routes of the different *C. maenas* colonies in overseas. Two recent publications have set out to remedy this problem, using very different methodologies (DARLING et al. 2008; COMPTON et al. 2010).

DARLING et al. (2008) investigated genetic patterns in Europe and in invasive areas. They note that there is very little geographic structure within Europe, possibly due to gene flow caused by infrequent long-distance dispersal. For this reason their results on the origin of the primary invasions of *C. maenas* - Australia, South Africa and Atlantic North America - were not conclusive. They did find some evidence for a northern European origin of at least part of the founder population in South Africa. However, they could also show that in this area hybridisation with the closely related Mediterranean crab *C. aestuarii* is very likely. This sibling species was first recorded at a low percentage in Cape Town harbour by GELLER et al. (1997). However, a later survey (ROBINSON 2005), as well as the present study, found no trace of *C. aestuarii*, which can be easily explained by a hybridisation process.

Hybridisation may be a benefit for invasion success (ELLSTRAND & SCHIERENBECK 2006; HANFLING 2007), simply by providing more genetic diversity to invaders, which are typically considered to have poor genetic diversity as a consequence of founder effects and bottlenecks during establishment (VELLEND et al. 2007). In addition, this hybridisation complicates the search for the reasons for the invasion success of the European shore crab, as the effect of hybridisation on physiological and morphological traits - as well as the reactions to parasites - is not yet clear.

COMPTON et al. (2010) chose another approach with the aim of predicting the global range of *C. maenas*. While modelling the native range of the shore crab, they found that it does not accurately predict the current invasive distribution. However, they could show that separating populations into a northern and a southern European group based on genetic differences (ROMAN & PALUMBI 2004) gave much better results. They conclude that most invasions in areas with comparably warmer waters, including South Africa and Australia, have a southern European origin.

These results are partly at odds with the results obtained from genetic studies (DARLING et al. 2008), but if true it might be necessary to focus more on the parasite community of crabs in areas west of the genetic divide between Hoek van Holland and Bremerhaven (ROMAN & PALUMBI 2004). My study was limited to crabs of the northern subpopulation of *C. maenas*,

which showed no change in defence mechanisms or life-history trade-offs. Due to the genetic differences between the two subpopulations, however, it is not possible to extend these findings to crabs from southern Europe. These may have a more pronounced reaction to the presence of and infection by parasites. As a consequence, the rather limited evidence for parasite release in northern crabs cannot necessarily be attributed to the southern subpopulation. In any case, more details both on the origin of the primary invasions and on the parasites present in the origin population will be needed in the future to come to a better understanding of the relevance of parasite release for *C. maenas*.

4.3.5 The effect of native parasites on invasive shore crabs

Non-indigenous species not only lose parasites during the invasion process, they frequently also acquire new parasites native to the colonized areas (TOMPKINS & POULIN 2006). These cases are of particular interest, because host-parasite interactions can be profoundly different as both host and parasite are naive and lack a history of co-evolution (TARASCHEWSKI 2006). The lack of co-evolution can have different results.

On the one hand, native parasites may achieve higher fitness in the invasive host. As a consequence, the new host will serve as a reservoir, thereby possibly increasing parasite pressure on native hosts (RAUQUE et al. 2003). This can result in a competitive benefit for the invader, thereby promoting invasion success. On the other hand, the invasive host may be more susceptible and suffer from higher pathogenicity than the native hosts that have had time to develop suitable defence mechanisms (BRYAN-WALKER et al. 2007; BATES et al. 2010). This could slow down invasion considerably.

Australia including Tasmania, where two native trypanorhynch tapeworms and a nematode infect *C. maenas* (GURNEY et al. 2004; present study), offers the opportunity to test these two scenarios. Previous research observed apparently lower *C. maenas* abundance in areas where these parasites were particularly common, as well as pathological effects as a consequence of high trypanorhynch intensity in individual crabs (GURNEY 2006). Unfortunately, my attempts to sample different *C. maenas* populations in Port Phillip Bay, Victoria, Australia, were in large part unsuccessful. In fact, anecdotal reports from fishers and divers indicate that populations may generally be declining in the Bay. If the hypothesis of GURNEY (2006) holds true this might be caused by an infestation by native trypanorhynch parasites. However, the population I could sample had a very low prevalence and intensity of trypanorhynchs. Yet without further data on other areas of the Bay it cannot be concluded that parasites are too rare in this area to be responsible for the decline in crab populations. It is possible that the estuarine population sampled in my study was abundant specifically

because these parasites were absent from the location, maybe due to environmental factors like salinity fluctuations.

Despite the low parasite prevalence and intensity in the Australian sampling location, mean size in both males and females was considerably lower than in South Africa, and not significantly different from European populations. Once again, this questions the relevance of parasite release, at least for the Australian crab population. However, the estuarine habitat may lead to an overabundance of green colour morph crabs, which are usually smaller than red crabs and which may skew the results on mean crab size. Clearly, data from a single population is not enough to draw final conclusions on the Australian crabs generally. A closer investigation of the different invasion sites is imperative for the evaluation of invasion success and the role of parasites.

4.3.6 Implications for the control of invasive populations

Overall, the results of my study do not support the parasite release hypothesis for invasive populations of *C. maenas*. There is no indication for compensatory release, and the parasitic castrators appear to be the only parasites whose loss could lead to meaningful regulatory release (TORCHIN et al. 2001; COLAUTTI et al. 2004). In fact, most of my results show only very limited support to the hypothesis that the shore crab is more successful in the invasive populations compared to its native range. This hypothesis was based mostly on the observation of larger mean size of crabs, as this indicates either faster growth or longer survival (TORCHIN et al. 2001). I found that females benefit from the invasion, with a more distinct size increase and generally larger ovaries resulting in higher individual reproductive fitness. However, the other traits investigated in my study showed no consistent trend in the invasive populations and thus suggest that the crabs derive no benefit with regard to immunocompetence or male reproduction.

In any case, crab size is an important trait for the invaded ecosystems, as a larger size range increases the range of possible prey for *C. maenas*. Yet if parasites do indeed have a considerably negative effect on population dynamics within Europe, one would also expect an effect of parasite release on crab density. However, what little data there is on density does not support this prediction.

In fact, crab densities in South Africa appear to be very much at the lower end of the scale of densities observed in Europe, despite the benefit of bigger size and higher female fecundity. ROBINSON (2005) reports densities ranging from 0.03 to 0.06 crabs/m^2 in three different

sampling locations in South Africa. In contrast, MUNCH-PETERSEN et al. (1982) observed densities between 0.001 and 5.21/m^2 in Denmark, with a mean of 0.87/m^2 over seven populations using the same mark-recapture method as ROBINSON (2005). Similarly, other authors report densities of up to 2.5/m^2 in Europe (DARE & EDWARDS 1981; BAETA et al. 2005). In New England, *C. maenas* densities closely resemble values observed in Europe, ranging between 0.1/m^2 and 7.9/m^2 (GRIFFEN & DELANEY 2007). There are no similar reports from other invasive areas, but the data we have casts considerable doubt on the idea that *C. maenas* is, in fact, more successful in invaded areas.

This is surprising as both increased size and larger ovaries are known to increase fecundity in female crabs (THESSALOU-LEGAKI 1992; D'UDEKEM-D'AKOZ 1994; LATHAM & POULIN 2002a). However, while higher fecundity does increase individual fitness, this does not necessarily have consequences for population dynamics. *C. maenas* suffers from very high settlement mortality in megalopae and juvenile crabs (MOKSNES 2002), and the same probably applies to the planktonic larval stages. In addition, MOKSNES (2002) found that settlement mortality can be density dependant. In a later study, the same author found strong density-dependent mortality among juvenile *C. maenas* caused by cannibalism (MOKSNES 2004). In older crabs, intraspecific interference and aggression was found to be a limiting factor for crab densities in the invasive populations of New England (GRIFFEN & DELANEY 2007). As a consequence, larval and juvenile mortality, the drift of larvae with unfavourable currents, the availability of refuges for early benthic stages (THIEL & DERNEDDE 1994) and intraspecific competition may be more relevant for recruitment and population dynamics than female fecundity.

There is already a case of a host-parasite system that appears to confirm this hypothesis, in the invasive crab *Charybdis longicollis*. This portunid crab was introduced to the Mediterranean in the 1950s and spread rapidly along the coasts of the southern Mediterranean (HOLTHUIS 1961; LEWINSOHN & HOLTHUIS 1986). In 1986, a study found that this crab constitutes up to 70% of the benthic biomass in appropriate habitats (GALIL 1986). Shortly afterwards, a parasitic castrator from the native distribution of the crab, the cirriped *Heterosaccus dollfusi*, was introduced to the area and rapidly spread among the invasive range of its host (ØKSNEBJERG et al. 1997). The effects of this castrator are very similar to those of *S. carcini*, but although very high prevalences were reported, they had no observable effect on crab populations (GALIL & INNOCENTI 1999; GALIL & ZENETOS 2002).

The case study of this very similar system casts further doubt on the ability of parasitic castrators to regulate or reduce host population density, as well as the question of parasite release with regards to this specific group of parasites.

My results and the general observations on crab density discussed above also have implications for the possible control of invasive *C. maenas* populations. I suggest that parasites of adult crabs, while possibly leading to reduced individual fitness, do not significantly reduce population density in Europe. As a consequence, parasites of the native population that target adult crabs are probably not particularly suited for control measures in invasive areas. This observation is independent of possible additional problems of such control measures, such as specificity and issues regarding trophically transmitted parasites which require one or more additional hosts (THRESHER et al. 2000).

I hypothesize that larval and juvenile mortality contribute more to the population dynamics within Europe, which is supported by reports on density dependent predation as well as the occurrence of mass mortalities among these developmental stages (LAUCKNER 1986; MOKSNES 2002). Parasites or pathogens which specifically target juvenile crabs or even larvae could therefore be more promising in the search for a biological control agent.

One example of such a parasite is the parasitoid *Fecampia erythrocephala*, which infects juvenile crabs and kills them during its own development. It was suggested as a possible biological control tool by KURIS et al. (2002), but due to a general focus of *C. maenas* research on adult specimens, comparatively little is known about its distribution, abundance and impact on host population dynamics. More research into this platyhelminth could yield interesting results.

An alternative option for the control of the invasive shore crab population is commercial fisheries. The crabs are edible and are considered a local food in some areas of Europe. My sampling efforts in both Australia and South Africa never yielded native crabs, fisheries specifically targeting *C. maenas* are therefore a realistic option, if a demand could be created for such a product. An ongoing pilot project in Denmark is investigating the exploitation of the shore crab for sauces for Asian markets (FRANS HOYER, Dansk Skaldyrcenter, pers. comm.). If such a project proves to be profitable, commercial fisheries have the potential to significantly reduce crab populations, both in Europe (where it is considered a pest) and overseas.

4.4 Conclusions

My study shows that knowledge of the source population is crucial for an evaluation of the impact of parasite loss on invasion success in *C. maenas*. Nevertheless, even without extensive knowledge on the population genetics of native and invasive populations of this crab, my results offer very limited support for the parasite or enemy release hypothesis. I

found little evidence for a prominent role of parasites aside from the significant effect of parasitic castrators on individual crab fitness - an effect which is apparently not reflected in *C. maenas* demographics. Despite their great diversity and ubiquitous prevalence, a major role of helminth parasites for shore crab population dynamics is not supported by my data. I demonstrated that parasites are unlikely to be a deciding factor for the control of native crab populations. Rather, it is probable that multiple factors are responsible for population control, including chemical and physical environment properties as well as community interactions.

For all future measures against the invasive populations of the shore crab, more research on the population dynamics, both in its home range and in overseas colonies, will be invaluable. In addition, the effect of parasites native to the invasive areas is in dire need of more research, as such parasites have the potential to cause considerable harm to naive shore crab populations.

5 References

Abelló P & Corbera J (1996). Epibiont bryozoans (Bryozoa, Ctenostomatida) of the crab *Goneplax thomboides* (Brachyura, Goneplacidae) off the Ebro delta (western Mediterranean). *Miscellània Zoològica* **19**, 43-52.

Abelló P, Aagaard A, Warman CG & Depledge MH (1997). Spatial variability in the population structure of the shore crab *Carcinus maenas* (Crustacea: Brachyura) in a shallow-water, weakly tidal fjord. *Marine Ecology Progress Series* **147**, 97-103.

Abelló P, Warman CG, Reid DG & Naylor E (1994). Chela loss in the shore crab *Carcinus maenas* (Crustacea: Brachyura) and its effect on mating success. *Marine Biology* **121**, 247-252.

Adamo SA (1999). Evidence for adaptive changes in egg laying in crickets exposed to bacteria and to parasites. *Animal Behaviour* **57**, 117-124.

Adamo SA (2004). Estimating disease resistance in insects: phenoloxidase and lysozyme-like activity and disease resistance in the cricket *Grylus texensis*. *Journal of Insect Physiology* **50**, 209-216.

Adelung D (1971). Untersuchungen zur Häutungsphysiologie der dekapoden Krebse am Beispiel der Strandkrabbe *Carcinus maenas.* Habilitationsschrift, Universität Gießen.

Agnew P, Koella JC & Michalakis Y (2000). Host life history responses to parasitism. *Microbes and Infection* **2**, 891-896.

Albon SD, Stien A, Irvine RJ, Langvatn R, Ropstad E & Halvorsen O (2002). The role of parasites in the dynamics of a reindeer population. *Proceedings of the Royal Society of London B* **269**, 1625-1632.

Anderson RM & Gordon DM (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373-398.

Anderson RM & May RM (1978). Regulation and stability of host-parasite population interactions: I. Regulatory processes. *The Journal of Animal Ecology* **47**, 219-247.

Angelo C, Gôlo PS, Camargo MG, Kluck GEG, Folly E & Bittencourt VRE (2010). Haemolymph protein and lipid profile of *Rhipicephalus (Boophilus) microplus* infected by fungi. *Transboundary and Emerging Diseases* **57**, 79-83.

Armonies W & Hartke D (1995). Floating of mud snails *Hydrobia ulvae* in tidal waters of the Wadden Sea, and its implications in distribution patterns. *Helgoländer Meeresuntersuchungen* **49**, 529-538.

Armstrong S (1995). Rare plants protect Cape's water supplies. *New Scientist* **145**, 8.

Ash LR, Orihel TC & Savioli L (1994). *Bench aids for the diagnosis of intestinal parasites*. World Health Organization, Geneva.

Atkinson D & Sibly RM (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology and Evolution* **12**, 235-239.

Atkinson RJA & Parsons AJ (1973). Seasonal patterns of migration and locotomor rhythmicity in populations of *Carcinus*. *Netherlands Journal of Sea Research* **7**, 81-93.

Bachère E, Gueguen Y, Gonzalez M, de Lorgeril J, Garnier J & Romestand B (2004). Insights into the anti-microbial defense of marine invertebrates: the penaeid shrimps and the oyster *Crassostrea gigas*. *Immunological reviews* **198**, 149-168.

Baeta A, Cabral HN, Neto JM, marques JC & Pardal MA (2005). Biology, population dynamics and secondary production of the green crab *Carcinus maenas* (L.) in a temperate estuary. *Estuarine, Coastal and Shelf Science* **65**, 43-52.

Bakker TCM, Mazzi D & Zala S (1997). Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology* **78**, 1098-1104.

Bates AE, Poulin R & Lamare MD (2010). Spatial variation in parasite-induced mortality in an amphipod: shore height versus exposure history. *Oecologia* **163**, 651-659.

Baudoin M (1975). Host castration as a parasite strategy. *Evolution* **29**, 335-352.

Bauer A, Haine ER, Perrot-Minnot MJ & Rigaud P (2005). The acanthocephalan parasite *Polimorphus minutus* alters the geotactic and clinging behaviours of two sympatric amphipod hosts: the native *Gammarus pulex* and the invasive *Gammarus roeseli*. *Journal of Zoology* **267**, 39-43.

Behrens Yamada S & Hauck L (2001). Field identification of the European green crab species: *Carcinus maenas* and *Carcinus aestuarii*. *Journal of Shellfish Research* **20**, 905-912.

Benesh DP, Duclos LM & Nickol BB (2005). The behavioural response of amphipods harboring *Corynosoma constrictum* (Acanthocephala) to various components of light. *Journal of Parasitology* **91**, 731-736.

Berril M (1982). The life cycle of the green crab *Carcinus maenas* at the northern end of its range. *Journal of Crustacean Biology* **2**, 31-39.

Bertness MD (1981). Pattern and plasticity in tropical hermit crab growth and reproduction. *American Naturalist* **117**, 754-773.

Bethel WM & Holmes JC (1973). Altered evasive behavior and responses to light in amphipods harboring acanthocephalan cystacanths. *Journal of Parasitology* **59**, 945-956.

Bleil M (2006). Untersuchungen zur Laicherbestandskultur, zum Anteil aktiver Laicher, zu Laichaktivitäten und zur Reifeentwicklung von Dorschen in Beziehung zur hydrographischen Situation. Bericht der Bundesforschungsanstalt für Fischerei, Institut für Ostseefischerei Rostock.

Blumenthal DM (2006). Interactions between resource availability and enemy release in plant invasions. *Ecology Letters* **9**, 887-895.

Blumenthal DM, Mitchell CE, Pyšek P & Jarošík V (2009). Synergy between pathogen release and resource availability in plant invasions. *Proceedings of the National Academy of Sciences* **106**, 7899-7904.

Boots M & Begon M (1993). Trade-offs with resistance to a granulosis virus in the Indian mean moth. *Functional Ecology* **7**, 528-534.

Brattey J, Elner RW, Uhazy LS & Bagnall AE (1985). Metazoan parasites and commensals of five crab (Brachyuran) species from eastern Canada. *Canadian Journal of Zoology* **63**, 2224-2229.

Bright C (1999). Invasive species: pathogens of globalisation. *Foreign Policy* 1999, 51-64.

Brockerhoff AM & Smales LR (2002). *Profilicollis novaezelandensis* n. sp. (Polymorphidae) and two other acanthocephalan parasites from shore birds (Haematopodidae and Scolopacidae) in New Zealand, with records of two species in intertidal crabs (Decapoda: Grapsidae and Ocypodidae). *Systematic Parasitology* **52**, 55–65.

Brown JKM (2003). A cost of disease resistance: Paradigm or peculiarity? *Trends in Genetics* **19**, 667-671.

Bryan-Walker K, Leung TLF & Poulin R (2007). Local adaption of immunity against a trematode parasite in marine amphipod populations. *Marine Biology* **152**, 687-695.

Carlisle DB (1957). On the hormonal inhibition of moulting in decapod crustacea. II. The terminal anecdysis in crabs. *Journal of the Marine Biological Association of the UK* **36**, 291-307.

Carlton JT (1987). Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Science* **41**, 452-465.

Carlton JT (1989). Man's role in changing the face of the ocean: biological invasions and implications for conservation. *Conservation Biology* **3**, 265-273.

Carlton TC & Cohen AN (2003). Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. *Journal of Biogeography* **30**, 1809-1820.

Carney WP (1969). Behavioral and morphological changes in carpenter ants harboring dicrocoeliid metacercariae. *American Midland Naturalist* **82**, 605-611.

Cerenius L & Söderhäll K (2004). The prophenoloxidase-activating system in invertebrates. *Immunological Reviews* **198**, 116-126.

Cézilly F, Thomas F, Médoc V & Perrot-Minnot M-J (2010). Host-manipulation by parasites with complex life-cycles: adaptive or not? *Trends in Parasitology* **26**, 311-316.

Cheung TS (1966). The interrelations among three hormonal-controlled characters in the adult female shore crab, *Carcinus maenas* (L.). *The Biological Bulletin* **130**, 59-66.

Christiansen ME (1969). Crustacea Decapoda Brachyura. *Marine Invertebrates of Scandinavia* **2**, 49-51.

Clavero M & García-Berthou E (2005). Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution* **20**, 110.

Clay K (2003). Parasites lost. *Nature* **421**, 585-586.

Cohen AN, Carlton JT & Fountain MC (1995). Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. *Marine Biology* **122**, 225-237.

Colautti RI & MacIsaac HJ (2004). A neutral terminology to define 'invasive' species. *Diversity and Distributions* **10**, 135-141.

Colautti RI, Ricciardi A, Grigorovich IA & MacIsaac HJ (2004). Is invasion succcess explained by the enemy release hypothesis? *Ecology Letters* **7**, 721-733.

Compton TJ, Leathwick JR & Inglis GJ (2010). Thermogeography predicts the potential global range of the invasive European green crab (*Carcinus maenas*). *Diversity and Distribution* **16**, 243-255.

Cornet S, Franceschi N, Bauer A, Rigaud T & Moret Y (2009). Immune depression induced by acanthocephalan parasites in their intermediate crustacean host: Consequences for the risk of super-infection and links with host behavioural manipulation. *International Journal for Parasitology* **39**, 221-229.

Coustau C, Chevillon C & ffrench-Constant R (2000). Resistance to xenobiotics and parasites: Can we count the costs? *Trends in Ecology and Evolution* **15**, 378-383.

Crawley MJ (1986). The population biology of invaders. *Philosophical Transactions of the Royal Society of London B* **314**, 711-731.

Cribb TH (2005). Digenea (endoparasitic flukes). In: *Marine Parasitology*, pp. 76-86. Rohde K (Ed.). CSIRO Publishing, Collingwood.

Crothers JH (1967). The biology of the shore crab *Carcinus maenas* (L.): 1. The background - anatomy, growth and life history. *Field Studies* **2**, 407-434.

Crothers JH (1968). The biology of the shore crab *Carcinus maenas* (L.): 2. The life of the adult crab. *Field Studies* **2**, 579-614.

Cunningham AA & Daszak P (1998). Extinction of a species of land snail due to infection with a microsporidian parasite. *Conservation Biology* **12**, 1139-1141.

Damian RT (1964). Antigen sharing by parasite and host and its consequences. *The American Naturalist* **98**, 129-149.

Dare PJ & Edwards DB (1976). Experiments on the survival, growth and yield of relaid seed mussels (*Mytilus edulis* L.) in the Menai Straits, North Wales. *Journal du Conseil International pour l'Exploration de la Mer* **37**, 16-28.

Dare PJ & Edwards DB (1981). Underwater television observations on the intertidal movements of shore crabs, *Carcinus maenas*, across a mudflat. *Journal of the Marine Biological Association of the United Kingdom* **61**, 107-116.

Darling JA, Bagley MJ, Roman J, Tepolt CK & Geller JB (2008). Genetic patterns across multiple introductions of the globally invasive crab genus *Carcinus*. *Molecular Ecology* **17**, 4992-5007.

Davies MA (2009). *Invasion Biology*. Oxford University Press, Oxford.

Dawkins R & Krebs JR (1979). Arms races between and within species. *Proceedings of the Royal Society of London B* **205**, 489-511.

Depledge MG & Bjerregaard P (1989). Haemolymph protein composition and copper levels in decapod crustaceans. *Helgoländer Meeresuntersuchungen* **43**, 207-223.

Destoumieux D, Bulet P, Loew D, Van Dorsselaer A, Rogriguez J & Bachère E (1997). Penaeidins, a new family of antimicrobial peptides isolated from the shrimp *Penaeus vannamei* (Decapoda). *The Journal of Biological Chemistry* **272**, 28398-28406.

Destoumieux-Garzón D, Saulnier D, Garnier J, Jouffrey C, Bulet P & Bachère E (2001). Crustacean immunity. *The Journal of Biological Chemistry* **276**, 47070-47077.

DMU (2006). Salinity and water temperature data for the Limfjord. Danmarks Miljøundersøgelser (National Environmental Research Institute), University of Aarhus.

Dobson AP (1988). Restoring island ecosystems: the potential of parasites to control introduced mammals. *Conservation Biology* **2**, 31-39.

Döös K, Meler HEM & Döscher R (2004). The Baltic haline conveyor belt or the overturning circulation and mixing in the baltic. *Ambio* **33**, 261-266.

d'Udekem-d'Acoz C (1994). Activités reproductrices saisonnières des différentes classes de tailles d'une population de crabes verts Carcinus maenas (Linnaeus, 1758) dans le sud de la mer du Nord. *Cahiers de Biologie Marine* **35**, 1-13.

Ebert D, Carius HJ, Little T & Decaestecker E (2004). The evolution of virulence when parasites cause host castration and gigantism. *The American Naturalist* **164**, S19-S32.

Ebert D, Lipsitch M & Mangin KL (2000). The effect of parasites on host population density and extinction: Experimental epidemiology with *Daphnia* and six microparasites. *The American Naturalist* **156**, 459-477.

Ebling FJ, Kitching JA, Muntz L & Taylor CM (1964). The ecology of Lough Ine. XIII. Experimental destruction of *Mytilus edulis* and *Nucella lapillus* crabs. *Journal of Animal Ecology* **33**, 73-82.

Ellstrand NC & Schierenbeck KA (2006). Hybridization as a stimulus for the evolution of invasiveness in plants? *Euphytica* **148**, 35-46.

Elton CS (1958). *The ecology of invasions by plants and animals*. Methuen, London.

Eslin P & Prevost G (1996). Variation in *Drosophila* concentration of haemocytes associated with different ability to encapsulate *Asobara tabida* larval parasitoid. *Journal of Insect Physiology* **42**, 549-555.

Fenchel T (1975). Factors determining the distribution patterns of the mud snails (Hydrobiidae). *Oecologia* **20**, 1-17.

Forbes MRL (1993). Parasitism and host reproductive effort. *OIKOS* **67**, 444-450.

Forest J & Gantes H (1960). Sur une collection de Crustaces Decapodes marcheurs du Maroc. *Bulletin of the Museum National d'Histoire Naturelle (Paris) Ser. 2* **32**, 346-358.

Fredensborg BL, Mouritsen KN & Poulin R (2004). Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda: Crustacea) infected by a trematode: experimental

infections and field observations. J*ournal of Experimental Marine Biology and Ecology* **311**, 253-265.

Freeman S & Herron JC (2004). *Evolutionary analysis*. Pearson Prentice Hall, Upper Saddle River.

Fulton SW & Grant FE (1900). Note on the occurrence of the European crab, *Carcinus maenas*, Leach, in Port Phillip. *Victorian Naturalist* **27**, 147.

Gage MJG (1994). Associations between body size, mating pattern, testis size and sperm lengths across butterflies. *Proceedings of the Royal Society of London B* **258**, 247-254.

Gage MJG (1995). Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proceedings of the Royal Society of London B* **261**, 25-30.

Galaktionov KV, Malkova II, Irwin SWB, Saville DH & Maguire JG (1996). Developmental changes in the tegument of four microphallid metacercariae in their second (crustacean) intermediate hosts. *Journal of Helminthology* **70**, 201-210.

Galil BS & Innocenti G (1999). Notes on the population structure of the portunid crab *Charybdis longicollis* Leene, parasitized by the rhizocephalan *Heterosaccus dollfusi* Boschma, off the Mediterranean coast of Israel. *Bulletin of Marine Science* **64**, 451-463.

Galil BS & Zenetos A (2002). A sea change - exotics in the eastern mediterranean. In: *Invasive aquatic species of Europe: distribution, impacts and management*, pp. 325-336. Leppäkoski E et al. (Eds.). Kluwer Academic, Dordrecht.

Galil BS (1986). Red Sea decapods along the Mediterranean coast of Israel: ecology and distribution. In: *Environmental Quality and ecosystem Stability, vol. IIIA/B*, pp. 179-183. Dubinski Z & Steinberger Y (Eds.). Bar-ilan University Press, Ramat Gan.

Gardner NC, Kwa S & Paturusi A (1994). First recording of the European shore crab *Carcinus maenas* in Tasmania. *Tasmanian Naturalist* **116**, 26-28.

Geller JB, Walton ED, Grosholz ED & Ruiz GM (1997). Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Molecular Ecology* **6**, 901-906.

Giard A (1887). Parasitic castration and its influence upon the external characters of the male sex, in the decapod crustaceans. *Annals and Magazine of Natural History Series* **19**, 5, 325-345.

Glenner H (2001). Cypris Metamorphosis, injection and earliest internal development of the Rhizocephalan *Loxothylacus panopaei* (Gissler). Crustacea: Cirripedia: Rhizocephala: Sacculinidae. *Journal of Morphology* **249**, 43-75.

Glude JB (1955). The effects of temperature and predators on the abundance of the soft-shell clam, *Mya arenaria*, in New England. *Transactions of the American Fisheries Society* **84**, 13-26

Griffen BD & Delaney DG (2007). Species invasion shifts the importance of predator dependence. *Ecology* **88**, 3012-3021.

Griffiths RW, Schloesser DW, Leach JH & Kovalak WP (1991). Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 1381-1388.

Grosholz ED & Ruiz GM (1995). Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. *Marine Biology* **122**, 239-247.

Grosholz ED & Ruiz GM (2002). Management plan for the European green Crab, submitted to the Aquatic Nuisance Species Task Force.

Grosholz ED & Ruiz GM (2003). Biological invasions drive size increases in marine and estuarine invertebrates. *Ecology Letters* **6**, 700-705.

Grosholz ED (2000). The impacts of a nonindigenous marine predator in a California bay. *Ecology* **81**, 1206-1224.

Grosholz ED (2002). Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology & Evolution* **17**, 22-27.

Gurney RH (2006). Potential biological control agents for the European green crab, *Carcinus maenas*, in Australian waters. PhD thesis, University of Tasmania.

Gurney RH, Nowak BF, Dykova I & Kuris AM (2004). Histopathological effects of trypanorhynch metacestodes in the digestive gland of a novel host, Carcinus maenas (Decapoda). *Diseases of Aquatic Organisms* **58**, 63-69.

Hagerman L (1983). Haemocyanin concentration of juvenile lobsters (*Homarus gammarus*) in relation to moulting cycle and feeding conditions. *Marine Biology* **77**, 11-17.

Hampton SL & Griffiths CL (2007). Why *Carcinus maenas* cannot get a grip on South Africa's wave-exposed coastline. *African Journal of Marine Science* **29**, 123-126.

Hanfling B (2007). Understanding the establishment of non-indigenous fishes: lessons from population genetics. *Journal of Fish Biology* **71**, 115-135.

Harcourt AH, Harvey PH, Larson SG & Short RV (1981). Testis weight, body weight and breeding system in primates. *Nature* **293**, 55-57.

Hartnoll RG (2001). Growth in crustacea - twenty years on. *Hydrobiologia* **449**, 111-122.

Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, Hofmann EE, Lip EK, Osterhaus ADME, Overstreet RM, Porter JW, Smith GW, Vasta GR (1999) Emerging marine diseases - climate links and anthropogenic factors. *Science* **285**,1505–1510.

Hatcher MJ, Dick JTA & Dunn AM (2006). How parasites affect interactions between competitors and predators. *Ecology Letters* **9**, 1253-1271.

Hauton C, Williams JA & Hawkins LE (1997). The effects of a live in vivo pathogenic infection on aspects of the immunocompetence of the common shore crab, *Carcinus maenas* (L). *Journal of Experimental Marine Biology and Ecology* **211**, 115-128.

Haye PA & Ojeda FP (1998). Metabolic and behavioural alterations in the crab *Hemigrapsus crenulatus* (Milne-Edwards 1837) induced by its acanthocephalan parasite *Profilicollis antarcticus* (Zdzitowiecki 1985). *Journal of Experimental Marine Biology and Ecology* **228**, 73-82.

Helluy S (1983). Relations hôtes-parasite du trématode *Microphallus papillorobustus* (Rankin, 1940). II - Modifications du comportement des *Gammarus* hôtes intermédiaires et localisation des métacercaires. *Annales de Parasitologie Humaine et Comparée* **58**, 1-17.

Helluy S (1984). Relations hôtes-parasite du trématode *Microphallus papillorobustus* (Rankin, 1940). III - facteurs impliqués dans les modifications du comportement des *Gammarus* hôtes intermédiaires et tests de prédation. *Annales de Parasitologie Humaine et Comparée* **59**, 41-56.

Henke GA (1985). Variations of hemolymph proteins of the common shore crab *Carcinus maenas* after a bacterial infection. *Marine Biology* **87**, 181-183.

Hidalgo FJ, Barón PJ & Orensanz JM (2005). A prediction come true. The green crab invades the Patagonian coast. *Biological Invasions* **7**, 547-552.

Høeg JT (1995). The biology and life cycle of the Rhizocephala (Cirripedia). *Journal of the Marine Biological Association of the United Kingdom* **75**, 517-550.

Høeg JT, Glenner H & Shields JD (2005). Cirripedia Thoracica and Rhizocephala (barnacles). In: *Marine Parasitology,* pp. 154-165. Rohde K (Ed.). CSIRO Publishing, Collingwood.

Holthuis LB (1961). Report on a collection of Crustacea Decapoda and Stomatopoda from Turkey and the Balkans. *Zoologische Verhandelingen (Leiden)* **47**, 1-67.

Hosken DJ (1997). Sperm competition in bats. *Proceedings of the Royal Society of London B* **264**, 385-392.

Hosken DJ (2001). Sex and death: microevolutionary trade-offs between reproductive and immune investment in dung flies. *Current Biology* **11**, R379-R380.

Howard RD & Minchella DJ (1990). Parasitism and mate competition. *Oikos* **58**, 120-122.

Hudson PJ & Dobson AP (1995). Macroparasites: observed patterns. In: *Ecology of infectious diseases in natural populations,* pp. 144-176. Greenfell BT & Dobson AP (Eds.). Cambridge University Press, Cambridge.

Hudson PJ, Dobson AP & Newborn D (1998). Prevention of population cycles by parasite removal. *Science* **282**, 2256-2258.

Hudson PJ, Rizzoli AP, Grenfell BT, Heesterbeek JAP & Dobson AP (2002). The Ecology of Wildlife Diseases. In: *The Ecology of Wildlife Diseases,* pp. 1-5. Hudson PJ et al. (Eds.). Oxford University Press, Oxford.

Hunter E & Naylor E (1993). Intertidal migration by the shore crab *Carcinus maenas*. *Marine Ecology Progress Series* **101**, 131-138.

Hurd H (2001). Host fecundity reduction: a strategy for damage limitation? *Trends in Parasitology* **17**, 363-368.

Jaenike J (1988). Parasitism and male mating success in *Drosophila testacea*. *The American Naturalist* **131**, 774-780.

Jensen KT & Mouritsen KN (1992). Mass mortality on two common soft-bottom invertebrates, *Hydrobia ulvae* and *Corophium volutator* - the possible role of trematodes. *Helgoländer Meeresuntersuchungen* **46**, 329-339.

Jensen KT, Ferreirea SM & Pardal MA (2004). Trematodes in a *Cyathura carinata* population from a temperate intertidal estuary: infection patterns and impact on host. *Journal of the Marine Biological Association of the United Kingdom* **84**, 4647.1-4647.8.

Jensen T, Jensen KT & Mouritsen KN (1998). The influence of the trematode *Microphallis claviformis* on two congeneric intermediate host species (*Corophium*): infection characteristics and host survival. *Journal of Experimental marine Biology and Ecology* **227**, 35-48.

Johansson MW, Keyser P, Sritunyalucksana K & Söderhäll K (2000). Crustacean haemocytes and haematopoiesis. *Aquaculture* **191**, 45-52.

Johnson MW (1957). The copepod *Chioniosphaera cancrorum* parasitizing a new host, the green crab *Carcinus maenas*. *Journal of Parasitology* **43**, 470-473.

Jokela J & Lively CM (1995). Parasites, sex and early reproduction in a mixed populations of freshwater snails. *Evolution* **49**, 1268-1271.

Kaiser MJ, Hughes RN & Reid DG (1990). Chelal morphometry, prey size selection and aggressive competition in green and red forms of *Carcinus maenas*. *Journal of Experimental Marine Biology* **140**, 121-134.

Kashenko SD & Korn OM (2002). Effects of temperature and salinity on the larvae of two species of Rhizocephalan (Crustacea: Cirripedia). *Invertebrate Reproduction and Development* **41**, 179-184.

Key MM Jr., Jeffries WB, Voris HK & Yang CM (1996). Epizoic bryozoans, horseshoe crabs, and other mobile benthic substrates. *Bulletin of Marine Science* **58**, 368-384.

Keymer A (1981). Dynamics of *Hymenolepis diminuta* in the intermediate host. *Journal of Animal Ecology* **50**, 941-950.

Khoo L, Robinette DW & Noga EJ (1999). Callinectin, an antibacterial peptide from blue crab, *Callinectes sapidus*, hemocytes. *Marine Biotechnology* **1**, 44-51.

Klein-Breteler WCM (1975). Laboratory experiments on the influence of environmental factors on the frequency of moulting and the increase in size at moulting in juvenile shore crabs, *Carcinus maenas*. *Netherlands Journal of Sea Research* **9**, 100-120.

Kraaijeveld AR & Godfray HCJ (1997). Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature* **389**, 278-280.

Kraaijeveld AR, Limentani EC & Godfray HCJ (2001). Basis of the trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Proceedings of the Royal Society of London B* **268**, 259-261.

Kuris AM, Lafferty KD & Torchin ME (2005). Biological control of the European Green Crab, *Carcinus maenas*: Natural enemy valuation and analysis of host specificity. *Second International Symposium on Biological Control of Arthropods*, 102-115.

Kuris AM, Torchin ME & Lafferty KD (2002). *Fecampia erythrocephala* rediscovered: prevalence and distribution of a parasitoid of the European shore crab, *Carcinus maenas*. *Journal of the Marine Biological Association of the UK* **82**, 955-960

Lack D (1954). *The natural regulation of animal number*. Clarendon Press, Oxford.

Lafferty KD & Holt RD (2003). How should environmental stress affect the population dynamics of disease? *Ecology Letters* **6**, 654-664.

Lafferty KD & Kuris AM (1996). Biological control of marine pests. *Ecology* **77**, 1989-2000.

Lafferty KD & Kuris AM (1999). How environmental stress affects the impacts of parasites. *Limnology and Oceanography* **44**, 925-931.

Lafferty KD (1993). Effects of parasitic castration on growth, reproduction and population dynamics of the marine snail *Cerithidea californica*. *Marine Ecology Progress Series* **96**, 229-237.

Latham ADM & Poulin R (2001). Effect of acanthocephalan parasites on the behaviour and coloration of the mud crab *Macrophthalmus hirtipes* (Brachyura: Ocypodidae*). Marine Biology* **139**, 1147-1154.

Latham ADM & Poulin R (2002a). Field evidence of the impact of two acanthocephalan parasites on the mortality of three species of New Zealand shore crabs (Brachyura). *Marine Biology* **141**, 1131–1139.

Latham ADM & Poulin R (2002b). Effect of acanthocephalan parasites on hiding behaviour in two species of shore crabs. *Journal of Helminthology* **76**, 323–326.

Lauckner G & Söhl M (1990). Populationsdynamik parasitengeschädigter Strandkrabben (*Carcinus maenas*) im Nordsylter Wattenmeer. *Jahresbericht der Biologischen Anstalt Helgolands*, 19-22.

Lauckner G (1986). Einfluss von Trematodeninfestationen auf Überleben und Wachstum der Strandkrabbe *Carcinus maenas*. *Jahresbericht der Biologischen Anstalt Helgolands*, 11-12.

Lauckner G (1987). Effects of parasites on juvenile Wadden Sea invertebrates. In: *Proceedings of the 5th International Wadden Sea Symposium*, pp. 103-212. Tougaard S & Esbjerg AS (Eds.). The National Forest and Nature Agency and the Museum of Fisheries and Shipping.

Le Moullac G & Haffner P (2000). Environmental factors affecting immune responses in Crustacea. *Aquaculture* **191**, 121-131.

Le Moullac G, Soyez C, Saulnier D, Ansquer D, Avarre JC & Levy P (1998). Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Pennaeus stylirostris*. *Fish and Shellfish Immunology* **8**, 621-629

Le Roux PJ, Branch GM & Joska MAP (1990). On the distribution, diet and possible impact of the invasive European shore crab *Carcinus maenas* (L.) along the South African coast. *South African Journal of Marine Science* **9**, 85-93.

Lee KT, McKnight A, Kellogg K & Juanes F (2003). Salinity tolerance in color phases of female green crabs, *Carcinus maenas* (Linnaeus, 1758). *Crustaceana* **76**, 247-253.

Lester RJG (2005). Cirripedia Isopoda (isopods). In: *Marine Parasitology,* pp. 138-144. Rohde K (Ed.). CSIRO Publishing, Collingwood.

Lewinsohn C & Holthuis LB (1986). The Crustacea Decapoda of Cyprus. *Zoologische Verhandelingen (Leiden)* **230**, 1-64.

Liat LB & Pike AW (1980). Incidence and distribution of *Profilicollis botulus* (Acanthocephala), in the eider duck, *Somateria mollissima*, and in its intermediate host the shore crab, *Carcinus maenas*, in northeast Scotland. *Journal of Zoology* **190**, 39-51.

Lillebø AI, Pardal MÂ & Marques JC (1999). Population structure, dynamics and production of *Hydrobia ulvae* (Pennant) (Molluscae: Prosobranchia) along an eutrophic gradient in the Mondego estuary (Portugal). *Acta Oecologica* **20**, 289-304.

Liu H, Jiravanichpaisal P, Cerenius L, Luel Lee B, Söderhäll I & Söderhäll C (2007). Phenoloxidase is an important component of the defense against *Aeromonas hydrophila* infection in a crustacean, *Pacifastacus leniusculus*. *The Journal of Biological Chemistry* **282**, 33593-33598.

Lockwood S, Hoopes M & Marchetti M (2007). *Invasion Ecology*. Blackwell Publishing, Oxford.

Loker ES (1994). On being a parasite in an invertebrate host: a short survival guide. *The Journal of Parasitology* **80**, 728-747.

Lorenzon S, de Guarrini S, Smith VJ & Ferrero EA (1999). Effects of LPS injection on circulating haemocytes in crustaceans *in vivo*. *Fish and Shellfish immunology* **9**, 31-50.

Lowe S, Browne M, Boudjelas S & De Poorter M (2004). 100 of the World's Most Invasive Alien Species. A selection from the Global Invasive Species database. Published by the Invasive Species Specialist Group (ISSG) of the IUCN.

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M & Bazzaz FA (2000). Biotic invasions: causes, epidemiology, global consequences and control. *Ecological Applications* **10**, 689-710.

Maizels RM & Yazdanbakhsh M (2003). Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology* **3**, 733-744.

Marden JH & Cobb JR (2004). Territorial and mating success of dragonflies that vary in muscle power output and presence of grgarine gut parasites. *Animal Behaviour* **68**, 657-665.

Mathews LM, McKnight AE, Avery R & Lee KT (1999). Incidence of autotomy in New England populations of green crabs, *Carcinus maenas*, and an examination of the effect of claw autotomy on diet. *Journal of Crustacean Biology* **19**, 713-719.

Mathieson S, Berry AJ & Kennedy S (1998). The parasitic rhizocephalan barnacle *Sacculina carcini* in crabs of the Forth Estuary, Scotland. *Journal of the Marine Biological Association of the UK* **78**, 665-667.

May RM & Anderson RM (1978). Regulation and stability of host-parasite population interactions: II. Destabilizing processes. *The Journal of Animal Ecology* **47**, 249-267.

McGaw IJ, Kaiser MJ, Naylor E & Hughes RN (1992). Intraspecific morphological variation related to the moult-cycle in colour forms of the shore crab *Carcinus maenas*. *Journal of Zoology* **228**, 351-359.

McGaw IJ & Naylor E (1992). Salinity preference of the shore crab *Carcinus maenas* in relation to coloration during intermoult and to prior acclimation. Journal of Experimental Marine Biology and Ecology **155,** 145-159.

McKnight AE, Mathews LM, Avery R & Lee KT (2000). Distribution is correlated with colour phase in green crabs, *Carcinus maenas* (Linnaeus, 1758) in southern New England. *Crustaceana* **73**, 763-768

McNeil WH (1976). *Plagues and People*. Monticello, New York.

McVean A & Findlay I (1979). The incidence of autotomy in an estuarine population of the shore crab *Carcinus maenas*. *Journal of the Marine Biological Association of the United Kingdom* **59**, 341-354.

McVean A (1976). The incidence of autotomy in *Carcinus maenas* (L.). *Journal of Experimental Marine Biology and Ecology* **24**, 177-187.

Meißner K & Bick A (1999). Mortality of *Corophium volutator* (Amphipoda) caused by infection with *Maritrema subdolum* (Digenea, Microphallidae) - laboratory studies. *Diseases of Aquatic Organisms* **35**, 47-52.

Menge BA (1983). Components of predation intensity in the low zone of the New England rocky intertidal region. *Oecologia* **58**, 141-155.

Menge BA (1985). Indirect effects in marine rocky intertidal interaction webs: patterns and importance. *Ecological monographs* **65**, 12-74.

Mills EL, Leach JH, Carlton JT & Secor CL (1994). Exotic species and the integrity of the Great Lakes: lessons from the past. *BioScience* **44**, 666-676.

Minchella DJ & Loverde PT (1981). A cost of icnreased early reproductive effort in the snail *Biomphalaria glabrata*. *The American Naturalist* **118**, 876-881.

Mitchell CE & Power AG (2003). Release of invasive plants from fungal and viral pathogens. *Nature* **421**, 625-627.

Mohamedeen H & Hartnoll RG (1989a). The effect of variation in temperature and food supply on the growth of post-larval *Carcinus maenas*. In: *Proceedings of the 21st European Marine Biology Symposium, Gdansk, 14–19 September, 1986, Poland*, pp. 115-122. Klekowski RZ, Styczynska-Jurewicz E & Falkowski L (Eds.). Polish Academy of Sciences, Wroclaw.

Mohamedeen H & Hartnoll RG (1989b). Larval and postlarval growth of individually reared specimens of the common shore crab *Carcinus maenas*. *Journal of Experimental Marine Biology and Ecology* **134**, 1-24.

Moksnes PO (2002). The relative importance of habitat-specific sttlement, predation and juvenile dispersal for distribution and abundance of young juvenile shore crabs *Carcinus maenas* L. *Journal of Experimental Marine Biology and Ecology* **271**, 41-73.

Moksnes PO (2004). Self-regulating mechanisms in cannibalistic populations of juvenile shore crabs *Carcinus maenas*. *Ecology* **85**, 1343-1354.

Moore J (2002). *Parasites and the Behaviour of Animals*. Oxford University Press, Oxford.

Morgan DE, Goodsell J, Mathiessen GC, Garey J & Jacobson P (1980). Release of hatchery-reared bay scallops (*Argopecten irradians*) onto a shallow coastal bottom in Waterfor, Connecticut. *Proceedings of the World Mariculture Society* **11**, 247-261.

Mouritsen KN & Jensen KT (1997). Parasite transmission between soft-bottom invertebrates: temperature mediated infection rates and mortality in *Corophium volutator*. *Marine Ecology Progress Series* **151**, 123-134.

Mouritsen KN & Jensen KT (2006). The effect of *Sacculina carcini* infections on the fouling, burying behaviour and condition of the shore crab, *Carcinus maenas*. *Marine Biology Research* **2**, 270-275.

Mouritsen KN (2002). The *Hydropbia ulvae-Maritrema subdolum* association: cercarial emergence controlled by host activity. *Journal of Helminthology* **76**, 349-353.

Mouritsen KN, Jensen T & Jensen KT (1997). Parasites on an intertidal Corophium-bed: factors determining the phenology of microphallid trematodes in the intermediate host populations of the mud snail *Hydrobia ulvae* and the amphipod *Corophium volutator*. *Hydrobiologica* **355**, 61-70.

Mucklow PT, Vizoso DB, Jensen KH, Refardt D & Ebert D (2004). VAriation in phenoloxidase activity and its relation to parasite resistance within and between two populations of *Daphnia magna*. *Proceedings of the Royal Society of London B* **271**, 1175-1183.

Munch-Petersen S, Sparre P & Hoffmann E (1982). Abundance of the shore crab, *Carcinus maenas* (L.), estimated from mark-recapture experiments. *Dana* **2**, 97-121.

Muntz L, Ebling FJ & Kitching JA (1965). The ecology of Lough Ine. XIV. Predatory activity of large crabs. *Journal of Animal Ecology* **34**, 315-329.

Nigam Y, Maudlin I, Welburn S & Ratcliffe NA (1997). Detection of phenoloxidase activity in the hemolympf of tsetse flies, refractory and susceptible to infection with *Trypanosoma brucei rhodesiense*. *Journal of Invertabrate Pathology* **69**, 279-281.

Øksnebjerg B (2000). The Rhizocephala (Crustacea: Cirripedia) of the Mediterranean and Black seas: taxonomy, biogeography, and ecology. *Israel Journal of Zoology* **46**, 1-102.

Øksnebjerg B, Enzenross R & Enzenross L (1997). First record of Rhizocephala (Crustacea, Cirripedia) from Turkish waters, with notes on lessespian migration. *Stuttgarter Beiträge zur Naturkunde (Biologie)* **557**, 1-7.

Onn Lee F & Cheng TC (1972). *Schistosoma mansoni*: Alterations in total protein and hemoglobin in the hemolymph of infected *Biomphalaria glabrata*. *Experimental Parasitology* **31**, 203-216.

Pampoulie C, Morand S, Lambert A, Rosecchi E, Bouchereau JL & Crivelli AJ (1999). Influence of the trematode *Aphalloides caelomicola* Dollfus, Chabaud & Golvan, 1957 on the fecundity and survival of *Pomatoschistus microps* (Krøyer, 1838) (Teleostei: Gobiidae). *Parasitology* **119**, 61-70.

Persson M, Cerenius L & Söderhäll K (1987). The influence of haemocyte number on the resistance of the freshwater crayfish, *Pacifastacus leniusculus* Dana, to the parasitic fungis *Aphanomyces astaci*. *Journal of Fish Diseases* **10**, 471-477.

Petersen IK, Pihl S, Hounisen JP, Holm TE, Clausen P, Therkildsen O & Christensen TK (2006). Landsdækkende optaellinger af vandfugle, januar og februar 2004. In: *Danmarks Miljøundersøgelser*, p. 76. Faglig rapport fra DMU nr. 606. http://www2.dmu.dk/Pub/FR606.pdf

Pietrock M & Marcogliese DJ (2003). Free-living endohelminth stages: at the mercy of environmental conditions. *TRENDS in Parasitology* **19**, 293-299.

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connel C, Wong E, Russel L, Zern J, Aquino T & Tsomondo T (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment* **84**, 1-20.

Plaistow SJ, Troussard JP & Cézilly F (2001). The effect of the acanthocephalan parasite *Pomphorhynchus laevis* on the lipid and glycogen content of its intermediate host *Gammarus pulex*. *International Journal for Parasitology* **31**, 346-351.

Poinar GO & Kuris AM (1975). Juvenile *Ascarophis* (Spirurida : Nematoda) parasitizing intertidal decapod crustacea in California: with notes on prevalence and effects on host growth and survival. *Journal of Invertebrate Pathology* **26**, 375-382.

Polak M & Starmer WT (1998). Parasite-induced risk of mortality elevates reproductive effort in male *Drosophila*. *Proceedings of the Royal Society of London B* **265**, 2197-2201.

Poulin R, Nichol K & Latham ADM (2003). Host sharing and host manipulation by larval helminths in shore crabs: cooperation or conflict? *International Journal for Parasitology* **33**, 425–433.

Prevost G, Davis DH & Vinson SB (1990). Evasion of encapsulation by parasitoid correlated with the extent of host hemocyte pathology. *Entomologia Experimentalis et Applicata* **55**, 1-10.

Pung OJ, Khan RN, Vives SP & Walker CB (2002). Prevalence, geographic distribution, and fitness effects of *Microphallus turgidus* (Trematoda: Microphallidae) in grass shrimp (*Palaemonetes* spp.) from coastal Georgia. *Journal of Parasitology* **88**, 89-92.

Rainbow PS, Ford MP & Heppelwhite I (1979). Absence of gregarious settling behaviour by female larvae of British parasitic rhizocephalan barnacles. *Journal of the Marine Biological Association of the UK* **59**, 591-196.

Rasmussen E (1959). Behaviour of sacculinized shore crabs (*Carcinus maenas* Pennant). *Nature* **183**, 479-480.

Rauque CA, Viozzi GP & Semenas LG (2003). Component population study of *Acanthocephalus tumescens* (Acanthocephala) in fishes from Lake Moreno, Argentina. *Folia Parasitologica*, **50**, 72-78.

Rayski C & Garden EA (1961). Life-cycle of an Acanthocephalan parasite of the eider duck. *Nature* **192**, 185-186.

Reeson AF, Wilson K, Gunn A, Hails RS & Goulson D (1998). Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. *Proceedings of the Royal Society of London B* **265***,* 1787-1791.

Reid DG & Aldrich JC (1989). Variation in responses to environmental hypoxia of different colour forms of the shore crab *Carcinus maenas* (L.). *Comparative Biochemistry and Physiology* **92A**, 535-539.

Reid DG, Abelló JC, Kaiser MJ & Warman CG (1997). Carapace colour, inter-moult duration and the behavioural and physiological ecology of the shore crab *Carcinus maenas*. *Estuarine, Coastal and Shelf Science* **44**, 211-302.

Reid DG, Abelló P, McGaw IJ & Naylor E (1989). Phenotypic variation in sympatric crab populations. In: *Phenotypic Responses and Individuality in Aquatic Ectotherms*, pp.89-06. Aldrich JC (Ed.). JAPAGA, Dublin.

Rigaud T & Moret Y (2003). Differential phenoloxidase activity between native and invasive gammarids infected vy local acanthocephalans: differential immunosuppression? *Parasitology* **127**, 571-577.

Rigby MC & Jokela J (2000). Predator avoidance and immune defence: costs and trade-offs in snails. *Proceedings of the Royal Society of London* **B 267**, 171-176.

Rigby MC, Hechinger RF & Stevens L (2002). Why should parasite resistance be costly? *Trends in Parasitology* **18**, 116-120.

Robinson TB (2005). Marine alien species of South Africa: threats and opportunities. PhD thesis, University of Cape Town.

Rojas JM & Ojeda FP (2005). Altered dopamine levels induced by the parasite *Profilicollis antarcticus* on its intermediate host, the crab *Hemigrapsus crenulatus*. *Biological Research* **38**, 259-266.

Roman J & Palumbi SR (2004). A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Molecular Ecology* **13**, 2891-2898.

Ropes JW (1968). The feeding habits of the green crab *Carcinus maenas*. *Journal of Experimental Marine Biology and Ecology* **67**, 183-201.

Rubiliani C (1983). Action of a rhizocephalan on the genital activity of host male crabs: characterization of a parasitic secretion inhibiting spermatogenesis. *International Journal of Invertebrate Reproduction* **6**, 137-147.

Rubiliani C (1985). Response by two species of crabs to a rhizocephalan extract. *Journal of Invertebrate Pathology* **45**, 304-310.

Rubiliani-Durozoi M, Rubiliani C, Payen GG (1980). Déroulement des gamétogenèses chez les crabes *Carcinus maenas* (L.) et *C. mediterraneus* Czerniavsky parasités par la Sacculine. *International Journal of Invertebrate Reproduction* **2**, 107-120.

Ruiz GM, Carlton JT, Grosholz ED & Hines AH (1997). Global invasions of marine and estuarine habitats by non-indigenous species: Mechanisms, extent, and consequences. *American Zoologist* **37**, 621-632.

Salzet M, Capron A & Stefano GB (2000). Molecular crosstalk in host–parasite relationships: schistosome- and leech-host interactions. *Parasitology Today* **16**, 536–540.

Saville DH & Irwin SWB (2005). A study of the mechanisms by which the cercariae of *Microphallus primas* (Jag, 1909) Stunkard, 1957 penetrate the shore crab, *Carcinus maenas*. *Parasitology* **131**, 521-529.

Say T (1817). An account of the Crustacea of the United States. *Journal of the Academy of Natural Sciences of Philadelphia* **1**, 57-63.

Schärer L, Ladurner P & Rieger RM (2004). Bigger testes do work more: experimental evidence that testis size reflects testicular cell proliferation activity in the marine invertebrate, the free-living flatworm *Macrostomum* sp. *Behavioral Ecology and Sociobiology* **56**, 420-425.

Schmid-Hempel P (2005). Evolutionary ecology of insect immune defenses. *Annual Review of Entomoloy* **50**, 529-551.

Schnapp D, Kemp GD & Smith VJ (1996). Purification and characterization of a proline.rich antibacterial peptide with sequence similarity to bactenecin-7, from the haemocytes of the shore crab, *Carcinus maenas*. *European Journal of Biochemistry* **240**, 532-539.

Schwarzenbach GA & Ward PI (2006). Responses to selection on phenoloxidase activity in yellow dung flies. *Evolution* **60**, 1612-1621.

Scott ME & Dobson A (1989). The role of parasites in regulating host abundance. *Parasitology Today* **5**, 176-183.

Scott ME (1987). Regulation of mouse colony abundance by *Heligmosoides polygyrus. Parasitology* **95**, 111-124.

Shelby KS, Adeyeye OA, Okto-Kotber BM & Webb BA (2000). Parasitism-linked block of host plasma melanization. *Journal of Invertabrate Pathology* **75**, 218-225.

Sheldon BC & Verhulst S (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11**, 317-321.

Shiao SH, Higgs S, Adelman Z, Christensen BM, Liu SH & Chen CC (2001). Effect of prophenoloxidase expression knockout on the melanization of microfilariae in the mosquito *Armigeres subalbatus*. *Insect Molecular Biology* **10**, 315-321.

Shields JD & Wood FEI (1993). IMpact of parasites on the reproduction and fecundity of the blue snd crab *Portununs pelagicus* from Moreton Bay, Australia. *Marine Ecology Progress Series* **92**, 159-170.

Shudo E & Iwasa Y (2001). Inducible defense against pathogens and parasites: optimal choice among multiple options. *Journal of Theoretical Biology* **209**, 233-247.

Sloan NA (1984). Incidence and effects of parasitism by the rhizocephalan barnacle, *Briarosaccus callosus* Boschma, in the golden king crab, *Lithodes aequispina* Benedict, from deep fjords in northern British Columbia, Canada. *Journal of Experimental Marine Biology and Ecology* **84**, 111-131.

Smith LD & Hines AH (1991). Autotomy in blue crab (*Callinectes sapidus* Rathbun) populations: geographic, temporal, and ontogenetic variation. *Biological Bulletin* **180**, 416-431.

Smith VJ (1996). The prophenoloxidase activating system: A common defence pathway for deuterostomes and protostomes? *Advances in Comparative and Environmental Physiology* **23**, 75-114.

Söderhäll K & Smith VJ (1986). The prophenoloxidase activating system : The biochemistry of its activation and role in arthropod cellular immunity, with special reference to crustaceans. In: *Immunity in Invertebrates,* pp. 208-223. Brehélin M (Ed.). Springer-Verlag, Heidelberg.

Spindler-Barth M (1976). A bacterial infection in the Common Shore Crab *Carcinus maenas* and the fiddler crab *Uca pugilator*. *Marine Biology* **36**, 1-4.

Storch V & Welsch U (1999). *Kükenthals Leitfaden für das zoologische Praktikum*, 23. Auflage. Spektrum Verlag, Berlin.

Stunkard HW (1957). The morphology and life-history of the digenetic trematode, *Microphallus similis* (Jägerskjöld, 1900) Baer, 1943. *Biological Bulletin* **112**, 254-266.

Styrishave B, Rewitz K & Andersen O (2004). Frequency of moulting by shore crabs *Carcinus maenas* (L.) changes their colour and their success in mating and physiological performance. *Journal of Experimental Marine Biology and Ecology* **313**, 317-336.

Swinton J, Woolhouse MEJ, Begon ME, Dobson AP, Ferroglio E, Grenfell BT, Guberti V, Hails RS, Heesterbeek JAP, Lavazza A, Roberts MG, White PJ & Wilson K (2002). Microparasite transmission and persistance. In: *The Ecology of Wildlife Diseases,* pp. 83-101. Hudson PJ et al. (Eds.). Oxford University Press, Oxford.

Taraschewski H (2000). Host-parasite interactions in Acanthocephala: a morphological approach. *Advances in Parasitology* **46**, 1-179.

Taraschewski H (2006). Hosts and parasites as aliens. *Journal of Helminthology* **80**, 99-128.

Taylor MI, Turner GF, Robinson RL & Stauffer JR Jr (1998). Sexual selection, parasites and bower height skew in a bower-building cichlid fish. *Animal Behaviour* **56**, 379-384.

Tettlebach ST (1986). Dynamics of crustacean predation on the northern bay scallop *Argopecten irradians irradians*. PhD dissertation, University of Connecticut.

Thessalou-Legaki M (1992). Reproductive variability of *Parapandalus narval* (crustacea: decapoda) along a depth gradient. *Estuarine, Coastal and Shelf Science* **35**, 593-603.

Thiel M & Dernedde T (1994). Recruitment of shore crabs *Carcinus maenas* on tidal flats: Mussel clumps as an important refuge for juveniles. *Helgoland Marine Research* **48**, 321-332.

Thieltges DW (2006). Parasite induced summer mortality in the cockle *Cerastoderma edule* by the trematode *Gymnophallus choledochus*. *Hydrobiologia* **559**, 455-461.

Thieltges DW, Hussel B, Herrmann J, Jensen KT, Krakau M, Taraschewski H & Reise K (2008a). Parasites in the northern Wadden Sea: a conservative ecosystem component over 4 decades. *Helgoland Marine Research* **62**, 37-47.

Thieltges DW, Jensen KT & Poulin R (2008b). The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology* **133**, 407-426.

Thomas F, Renaud F, Rousset F, Cezilly F & de Meeûs T (1995). Differential mortality of two closely related host species induced by one parasite. *Proceedings of the Royal Society of London* **B 260**, 349-352.

Thompson AB (1985a). Analysis of *Profilicollis botulus* (Acanthocephala: Echinorhynchidae) burdens in the shore crab, *Carcinus maenas*. *Journal of Animal Ecology* **54**, 595-604.

Thompson AB (1985b). Transmission dynamics of *Profilicollis botulus* (acanthocephala) from crabs (*Carcinus maenas*) to eider ducks (*Somateria mollissima*) on the Ythan Estuary, New Scotland. *Journal of Animal Ecology* **54**, 605-616.

Thornhill JA, Jones JT & Kusel JR (1986). Increased oviposition and growth in immature *Biomphalaria glabrata* after exposure to *Schistosoma mansoni*. *Parasitology* **93**, 443-450.

Thresher RE (1997). Proceedings on the first international Workshop on the Demography, Impacts and Management of introduced Populations of the European Crab, *Carcinus maenas*. Thresher RE (Ed.). Center for Research in introduced Marine Pests (Australia), Technical Report 11.

Thresher RE, Werner M, Høeg JT, Svane I, Glenner H, Murphy NE & Wittwer C (2000). Developing the options for managing marine pests: specificity trials on the parasitic castrator, *Sacculina carcini*, against the European crab, *Carcinus maenas*, and related species. *Journal of Experimental Marine Biology and Ecology* **254**, 37–51.

Timm RM & Cook EF (1979). The effect of bot fly larvae on reproduction in white-footed mice, *Peromyscus leucopus*. *The American Midland Naturalist* **101**, 211-217.

Tolley, S. G., Winstead, J. T., Haynes, L. and Volety, A. K. (2006). Influence of salinity on prevalence of the parasite *Loxothylacus panopaei* in the xanthid *Panopeus obesus* in SW Florida. *Diseases of Aquatic Organisms* **70**, 243-250.

Tompkins DM & Poulin R (2006). Parasites and biological invasions. *Ecological Studies* **186**, 67-84

Tompkins DM, Dobson AP, Arneberg P, Begon ME, Cattadori IM, Greenman JV, Heesterbeek JAP, Hudson PJ, Newborn D, Pugliese A, Rizzoli AP, Rosà R, Rosso F & Wilson K (2002). Parasites and Host Population Dynamics. In: *The Ecology of Wildlife Diseases,* pp. 45-62. Hudson PJ et al. (Eds.). Oxford University Press, Oxford.

Torchin ME, Lafferty KD & Kuris AM (1996). Infestation of an introduced host, the European green crab, *Carcinus maenas*, by a symbiotic nemertean egg predator, *Carcinonemertes epialti. Journal of Parasitology* **82**, 449-453

Torchin ME, Lafferty KD & Kuris AM (2001). Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions* **3**, 333–345.

Torchin ME, Lafferty KD & Kuris AM (2002). Parasites and marine invasions. *Parasitology* **124**, 137-151.

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ & Kuris AM (2003). Introduced species and their missing parasites. *Nature* **421**, 628-630.

Truscott R & White KN (1990). The influence of metal and temperature stress on the immune system of crabs. *Functional Ecology* **4**, 455-461.

Ubelaker JF, Cooper NB & Allison VF (1970). Possible defensive mechanism of *Hymenolepis diminuta* cysticercoids to hemocytes of the beetle *Tribolium confusum*. *Journal of Invertebrate Pathology* **16**, 310-312.

Uglow RF (1969a). Haemolymph protein concentrations in portunid crabs - I. Studies on adult *Carcinus maenas*. *Comparative Biochemistry and Physiology* **30**, 1083-1090.

Uglow RF (1969b). Haemolymph protein concentrations in portunid crabs - II. The effects of imposed fasting on *Carcinus maenas*. *Comparative Biochemistry and Physiology* **31***,* 595-967.

Van der Meeren (1994). Sex- and size-dependent mating tactics in a natural population of shore crabs *Carcinus maenas*. *Journal of Animal Ecology* **63**, 307-314.

Vass E & Nappi AJ (2000). Developmental and immunological aspects of *Drosophila*-parasitoid relationships. *Journal of Parasitology* **86**, 1259-1270.

Veillet A & Graf F (1958). Dégénérescence de la glande androgène des Crustacés Décapodes parasités par les Rhizocéphales. *Bulletin de la Société des Science de Nancy* **18**, 123-127.

Vellend M, Harmon LJ, Lockwood JL, Mayfield MM, Hughes AR, Wares JP & Sax DF (2007). Effects of exotic species on evolutionary diversification. *Trends in Ecology and Evolution* **22**, 481-488.

Vitousek PM, D'Antonio CM, Loope LL, Rejmánek M & Westbrooks R (1997). Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* **21**, 1-16.

Vivarès CP & Cuq JL (1981). Physiological and Metabolic Variations in *Carcinus mediterraneus* (Crustacea: Decapoda) Parasitized by *Thelohania maenadis* (Microspora: Microsporida): An Ecophysiopathological Approach. *Journal of Invertebrate Pathology* **37**, 38-46.

Wahl M (1989). Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Marine Ecology Progress Series* **58**, 175-189.

Wahl M, Hay ME & Enderlein P (1997). Effects of epibiosis on consumer-prey interactions. *Hydrobiologia* **355**, 49-59.

Walker G (1985). The cypris larvae of *Sacculina carcini* Thompson (Crustacea: Cirripedia: Rhizocephala). *Journal of Experimental Marine Biology and Ecology* **93**, 131-145.

Walne PR & Dean GJ (1972). Experiments on predation by the shore crab, *Carcinus maenas* L., on *Mytilus* und *Mercenaria*. *Journal du Conseil International pour l'Exploration de la Mer* **34**, 190-199.

Walne PR & Dean GJ (1977). The effect of mesh covers on the survival and growth of *Crassostrea gigas* grown on the sea bed. *Aquaculture* **11**, 313-322.

Wang LU & Chen JC (2005), The immune response of white shrimp L*itopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* at different salinity levels. Fish *and Shellfish Immunology* **18**, 269-278.

Webster JP & Woolhouse MEJ (1999). Cost of resistance: relationship between reduced fertility and increased resistance in a snail-schistosome host-parasite system. *Proceedings of the Royal Society of London B* **266**, 391-396.

Werner M (2001). Prevalence of the parasite *Sacculina carcini* Thompson 1836 (Crustacea, Rhizocephala) on its host crab *Carcinus maenas* (L.) on the west coast of Sweden. *Ophelia* **55**, 101-110.

Williamson MH & Fitter A (1996a). The varying success of invaders. *Ecology* **77**, 1661-1666.

Williamson MH & Fitter A (1996b). The characters of successful invaders. *Biological Conservation* **78**, 163-170.

Willis AJ, Memmott J & Forrester R (2000). Is there evidence for the post-invasion evolution of increased size among invasive plant species? *Ecology Letters* **3**, 275-283.

Wilson K, Bjørnstad ON, Dobson AP, Merler S, Poglayen G, Randolph SE, Read AF & Skorping A (2002). Heterogeneities in macroparasite infections: patterns and processes. In: *The Ecology of Wildlife Diseases,* pp. 6-44. Hudson PJ et al. (Eds.). Oxford University Press, Oxford.

Wolf F (1998). Red and green colour forms in the common shore crab *Carcinus maenas* (L.) (Crustacea: Brachyura: Portunidae): theoretical predictions and empirical data. *Journal of Natural History* **32**, 1807-1812.

Yeh ST, Liu CH & Chen JC (2004). Effect of copper sulfate on the immune response and susceptibility to *Vibrio alginolyticu*s in the white shrimp *Litopenaeus vannamei*. *Fish and Shellfish Immunology* **17**, 437-446.

Yoshino TP & Lodes MJ (1988). Secretory protein biosynthesis in snail hemocytes: *in vitro* modulation by larval schistosome excretory products. *Journal of Parasitology* **74**, 538-547.

Yoshino TP & Vasta GR (1996). Parasite-invertebrate host immune interactions. *Advances in Comparative and Environmental Physiology* **24**, 125-167.

Zaiou M (2007). Multifunctional antimicrobial peptides: therapeutic targets in several human diseases. *Journal of Molecular Medicine* **85**, 317-329.

Zambrano-Villa S, Rosales-Burjas D, Carrero J-C & Ortiz-Ortiz L (2002). How protozoan parasites evade the immune response. *Trends in Parasitology* **18**, 272-278.

Zuk M & Stoehr AM (2002). Immune defence and host life history. *The American Naturalist* **160**, 9-22.

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8 Appendix

Appendix table 1: Differences in *S. carcini* prevalence between European locations. Results of pair wise Fisher's exact tests (χ^2, p) are given for significant differences between pairs.

loc	1	2	3	4	5	6	7	8	9	10
1	-	9.816 0.013	20.836 0.000	19.743 0.000	17.055 0.000	n.s.	n.s.	n.s.	n.s.	11.105 0.009
2	9.816 0.013	-	81.468 0.000	79.398 0.000	71.540 0.000	16.437 0.000	6.998 0.013	22.108 0.000	n.s.	n.s.
3	20.836 0.000	81.468 0.000	-	n.s.	n.s.	28.397 0.000	46.667 0.000	16.338 0.000	52.603 0.000	90.860 0.000
4	19.743 0.000	79.398 0.000	n.s.	-	n.s.	27.402 0.000	45.561 0.000	15.287 0.000	51.237 0.000	88.564 0.000
5	17.055 0.000	71.540 0.000	n.s.	n.s.	-	21.360 0.000	37.723 0.000	12.174 0.000	44.345 0.000	80.079 0.000
6	n.s.	16.437 0.000	28.397 0.000	27.402 0.000	21.360 0.000	-	n.s.	n.s.	6.425 0.011	18.565 0.000
7	n.s.	6.998 0.013	46.667 0.000	45.561 0.000	37.723 0.000	n.s.	-	4.921 0.029	n.s.	7.917 0.009
8	n.s.	22.108 0.000	16.338 0.000	15.287 0.000	12.174 0.000	n.s.	4.921 0.029	-	9.526 0.003	24.958 0.000
9	n.s.	n.s.	52.603 0.000	51.237 0.000	44.345 0.000	6.425 0.011	n.s.	9.526 0.003	-	n.s.
10	11.105 0.009	n.s.	90.860 0.000	88.564 0.000	80.079 0.000	18.565 0.000	7.917 0.009	24.958 0.000	n.s.	-

Appendix table 2: Differences in microphallid prevalence between European locations. Results of pair wise Fisher's exact tests (χ^2, p) are given for significant differences between pairs.

loc	1	2	3	4	5	6	7	8	9	10
1	-	3.839 0.066	100.99 0.000	58.113 0.000	67.667 0.000	37.351 0.000	36.566 0.000	61.033 0.000	8.883 0.003	7.307 0.009
2	3.839 0.066	-	93.419 0.000	57.759 0.000	61.051 0.000	32.063 0.000	31.636 0.000	54.468 0.000	n.s.	n.s.
3	100.99 0.000	93.419 0.000	-	15.504 0.000	n.s.	19.477 0.000	22.557 0.000	n.s.	64.720 0.000	80.393 0.000
4	58.113 0.000	57.759 0.000	15.504 0.000	-	9.199 0.002	n.s.	n.s.	7.732 0.004	29.960 0.000	45.508 0.000
5	67.667 0.000	61.051 0.000	n.s.	9.199 0.002	-	11.910 0.000	13.764 0.000	n.s.	41.431 0.000	51.542 0.000
6	37.351 0.000	32.063 0.000	19.477 0.000	n.s.	11.910 0.000	-	n.s.	10.155 0.001	15.671 0.000	23.807 0.000
7	36.566 0.000	31.636 0.000	22.557 0.000	n.s.	13.764 0.000	n.s.	-	11.785 0.000	14.633 0.000	23.092 0.000
8	61.033 0.000	54.468 0.000	n.s.	7.732 0.004	n.s.	10.155 0.001	11.785 0.000	-	36.663 0.000	45.686 0.000
9	8.883 0.003	n.s.	64.720 0.000	29.960 0.000	41.431 0.000	15.671 0.000	14.633 0.000	36.663 0.000	-	n.s.
10	7.307 0.009	n.s.	80.393 0.000	45.508 0.000	51.542 0.000	23.807 0.000	23.092 0.000	45.686 0.000	n.s.	-

Appendix table 3: Differences in *P. botulus* prevalence between European locations. Results of pair wise Fisher's exact tests (χ^2, p) are given for significant differences between pairs.

loc	1	2	3	4	5	6	7	8	9	10
1	-	n.s.	49.737 0.000	n.s.	41.411 0.000	48.548 0.000	47.560 0.000	6.586 0.013	7.802 0.008	14.717 0.000
2	n.s.	-	70.076 0.000	105.73 0.000	53.093 0.000	63.637 0.000	65.630 0.000	8.497 0.005	16.572 0.000	32.483 0.000
3	49.737 0.000	70.076 0.000	-	n.s.	n.s.	n.s.	n.s.	99.729 0.000	21.723 0.000	16.680 0.000
4	n.s.	105.73 0.000	n.s.	-	n.s.	n.s.	n.s.	146.27 0.000	41.305 0.000	33.278 0.000
5	41.411 0.000	53.093 0.000	n.s.	n.s.	-	n.s.	n.s.	76.924 0.000	20.155 0.000	16.094 0.000
6	48.548 0.000	63.637 0.000	n.s.	n.s.	n.s.	-	n.s.	90.810 0.000	23.039 0.000	19.226 0.000
7	47.560 0.000	65.630 0.000	n.s.	n.s.	n.s.	n.s.	-	93.868 0.000	21.140 0.000	16.322 0.000
8	6.586 0.013	8.497 0.005	99.729 0.000	146.27 0.000	76.924 0.000	90.810 0.000	93.868 0.000	-	36.041 0.000	56.401 0.000
9	7.802 0.008	16.572 0.000	21.723 0.000	41.305 0.000	20.155 0.000	23.039 0.000	21.140 0.000	36.041 0.000	-	n.s.
10	14.717 0.000	32.483 0.000	16.680 0.000	33.278 0.000	16.094 0.000	19.226 0.000	16.322 0.000	56.401 0.000	n.s.	-

Appendix table 4: Differences in *P. maenadis* prevalence between European locations. Results of pair wise Fisher's exact tests (χ^2, p) are given for significant differences between pairs.

loc	1	2	3	4	5	6	7	8	9	10
1	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2	n.s.	-	n.s.	5.578 0.031	n.s.	n.s.	n.s.	n.s.	10.355 0.002	n.s.
3	n.s.	n.s.	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.204 0.040
4	n.s.	5.578 0.031	n.s.	-	n.s.	n.s.	n.s.	n.s.	n.s.	6.353 0.013
5	n.s.	n.s.	n.s.	n.s.	-	n.s.	n.s.	n.s.	5.073 0.043	n.s.
6	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.	n.s.	n.s.	n.s.
7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.	n.s.	7.917 0.009
8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-	n.s.	n.s.
9	n.s.	10.355 0.002	n.s.	n.s.	5.073 0.043	n.s.	n.s.	n.s.	-	11.782 0.001
10	n.s.	n.s.	4.204 0.040	6.353 0.013	n.s.	n.s.	7.917 0.009	n.s.	11.782 0.001	-

Appendix table 5: Parasite prevalence in male and female crabs of Europe. Results of Fisher's exact tests and significance level are given for significant differences between the sexes.

sample year	loc		*S. carcini* infection			Microphallid infection			*P. botulus* infection		
			no	yes	χ^2, p	no	yes	χ^2, p	no	yes	χ^2, p
2008	**1**	**male**	44	3	n.s.	14	33	n.s.	26	21	n.s.
		female	2	0		0	2		1	1	
2008	**2a**	**male**	25	0	n.s.	15	10	n.s.	10	15	n.s.
		female	11	0		4	7		4	7	
2008	**2b**	**male**	20	0	n.s.	0	20	n.s.	15	5	n.s.
		female	21	0		1	20		16	5	
2008	**2c**	**male**	16	0	n.s.	12	4	n.s.	11	5	n.s.
		female	19	0		14	5		10	9	
2009	**2a**	**male**	33	0	n.s.	21	12	n.s.	10	23	n.s.
		female	9	0		3	6		5	4	
2006	**3**	**male**	47	33	n.s.	80	0	5.015	78	2	n.s.
		female	28	21		46	3	0.053	47	2	
2006	**4**	**male**	14	22	n.s.	34	2	n.s.	36	0	n.s.
		female	7	17		19	5		23	1	
2007		**male**	63	18	n.s.	70	11	n.s.	81	0	n.s.
		female	24	14		27	11		38	0	
2006	**5**	**male**	21	23	n.s.	44	0	n.s.	44	0	n.s.
		female	6	5		11	0		11	0	
2007		**male**	19	2	-	19	2	-	21	0	-
		female	-	-		-	-		-	-	
2007	**6**	**male**	79	6	**5.447**	69	16	n.s.	84	1	n.s.
		female	11	4	**0.041**	11	4		15	0	
2006	**7**	**male**	76	5	n.s.	63	18	n.s.	80	1	n.s.
		female	34	0		27	7		32	2	
2005	**8**	**male**	41	6	n.s.	47	0	n.s.	16	31	n.s.
		female	17	3		18	2		5	15	
2008	**9**	**male**	24	14	n.s.	55	47	n.s.	80	22	n.s.
		female	87	32		4	3		4	3	
2006	**10**	**male**	174	0	n.s.	87	87	n.s.	141	33	n.s.
		female	5	0		3	2		5	0	

Appendix table 6: Parasite prevalence in green and red crabs of Europe. Results of Fisher's exact tests and significance level are given for significant differences between the morphs.

sample year	loc		*S. carcini* infection			Microphallid infection			*P. botulus* infection		
			no	yes	χ^2, p	no	yes	χ^2, p	no	yes	χ^2, p
2008	**1**	**green**	25	1	n.s.	10	16	n.s.	14	12	n.s.
		red	21	2		4	19		13	10	
2008	**2a**	**green**	20	0	n.s.	12	8	n.s.	7	13	n.s.
		red	16	0		7	9		7	9	
2008	**2b**	**green**	38	0	n.s.	1	37	n.s.	29	9	n.s.
		red	3	0		0	3		2	1	
2008	**2c**	**green**	30	0	n.s.	23	7	n.s.	20	10	n.s.
		red	5	0		3	2		1	4	
2009	**2a**	**green**	3	0	n.s.	2	1	n.s.	2	1	n.s.
		red	39	0		22	17		13	26	
2006	**3**	**green**	47	30	n.s.	76	1	n.s.	75	2	n.s.
		red	28	24		50	2		50	2	
2006	**4**	**green**	13	26	n.s.	34	5	n.s.	39	0	n.s.
		red	7	13		19	1		19	1	
2007		**green**	27	9	n.s.	31	5	n.s.	36	0	n.s.
		red	60	23		66	17		83	0	
2006	**5**	**green**	16	9	n.s.	25	0	n.s.	25	0	n.s.
		red	11	19		30	0		30	0	
2007		**green**	3	0	n.s.	2	1	n.s.	3	0	n.s.
		red	16	2		17	1		18	0	
2007	**6**	**green**	41	4	n.s.	36	9	n.s.	45	0	n.s.
		red	49	6		44	11		54	1	
2006	**7**	**green**	49	2	n.s.	34	17	**8.374**	49	2	n.s.
		red	60	3		56	7	**0.005**	62	1	
2005	**8**	**green**	44	3	**7.294**	47	0	n.s.	15	32	n.s.
		red	13	6	**0.013**	17	2		6	13	
2008	**9**	**green**	49	0	n.s.	24	25	n.s.	33	16	**4.755**
		red	58	2		35	25		51	9	**0.039**
2006	**10**	**green**	52	0	n.s.	24	28	n.s.	39	13	n.s.
		red	123	0		65	58		104	19	

Appendix table 7: Autotomy in *C. maenas*: number of missing legs in relation to *S. carcini* infection. Where sample sizes were sufficient, there was no significant difference between both groups

loc	*S. carcini* infection	number of missing legs					
		0	1	2	3	4	5
1	no	31	10	3	2		
	yes	2	1	0	0		
	total	33	11	3	2		
2	no	77	64	14	1	2	
	yes						
	total	77	64	14	1	2	
3	no	50	15	5	3	2	
	yes	33	15	5	1	0	
	total	83	30	10	4	2	
4	no	68	34	2	1	1	
	yes	52	15	2	1	1	
	total	120	49	4	2	2	
5	no	26	13	4	2	1	
	yes	23	6	1	0	0	
	total	49	19	5	2	1	
6	no	71	16	3			
	yes	9	1	0			
	total	80	17	3			
7	no	77	26	5	1		1
	yes	4	0	0	1		0
	total	81	26	5	2		1
8	no	21	21	7	5	1	3
	yes	5	3	1	0	0	0
	total	26	24	8	5	1	3
9	no	70	30	3	3	1	
	yes	2	0	0	0	0	
	total	72	30	3	3	1	
10	no	113	37	19	7	3	
	yes						
	total	113	37	19	7	3	
11	no	30	3	1		1	
	yes						
	total	30	3	1		1	
12	no	131	37	9	2	1	
	yes						
	total	131	37	9	2	1	
13	no	56	29	8	6	1	
	yes						
	total	56	29	8	6	1	
14	no	96	6	5			
	yes						
	total	96	6	5			

Appendix table 8: Autotomy in *C. maenas*: number of missing legs in relation to microphallid infection. Where sample sizes were sufficient, there was no significant difference between both groups.

loc	microphallid infection	number of missing legs					
		0	1	2	3	4	5
	no	10	3	1	0		
1	yes	23	8	2	2		
	total	33	11	3	2		
	no	32	29	8	0	1	
2	yes	45	35	6	1	1	
	total	77	64	14	1	2	
	no	80	30	10	4	2	
3	yes	3	0	0	0	0	
	total	83	30	10	4	2	
	no	101	39	4	2	2	
4	yes	19	10	0	0	0	
	total	120	49	4	2	2	
	no	48	18	5	2	1	
5	yes	1	1	0	0	0	
	total	49	19	5	2	1	
	no	65	13	2			
6	yes	15	4	1			
	total	80	17	3			
	no	65	20	4	1		0
7	yes	16	6	1	1		1
	total	81	26	5	2		1
	no	26	24	8	4	1	2
8	yes	0	0	0	1	0	1
	total	26	24	8	5	1	3
	no	37	19	1	1	1	
9	yes	35	11	2	2	0	
	total	72	30	3	3	1	
	no	56	17	10	4	3	
10	yes	57	20	9	3	0	
	total	113	37	19	7	3	
	no	30	3	1		1	
11	yes						
	total	30	3	1		1	
	no	131	37	9	2	1	
12	yes						
	total	131	37	9	2	1	
	no	56	29	8	6	1	
13	yes						
	total	56	29	8	6	1	
	no	96	6	5			
14	yes						
	total	96	6	5			

Appendix table 9: Autotomy in *C. maenas*: number of missing legs in relation to *P. botulus* infection. Where sample sizes were sufficient, there was no significant difference between both groups.

loc	P. botulus infection	number of missing legs					
		0	1	2	3	4	5
	no	19	5	2	1		
1	yes	14	6	1	1		
	total	33	11	3	2		
	no	44	33	5	0	1	
2	yes	33	31	9	1	1	
	total	77	64	14	1	2	
	no	81	29	9	4	2	
3	yes	2	1	1	0	0	
	total	83	30	10	4	2	
	no	119	49	4	2	2	
4	yes	1	0	0	0	0	
	total	120	49	4	2	2	
	no	49	19	5	2	1	
5	yes						
	total	49	19	5	2	1	
	no	79	17	3			
6	yes	1	0	0			
	total	80	17	3			
	no	80	24	5	2		1
7	yes	1	2	0	0		0
	total	81	26	5	2		1
	no	7	7	4	2	0	1
8	yes	19	17	4	3	1	2
	total	26	24	8	5	1	3
	no	52	25	3	3	1	
9	yes	20	5	0	0	0	
	total	72	30	3	3	1	
	no	92	30	16	5	3	
10	yes	21	7	3	2	0	
	total	113	37	19	7	3	
	no	30	3	1		1	
11	yes						
	total	30	3	1		1	
	no	131	37	9	2	1	
12	yes						
	total	131	37	9	2	1	
	no	56	29	8	6	1	
13	yes						
	total	56	29	8	6	1	
	no	96	6	5			
14	yes						
	total	96	6	5			

Appendix table 10: Parasite infections in relation to the presence of polychaetes. Results of Fishers exact tests are given for all significance values of p<0.07.

loc	polychaetes present	*S. carcini* present		χ^2, p	microphallids present		χ^2, p	*P. botulus* present		χ^2, p
		no	yes		no	yes		yes	no	
loc 1	no	46	3	-	14	35	-	22	27	-
	yes	0	0		0	0		0	0	
loc 2	no	156	0	-	69	87	n.s.	75	81	n.s.
	yes	2	0		1	1		0	2	
loc 3	no	67	47	n.s.	112	2	n.s.	4	110	n.s.
	yes	8	7		14	1		0	15	
loc 4	no	97	60	n.s.	130	27	n.s.	1	156	n.s.
	yes	11	11		20	2		0	22	
loc 5	no	43	25	n.s.	66	2	n.s.	0	68	-
	yes	3	5		8	0		0	8	
loc 6	no	80	7	n.s.	69	18	n.s.	0	87	n.s.
	yes	10	3		11	2		1	12	
loc 7	no	110	5	-	90	25	-	3	112	-
	yes	0	0		0	0		0	0	
loc 8	no	57	8	n.s.	63	2	n.s.	46	19	n.s.
	yes	1	1		2	0		0	2	
loc 9	no	107	2	-	59	50	-	25	84	-
	yes	0	0		0	0		0	0	
loc 10	no	178	0	-	90	88	n.s.	33	145	n.s.
	yes	1	0		0	1		0	1	

Appendix table 11: Parasite infections in relation to the presence of bryozoans. Results of Fishers exact tests are given for all significance values of p<0.07.

loc	bryozoans present	*S. carcini* present		χ^2, p	microphallids present		χ^2, p	*P. botulus* present		χ^2, p
		no	yes		no	yes		yes	no	
loc 1	**no**	46	3	-	14	35	-	22	27	-
	yes	0	0		0	0		0	0	
loc 2	**no**	152	0	-	66	86	n.s.	72	80	n.s.
	yes	6	0		4	2		3	3	
loc 3	**no**	67	47	n.s.	112	2	n.s.	4	110	n.s.
	yes	8	7		14	1		0	15	
loc 4	**no**	96	65	n.s.	133	28	n.s.	1	160	n.s.
	yes	12	6		17	1		0	18	
loc 5	**no**	42	25	n.s.	65	2	n.s.	0	67	-
	yes	4	5		9	0		0	9	
loc 6	**no**	74	8	n.s.	67	15	n.s.	1	81	n.s.
	yes	16	2		13	5		0	18-	
loc 7	**no**	110	5	-	90	25	-	3	112	-
	yes	0	0		0	0		0	0	
loc 8	**no**	57	7	**7.654, 0.045**	62	2	n.s.	46	18	**6.879, 0.028**
	yes	1	2		3	0		0	3	
loc 9	**no**	107	2	-	59	50	-	25	84	-
	yes	0	0		0	0		0	0	
loc 10	**no**	178	0	-	90	88	n.s.	33	145	n.s.
	yes	1	0		0	1		0	1	

Appendix table 12: Parasite infections in relation to the presence of barnacles. Results of Fishers exact tests are given for all significance values of p<0.07.

loc	barnacles present	*S. carcini* present		χ^2, p	microphallids present		χ^2, p	*P. botulus* present		χ^2, p
		no	yes		no	yes		yes	no	
loc 1	**no**	26	2	n.s.	11	17	3.675, 0.066	10	18	n.s.
	yes	20	1		3	18		12	9	
loc 2	**no**	150	0	-	67	83	n.s.	69	81	n.s.
	yes	8	0		3	5		6	2	
loc 3	**no**	67	47	n.s.	112	2	n.s.	4	110	n.s.
	yes	8	7		14	1		0	15	
loc 4	**no**	85	59	n.s.	118	26	n.s.	1	143	n.s.
	yes	23	12		32	3		0	35	
loc 5	**no**	40	26	n.s.	64	2	n.s.	0	66	-
	yes	6	4		10	0		0	10	
loc 6	**no**	63	4	n.s.	52	15	n.s.	0	67	n.s.
	yes	27	6		28	5		1	32	
loc 7	**no**	104	5	n.s.	85	24	n.s.	3	106	n.s.
	yes	6	0		5	1		0	6	
loc 8	**no**	57	6	**13.867, 0.007**	61	2	n.s.	45	18	n.s.
	yes	1	3		4	0		1	3	
loc 9	**no**	98	1	n.s.	55	44	n.s.	22	77	n.s.
	yes	9	1		4	6		3	7	
loc 10	**no**	177	0	-	90	87	n.s.	33	144	n.s.
	yes	2	0		0	2		0	2	

Appendix table 13: Statistical differences in reproduction index (RI) in male crabs. T-tests were conducted for single population (t, p), the Mann-Whitney test for polled data. Location numbers refer to Figure 13, 15 & 17.

Location	t	p	green morph		red morph	
			N	mean RI	N	mean RI
loc 1	-4.296	**<0.001**	23	0.9490	17	1.0714
loc 2	-1.627	n.s.	31	0.9763	37	1.0158
loc 5	-4.592	**<0.001**	62	0.9577	87	1.0362
loc 7	-0.836	n.s.	24	0.9932	40	1.0037
loc 9	-5.635	**<0.001**	47	0.9344	48	1.0717
loc 10	-7.404	**<0.001**	48	0.9609	120	1.0153
loc 12	-2.657	**0.009**	56	0.9768	30	1.0396
loc 13	-1.907	0.065	12	0.9666	24	1.0160
loc 14	1.611	n.s.	16	1.0193	15	0.9786
pooled	Z=-12.113	p<0.001	379	0.9532	418	1.0436

9 Curriculum vitae

Personal details

Name	Claudia M. Zetlmeisl
Date of Birth	December 18th, 1979
Place of Birth	Mannheim, Germany
Nationality	German

Education

1999	Abitur (matriculation), Carl-Friedrich-Gauß-Gymnasium Hockenheim (average grade 1.1)
2002	Intermediate Diploma, University of Heidelberg, Germany
2006	Diploma (Hons, distinction), University of Würzburg, Germany. Main focus: microbiology, ecology, socio-biology and behavioural physiology (final grade: "sehr gut") Thesis: The role of cuticular hydrocarbons as a queen signal and possible larval recognition cue in the ant *Camponotus floridanus.* Supervisors: Prof. Dr. Wolfgang Rössler, Dr. Jürgen Liebig
2006	Begin Doctoral studies, University of Karlsruhe, Germany Thesis: Host-parasite interactions in the European shore crab *Carcinus maenas* and their implications for the invasion success of this introduced species. Supervisor: Prof. Dr. Horst Taraschewski

Grants

2006	Doctoral grant from the Federal State Foundation for Graduate Students (Landesgraduiertenförderung, 5.000€).
2007-2009	Doctoral grant from the German Federal Environmental Foundation (Deutsche Bundesstiftung Umwelt (31.620€). http://www.dbu.de/stipendien_20007/912_db.html
2007	Grant from the German Academic Exchange Service (Deutscher Akademischer Austauschdienst) for research at the University of Cape Town, South Africa (1.500€).
2008	Grant from the German Academic Exchange Service (Deutscher Akademischer Austauschdienst) for research at the University of Melbourne, Australia (1.600€).

International experience

2006, 2007	Research trips to the Danish Scaldyrcenter, Mors, Denmark. Research aim: Parasite-induced morphological and physiological changes in *Carcinus maenas*
2007	Research trip to the University of Cape Town, South Africa Research aim: Investigation of the local introduced *C. maenas* population for parasites and parasite-induced morphological and physiological changes Collaboration partner: Prof. Charles Griffiths
2008	Research trip to the University of Melbourne, Australia Research aim: Investigation of the local introduced *C. maenas* population for parasites and parasite-induced morphological and physiological changes Collaboration partner: Dr. Nathan Bott
Languages	German (first language), English (fluent), French (good)

Congresses and publications

The German Society for Parasitology, 23rd Annual Meeting. Hamburg, March 05-07, 2008.

Talk: Zetlmeisl C, Hermann J, Griffiths C & Taraschewski H (2008). Host-parasite interactions in the invasive European shore crab, *Carcinus maenas*, in its European home range and in colonized areas.

European Congress of Conservation Biology. Prague, September 01-05, 2009.

Poster: Zetlmeisl C, Petney T, Griffiths C & Taraschewski H (2009). How invading species avoid infection: parasites and immunocompetence *Carcinus maenas*.

The German Society for Parasitology, 24th Meeting. Düsseldorf, 16.-20.03.2010.

Talk: Zetlmeisl C, Petney T, Griffiths C, Glenner H & Taraschewski H (2010). Parasites and immunocompetence in a marine invader, the green crab *Carcinus maenas*.

Zetlmeisl C, Hermann J, Petney T, Glenner H, Griffiths C & Taraschewski H (2010). Parasites in the shore crab *Carcinus maenas* (L.): implications for reproductive potential and invasion success. *Parasitology* 138, 394-401.

Zetlmeisl C, Petney T, Griffiths C & Taraschewski H (2011). Impact of parasite infection on *Carcinus maenas* (L.) haemolymph parameters. In preparation.