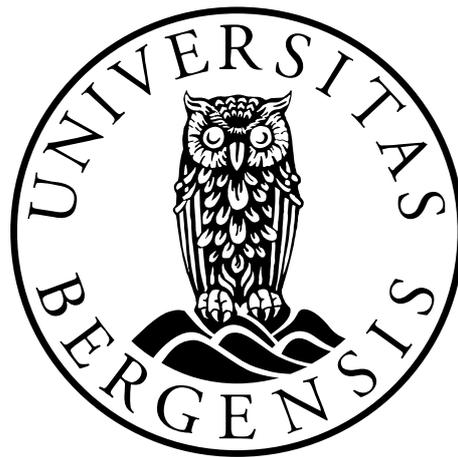


# **Environmental impact on host-parasite interactions**

*A study on the adaptive value of host castration and gigantism when hosts can regain reproduction*

**Jon H. Magerøy**



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## **Scientific environment**

During my PhD-studies I was employed as a research fellow by the Department of Biology at the University of Bergen. At the department, I was a member of the Aquatic Behavioural Ecology (ABE) and Evolutionary Ecology (EE) research groups, which provided financial support and supervision. My supervisors were Per J. Jakobsen (ABE) and Knut Helge Jensen (EE).

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## Summary

The aim of this thesis was to investigate how altering the external environment affects life history strategies of hosts and their parasites, and to gain a better understanding of evolutionary theories on castration and gigantism in parasitized hosts.

We found that maintaining *Daphnia magna* in a high resource growth medium resulted in increased reproduction and decreased survival, suggesting that reproduction is costly for this organism. However, within growth mediums we found a positive association between reproduction and survival, suggesting that high quality individual were able to invest more in both aspects of life history compared to low quality individuals. These findings may seem contradictory, but one would only expect to be able to detect cost of reproduction when the increase in reproductive investment outweighs the differences in individual quality. In addition, we found that increased resources led to reproduction more closely resembling reproduction under natural conditions than under standard laboratory conditions. This illustrates the importance of studying host-parasite interactions under differing resource levels.

When infecting the crustacean *D. magna* with the bacterial parasite *Pasteuria ramosa* we found that a substantial percentage of infected animals regained reproduction. Such a regain has never been reported before in this host-parasite system. We attribute this finding to the fact that we used a natural source of growth medium in our experiments, while previous experiments have used an artificial growth medium. In addition, our findings suggest that increasing the resources available to the host can be of great benefit to the host, while being of no or only a limited benefit to the parasite. This differs from previous findings, which have shown that both hosts and parasites benefit greatly from increased resources. The findings from the system suggest that the impact of additional resources on the life histories of the host and parasite is dependent upon the resource in question.

Castration of parasitized hosts has received substantial attention with respect to its adaptive value and has been suggested to be adaptive to the parasite, the host, both or neither. However, for castration to be a host adaptation the host has to be able to regain the ability to reproduce. Our finding that *P. ramosa* infected *D. magna* could regain reproduction enabled us to test for the adaptive value of castration in this system. For two different clones, one sympatric and one allopatric, we found that host castration was only adaptive to the parasite. This supports findings from previous studies, in which the hosts were permanently castrated. In

addition, our results support the existence of local adaptation in host-parasite interactions, as the parasite was more successful in the host it was locally adapted to.

Gigantism of the host often accompanies castration and has also been suggested to be adaptive to the parasite, the host, both or neither. In the *D. magna*-*P. ramosa* system, this phenomenon has been viewed as adaptive to the parasite since the parasite benefits from infecting large host, and there has been found a positive correlation between host size and parasite fitness. However, we found that the hosts which regained reproduction were larger and contained fewer spores than the hosts which were permanently castrated. This finding questions whether host gigantism is adaptive to the parasite when the host can regain reproduction. We also suggest that permanent castration is not the norm under natural conditions in this host-parasite system. It is therefore important to further investigate the adaptive value of host gigantism, especially when hosts can regain reproduction and under natural conditions.

Finally, the fact that these studies show that *P. ramosa* infected *D. magna* can regain reproduction opens for the possibility that the parasite could be transmitted vertically, which would alter our understanding of this host-parasite system.

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## List of papers

### Paper I

**Mageroy, J. H.** and Jensen, K. H. Cost of reproduction: An environmentally induced shift in reproductive output. (Manuscript.)

### Paper II

**Mageroy, J. H.**, Grepperud, E. J. and Jensen, K. H. in Press. Who benefits from reduced reproduction in parasitized hosts? An experimental test using the *Pasteuria ramosa*-*Daphnia magna* system. *Parasitology*. Available on CJO 2011.

### Paper III

**Mageroy, J. H.**, Wathne, I., Jakobsen, P.J., and Jensen, K. H. How does host resource availability alter host-parasite interactions? *Daphnia magna* infected with *Pasteuria ramosa*. (Manuscript.)

### Paper IV

**Mageroy, J. H.**, Wathne, I., Jakobsen, P.J., and Jensen, K. H. The adaptive value of castration and gigantism in parasitized hosts when hosts can regain reproduction. (Manuscript.)

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## Introduction

The fact that environmental conditions can greatly affect the lives of organisms is well known. Environmental conditions should therefore also greatly affect the lives of hosts and their parasites. When investigating the impact of environmental conditions upon host-parasite interactions it can be useful to divide the environment into two categories both for hosts and for parasites, the latter suggested by Thomas *et al.* (2002). The two categories are the environment internal to the host and the environment external to the host. The first category has received a great deal of attention. However, the impact of the environment external to the host (hereafter referred to only as environmental conditions) on the life histories of hosts and their parasites has only started to receive attention within the last decades (reviewed in Marcogliese 2001, Thomas *et al.* 2002, Sandland & Minchella 2003, Wolinska & King 2009).

Research on the topic has primarily focused on environmental conditions such as temperature (Marcogliese 2001, Ebert 2005, Wolinska & King 2009) and nutrient availability (Marcogliese 2001, Thomas *et al.* 2002, Sandland & Minchella 2003, Ebert 2005, Wolinska & King 2009), and shows that environmental conditions can greatly affect both hosts (Marcogliese 2001, Sandland & Minchella 2003, Wolinska & King 2009) and parasites (Patz *et al.* 2000, Marcogliese 2001, Thomas *et al.* 2002, Wolinska & King 2009). However, environmental conditions would never only affect one or the other antagonist, but also the interaction between them. Environmental conditions have been found to affect host-parasite interactions (Marcogliese 2001, Wolinska & King 2009) and it is therefore important to take such conditions into consideration when studying host-parasite interactions and the evolutionary theories based upon these interactions. In addition, such findings illustrate the importance of laboratory conditions being representative of the conditions found in nature.

*Daphnia* and their numerous parasites have been extensively used to study and develop a variety of evolutionary theories on host-parasite interactions (reviewed in Ebert 2005, 2008). Although previous research has primarily focused on the environment internal to the host, the external environment has received more attention lately (reviewed in Ebert 2005, 2008). This research has primarily focused on the impact of environmental conditions, such as food quantity and quality (Ebert *et al.* 2004, Frost *et al.* 2008), and temperature, on the interaction between *D. magna* and *P. ramosa* and has shown that environmental conditions can greatly affect the relationship between the host and parasite. Temperature has for example been shown

to greatly affect the life histories of both host and parasite (Mitchell *et al.* 2005, Vale *et al.* 2008, Vale & Little 2009, Allen & Little 2011). In addition it has also been shown to alter the competitive balance between different clones of *D. magna* (Mitchell *et al.* 2005) and different isolates of *P. ramosa* (Vale *et al.* 2008). However, to our knowledge there has been no attempt at experimentally investigating the interactions between these antagonists while trying to mimic natural resource conditions. Investigating the life histories of hosts and their parasites under natural conditions is of great importance, as there is a possibility that many of the nutrients necessary for host defences are not found under the standard laboratory conditions commonly used in this system (e.g. Ebert *et al.* 2004, Jensen *et al.* 2006).

Castration of parasitized hosts is one of the outcomes of host-parasite interactions that have received a great deal of attention. This phenomenon has been considered a parasite adaptation, since the parasite could use the resources released from host reproduction to increase its own fitness through increased growth and/or reproduction. In addition, these resources could also improve the survival of the host which also could benefit the parasite (Obrebski 1975, Dawkins 1982, Ebert & Herre 1996, Jaenike 1996, Hurd, 2001, O'Keefe and Antonovics 2002, Ebert *et al.* 2004, Hall *et al.* 2007, Lafferty & Kuris 2009). Castration has also been suggested to be a host adaptation since the use of resources released from reproduction could be used to increase host resistance to the parasite and/or host survival. Such a strategy would be adaptive to the host as long as the loss in current reproduction is outweighed by a gain in future reproduction (Hurd 2001, Sorensen & Minchella 2001, Day & Burns 2003, Lafferty & Kuris 2009). Host castration could potentially also be adaptive to both antagonists (Hurd 2001). Finally, it could be a non-adaptive side effect of parasitism (Hurd 2001). Although this phenomenon has gathered such interest, there has to our knowledge been no attempt at experimentally investigating how altering the environment would affect the adaptive outcome of such interactions.

The *D. magna*-*P. ramosa* system has been important in developing theories on castration of parasite infected hosts (reviewed in Ebert 2005, 2008). Host castration is considered to be adaptive to the parasite in this system since it has been shown that the parasite benefits from castration (Ebert *et al.* 2004). Although, host castration is considered permanent in this system (Ebert 2005), there is data that question this conclusion. If treated with antibiotics the infected hosts regain reproduction, showing that the parasite does not destroy the reproductive organs of the host (Little & Ebert 2000). In addition, findings from nature are contradictory with respect to the impact of the parasite infection on host fecundity and none of the studies from nature have shown that castration is permanent (Stirnadel & Ebert 1997,

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Decaestecker *et al.* 2005). If indeed infected hosts can regain reproduction castration could be a host adaptation. It is therefore important to investigate the nature of castration in this system while mimicking natural conditions.

Gigantism of parasitized hosts often accompanies host castration and has also received a great deal of attention. This phenomenon has also been considered adaptive to the parasite as it could be a way of storing the resources released from host reproduction. In addition the greater size of hosts could benefit the parasite through increased host survival (Baudoin 1975, Sousa 1983, Dawkins 1982). The fact that gigantism could increase host survival has also led to the suggestion that it is a host adaptation (e.g. Minchella 1985, Ballabeni 1995). In addition, gigantism has also been explained as adaptive to both of the antagonists (Karban & English-Loeb 1997). Finally, it has also been seen as a non-adaptive side effect of host castration (Wright 1971, Minchella *et al.* 1985, Keas & Esch 1997, Probst & Kube 1999). To our knowledge there has been no attempt at experimentally investigating how the impact of environmental conditions can alter our understanding of the adaptive value of this phenomenon.

*P. ramosa* infected *D. magna* exhibit gigantism and this system has been important in developing the temporal storage hypothesis (Ebert *et al.* 2004). This hypothesis aims to explain why it is adaptive for certain parasite to induce gigantism in their hosts, since resources released from host reproduction through castration of the host can be stored as host growth for later use by the parasite. The findings from the *D. magna-P. ramosa* system support this hypothesis, as it is known that infected hosts grow to a greater size than uninfected individuals (e.g. Ebert *et al.* 2004; Jensen *et al.* 2006), that the parasite benefits from infecting large hosts (Mageroy *et al.* in Prep.) and that there is a positive correlation between host size and parasite spore production (Ebert *et al.* 2004). However, nothing is known about the relationship between host size and parasite spore production under natural conditions.

Primarily, the aim of this thesis was to investigate how altering the external environment of the host affects the life histories of hosts and their parasites. We wished to investigate how altering the resources available to the host and how mimicking natural conditions (paper I), affected the life histories of the host and parasite (papers II-IV). Secondly, the aim of the thesis was to gain a better understanding of evolutionary theories on castration and gigantism in parasitized hosts. We wished to investigate how altering the environment, as described above, affected our understanding of the adaptive value of host castration (papers II & IV) and gigantism (paper IV).

## Experimental system and approach

### *The host-parasite system*

*Daphnia magna* Straus (Cladocera: Crustacea) is a commonly used model organism in evolutionary ecology. The fact that it can produce clonal offspring of both sexes in addition to sexual females, makes it suitable for testing a variety of ecological and evolutionary theories. Since it also is infected by a wide variety of parasites, it has been popular for testing various theories on host-parasite interactions. In these experiments we used three different *D. magna* clones: DG-1-106 from a pond in Garzerfeld northern Germany (papers I, III and IV), Fi-X from a rock pool in Tvärminne, southern Finland (paper II) and EL-75-69 from a pond in London, UK (paper III).

The bacterial parasite *Pasteuria ramosa* Metchnikoff 1888 is an obligate parasite of *Daphnia* (Ebert *et al.* 1996) found in many natural populations (Ebert 2005). Since it induces castration and gigantism of the host under laboratory conditions (e.g. Ebert *et al.* 2004, Jensen *et al.* 2006) it has been important in developing theories on such phenomena. However, less is known about the impact of this parasite on the host in nature (but see Stirnadel & Ebert 1997, Decaestecker *et al.* 2005). In these experiments we used *P. ramosa* collected from a pond in Garzerfeld, northern Germany (papers II-IV).

### *Experimental procedure*

The primary difference between the experiments performed in this thesis and previous experiments on the *D. magna*-*P. ramosa* system is in the use of growth mediums. To our knowledge, all other laboratory studies on the system have used the artificial growth medium ADaM (Klüttgen *et al.* 1994, or as modified by Ebert *et al.* 1996, or Ebert *et al.* 1998). The two growth mediums used in these experiments were based upon lake water from Lake Myravatn, in Bergen, Norway. The lake water medium was made by filtering the water through a rough filter (20  $\mu\text{m}$ ) and a fine filter (0.20 or 0.45  $\mu\text{m}$ ), and oxygenating it before use. To make the straw water medium, 95 % lake water was mixed with 5 % straw extract. The straw extract was made by immersing 1 g hay (Sluis Comfort Hay) in 500 ml of the lake water mentioned

above. After approximately 24 h (over night) the straw extract was filtered through a 60  $\mu\text{m}$  filter and added to the filtered lake water to complete the medium.

Eurofins Norwegian Environmental Analysis AS performed the analysis of the nutritional contents of the two growth mediums. The addition of straw led to an increase, measured in mg per liter, from 0.0079 to 4.1 total phosphorous, from 2.8 to 160 total organic carbon, from 6.5 to 12.5 sulphate, from 1.5 to 26 potassium, and from 1.2 to 3.5 magnesium. For the other water quality measures performed (Alkalinity, Ca, Cl, Cu, Fe, Na, and Tot. N), there were only minor changes.

## Summary of papers

### *Paper I*

#### Cost of reproduction:

An environmentally induced shift in reproductive output.

Mageroy, J. H. & Jensen, K. H.

There is evidence supporting reproduction being costly in the form of decreased investment in other life history traits. However, there is also evidence supporting high quality individuals being able to invest more in all life history traits compared to low quality individuals. Cost of reproduction should only be found when increased reproduction is the primary factor in determining investment in other life history traits. We investigated the investment in life history traits among *D. magna* in two growth mediums with differing resource levels. Our data show that the increased resources led to increased investment in reproduction in the form of earlier maturation and increased offspring production, which was accompanied by decreased survival. These findings suggest that the increase in reproduction was costly in the form of lowered survival. However, within the growth mediums we found that survival increased with increasing investment in reproduction, indicating that high quality individuals are able to invest more in both life history traits. These findings suggest that one is only able to detect cost of reproduction, when differences in reproductive investment outweigh differences in individual quality.

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*Paper II*

Who benefits from reduced reproduction in parasitized hosts?  
An experimental test using the *Pasteuria ramosa*-*Daphnia magna* system.

Mageroy, J. H., Grepperud, E. J. and Jensen, K. H.

We investigated whether parasites or hosts benefit from reduced reproduction in infected hosts. When parasites castrate their hosts, the regain of host reproduction is necessary for castration to be a host adaptation. When infecting *Daphnia magna* with *Pasteuria ramosa*, in a lake water based medium, 49.2% of the castrated females regained reproduction. We investigated the relationship between castration level, and parasite and host fitness proxies to determine the adaptive value of host castration. Hosts which regained reproduction contained less spores and had a higher lifetime reproduction than permanently castrated hosts. We also found a negative correlation between parasite and host lifetime reproduction. For hosts which regained reproduction we found no optimal level of castration associated with lifetime reproduction. These results support the view that host castration only is adaptive to the parasite in this system. In addition, we suggest that permanent castration might not be the norm under natural conditions in this system. Finally, we argue that a reduction in host reproduction is more likely to evolve as a property favouring parasites rather than hosts. To our knowledge this is the only experimental study to investigate the adaptive value of reduced host reproduction when castrated hosts can regain reproduction.

*Paper III*

How does host resource availability alter host-parasite interactions?

*Daphnia magna* infected with *Pasteuria ramosa*.

Mageroy, J. H., Wathne, I., Jakobsen, P. J. and Jensen, K. H.

We examined how altering the resources available to the host affect the life histories of hosts and their parasites. In this study, we maintained two clones of *Daphnia magna* exposed to the parasite *Pasteuria ramosa* in two growth media of differing resource levels. Our main finding is that the increased resources greatly benefited the host in the form of increased lifetime reproduction, while being of no or only limited benefit to the parasite. This differs from previous findings from the system, which have shown that both the host and the parasite benefits substantially from an increase in resources. In addition, we found that a substantial number of the castrated hosts regained the ability to reproduce. This is only the second study to show such a regain in this system. We attribute the regain of reproduction to the use of lake water based growth mediums, since experiments using artificial growth mediums have shown little or no regain of reproduction. Finally, we also found that the prevalence of the parasite in the two host clones followed expectations based upon local adaptations in host-parasite interactions. Overall, our study shows that both environmental and genetic factors are important in determining the outcome of host-parasite interactions.

*Paper IV*

The adaptive value of castration and gigantism in parasitized hosts when hosts can regain reproduction.

Mageroy, J. H., Wathne, I., Jakobsen, P.J. and Jensen, K. H.

Host castration and gigantism is often considered to be adaptive to the parasite. However, if the host can regain reproduction these phenomena could potentially be adaptive to the host. In this study, we found that, even when *Pasteuria ramosa* infected *Daphnia magna* can regain reproduction, host castration was only adaptive to the parasite. However, we question whether host gigantism always is adaptive to the host. These conclusions were reached based upon the fact that the hosts which regained reproduction contained fewer parasite spores when controlling for host age, produced more offspring and became larger than permanently castrated hosts. Overall, our conclusions were not affected by the resources available to the host. To our knowledge, this is the only study to investigate the effect of host regain of reproduction in a sympatric host-parasite combination.

## Discussion

### *The external environment and the host*

Our findings show that the resources available to the host (straw vs. lake water) affected the life history of *D. magna*. We found that increased resources led to higher levels of offspring production (papers I and III) and an earlier onset of reproduction (paper I). In addition, the animals maintained in straw water also grew to greater sizes than those maintained in lake water (papers I and III). This increase in size can also be viewed as an investment to increase reproduction, since size has been argued to directly limit offspring production in *Daphnia* (Green 1954). We also found that increased resources led to a shorter lifespan for uninfected animals (paper I). Contradictory to this finding, we found only a non-significant difference in paper III when investigating the effect of the resources available to the animals on survival in infected, exposed but uninfected and control animals. This lack of a significant difference is likely explained by the fact that the increase in reproduction was more limited in paper III than in paper I, which should result in a smaller difference in survival. Based upon these findings, I conclude that increased reproduction due to increased resources is costly in the form of decreased survival. However, within the growth mediums we found a different picture as there was a positive correlation between reproduction and survival (paper I). This finding suggests that even among genetically identical individuals there are differences in quality and that high quality individuals are able to invest more in both aspects of life history than low quality individuals

Increased reproduction in combination with decreased survival suggest that increased reproduction can be costly, which is known as the cost of reproduction hypothesis (Williams 1966). The existence of such a phenomenon is supported by both theoretical studies (Bell 1980, Roff 1992, Stearns 1992) and empirical data (reviewed in Bell & Koufopanou 1985, Roff 1992, Stearns 1992, Obeso 2002, Harshman & Zera 2007). There are also other findings from *Daphnia* supporting an environmentally induced cost of reproduction (Becker & Boersma 2003, Weber *et al.* 2003, *Ceriodaphnia* Rose *et al.* 2000). When comparing between growth mediums, our findings therefore lend further support to the existence of a trade-off between reproduction and longevity in this system. However, within growth mediums our findings suggest that high quality individuals are able to invest more in both reproduction and survival

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compared to low quality individuals. The view that high quality individuals should be able to invest more in all life history traits has received support from both theoretical (Van Noordwijk and de Jong 1986, de Jong and Van Noordwijk 1992, Reznick *et al.* 2000) and empirical studies (reviewed in Reznick 1985, Roff 1992). In addition, there are findings from *Daphnia* that show positive associations between reproduction and other life history traits (e.g. Bell 1984, Spitze 1991, Spitze *et al.* 1991, Lynch and Spitze 1994). Our findings from between and within growth mediums therefore seem to contradict each other. However, one should only expect to be able to detect cost of reproduction when the increase in reproductive investment outweighs the effect of differences in individual quality. It should therefore not be surprising that we found support for cost of reproduction when *D. magna* offspring production nearly doubled.

It is also interesting to note that the levels of reproduction observed in straw water are close to those observed in nature (Berg 1931, Edlén 1943, Green 1954), which are typically higher than those observed under standard laboratory conditions (e.g. Chadwick & Little 2005, Duncan *et al.* 2006, Coors & De Meester 2008). In addition, decreased host survival could also affect host-parasite interactions. These findings further illustrate the importance of studying host-parasite interactions under differing availability of resources to the host.

### *The external environmental and host-parasite interactions*

Our findings show that the external environment of the host can greatly alter the life histories of hosts and their parasites. We found that when maintained in growth mediums, based upon lake water, *D. magna* infected with *P. ramosa* exhibited substantial regain of reproduction both in the percentage of infected females which regain reproduction and in the number of offspring produced after regain (papers II-IV). This was found to be true for all the three clones (Fi-X in paper II, EL-75-69 in paper III and DG-I-106 in papers III-IV) we maintained in lake water based growth mediums, suggesting that this might be a common phenomenon under such conditions. However, when comparing between lake water based growth mediums with high (straw water) and low resources (lake water) (paper III), we found no significant difference in the frequency of regain or in the duration of the castration period. This finding indicates that the regain of reproduction did not occur due to differences in the resources available to the host, between the growth mediums.

No other studies on *D. magna*-*P. ramosa* interactions have reported regain of reproduction, although when reanalysing data from an experiment from a previously published study (Jensen *et al.* 2006), we found a very limited regain of reproduction (paper III). The major difference between our experiments and previous ones is that, to our knowledge, all previous studies on this system have been performed using an artificial growth medium, ADaM (Klüttgen *et al.* 1994, or as modified by Ebert *et al.* 1996, or Ebert *et al.* 1998). I therefore conclude that the substantial regain exhibited in our studies is due to the use of lake water based mediums. Although this parasite has been shown to substantially reduce the fecundity of the host in nature (Decaestecker *et al.* 2005, but see Stirnadel & Ebert 1997), it has not been shown that the host is permanently castrated in nature. Based upon these findings, I therefore suggest that permanent castration might not be the norm under natural conditions.

We also found that in a high resource growth medium, *D. magna* can utilize the increased resources to increase reproduction and growth (paper I) and that the increased resource can alter *D. magna*-*P. ramosa* interactions (paper III). Infected hosts significantly increased their lifetime reproduction in straw water compared to lake water (paper III). This increase in lifetime reproduction was due to the fact that controls, exposed but uninfected and infected animals produced more offspring per clutch in straw water than in lake water. In addition, the infected animals were also castrated at a significantly later time in straw water than in lake water. However, with respect to parasite spore production, we only found a trend towards a significantly higher number in straw water, when controlling for host age, and no such difference when not controlling for host age. In addition, there was no significant difference in the prevalence of the parasite between the two groups. These findings show that the additional resources found in straw water was of great benefit to the host, but was of no or only limited benefit to the parasite. The fact that a substantial regain of reproduction only has been shown to occur in an environment with more resources (see papers II-III) than the standardized artificial medium (Klüttgen *et al.* 1994, or as modified by Ebert *et al.* 1996, or Ebert *et al.* 1998), further supports the conclusion that increased resources can greatly benefit the host without necessarily benefitting the parasite.

Our finding that increased resources was of great benefit to *D. magna*, but was of no or only a limited benefit to *P. ramosa*, differs from other findings from the system. Previously it has been found that the addition of resources, in the form of increased amounts of the food algae *Scenedesmus* sp. and in the form of increased phosphorous content of the same algae, led to substantial increases in both host offspring production and parasite spore production (Ebert *et al.* 2004, Frost *et al.* 2008). The latter study also showed that the prevalence of the parasite

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increased with increased phosphorous content of the food. Our findings show that the increase in host resources does not necessarily benefit both the host and the parasite. The fact that infected hosts lived shorter in straw than in lake water (paper IV) would reduce parasite survival, and could partially explain the fact that the increase in resources was of no or only limited benefit to the parasite. Overall, findings from this system show that the impact of additional resources on the life histories of hosts and their parasites is dependent upon the resources in question.

Overall, our findings from the *D. magna*-*P. ramosa* system show that the environment can affect the life histories of hosts and their parasites. This is the first series of experiments to show that the host can regain reproduction when infected with the parasite. I suggest that the regain of reproduction occurred due to the use of growth mediums from a natural source. In addition, I suggest that permanent castration might not be the norm in this system. This illustrates the importance of investigating host-parasite interactions under conditions representative of those found in nature. The lake water based mediums used in this series of experiments are probably one step closer to natural conditions. However, through filtering the lake water we may still have removed a substantial portion of the resources that are available to *D. magna* in the wild. We tried to mitigate this by adding straw water to the mediums, but even so it is necessary to investigate the characteristics of the host-parasite interaction under natural conditions.

The regain of reproduction opens up for the possibility that the parasite could be transmitted vertically. If this is the case it would contradict previous findings (Ebert *et al.* 1996) and alter our understandings of host-parasite interactions in this system. Increased host reproduction under high resource availability, without a corresponding increase in parasite reproduction, can be viewed as life history resistance to the parasite, as it could prevent the parasite from gaining access to the additional resources.

### *The adaptive value of host castration*

Our finding that *D. magna* infected with *P. ramosa* can exhibit a substantial regain of reproduction, in the form of the frequency of regain and in the form of the number of offspring produced after regain (papers II-III), opens up for the possibility that castration could be adaptive to the host. However, our data show that only the parasite benefits from castration even when hosts can regain reproduction (papers II & IV). We found that the parasite lifetime

reproduction was significantly higher in the permanently castrated hosts (only when correcting for host age in paper IV) than in the hosts which regained reproduction. With respect to host lifetime reproduction we found the opposite relationship. In addition we found a significant negative correlation between host and parasite lifetime reproduction (paper II). These findings show that the parasite benefits from reduced host reproduction and that host castration therefore is adaptive to the parasite.

That we found that the *P. ramosa* infected *D. magna* which regained reproduction produced significantly more offspring than those which did not regain reproduction (papers II & IV) shows that the host benefited from the regain in itself. However, a substantial number of the infected hosts never regained reproduction (papers II & IV) and these animals would therefore completely fail at their strategy if castration was a host strategy. Even with this in mind castration could be adaptive for the hosts which regained reproduction. For this to be the case, the host should maximize its fitness by saving resources for future reproduction. In addition, the host lifetime reproduction should be maximized for a duration or age at castration intermediate between no castration and complete castration. However, we did not find that the hosts which regained reproduction invested less in current reproduction than the permanently castrated hosts (papers II & IV). In addition, we did not find host castration to be maximized for any intermediate duration or age at castration. The only significant finding with respect to the maximization of host reproduction was to be castrated as late as possible, suggesting that no castration is optimal for the host. I therefore conclude that host castration is only adaptive to the parasite in this system.

These findings suggest that it is generally true that castration only is adaptive to the parasite, even when hosts can regain reproduction (papers II & IV), in the *D. magna*-*P. ramosa* system. Although it is known that there are strong effects of the host clone and parasite isolate on host-parasite interactions in this system (e.g., Decaestecker et al. 2003, reviewed in Ebert, 2005, 2008), the fact that our findings are based upon one sympatric (paper IV) and one allopatric (paper II) host-parasite combinations supports this view. In addition, our findings also support previous findings from the system, which have shown that the parasite benefits from castration of the host when the host is permanently castrated (reviewed in Ebert 2005, 2008).

It is also interesting to note that our findings (paper I), along with other studies (Becker and Boersma 2003, Weber *et al.* 2003), support the existence of cost of reproduction in the form of decreased survival for *Daphnia*. This finding further illustrates the importance of

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castration as it would reduce host reproduction, which would aid in increasing host survival, which would also increase parasite survival.

### *The adaptive value of host gigantism*

Our findings question whether gigantism is adaptive to the parasite when hosts can regain reproduction. We found that the *D. magna* infected with *P. ramosa* which regained reproduction were larger and produced more offspring than those which did not regain reproduction (paper IV). In addition, the former group also contained fewer parasite spores than the latter, when correcting for the age of the host. These findings suggest that the host, and not the parasite, benefited from the increased size of the animals which regained reproduction.

This conclusion seems to be contradictory to expectations based upon the temporal storage hypothesis (Ebert *et al.* 2004). According to this hypothesis one would expect a positive association between host size and parasite fitness. Support for such an association has been found in that *P. ramosa* has been shown to benefit from infecting large *D. magna* (Mageroy *et al.* in Prep.). In addition, it has also been shown a positive correlation between host size and parasite spore production (Ebert *et al.* 2004). Unfortunately, our data is not suitable for testing for the existence of such a correlation, as the size of the host and parasite spore numbers were recorded at different times. Even so, our data clearly shows that there is no positive association between host size and parasite reproduction, when hosts can regain reproduction.

We cannot explain the seemingly contradictory nature of our findings compared to previous findings from the *D. magna*-*P. ramosa* system. Therefore, it is important to further investigate the relationship between host size and parasite spore production, when hosts can regain reproduction. It would be interesting to determine the direct relationship between these two factors by recording them at the same point in time. However, if the regain of reproduction among infected hosts leads to vertical transmission it could explain our findings, as the parasite could benefit from increased host size leading to the regain of reproduction. Whether this is the case or not, it is clear that the adaptive value of gigantism in this system needs further investigation.

### *Local adaptation in host-parasite interactions*

The fact that we used several host clones in our experiments to allow for the generalization of our results, also allows us to discuss local adaptation in host-parasite interactions. In papers II and IV we used an allopatric and a sympatric *D. magna*-*P. ramosa* combination, respectively. Although the conclusions we reached about the adaptive value of host castration were the same for both papers, there were also distinct differences in the findings. We found that a much lower percentage of the hosts regained reproduction for the sympatric than the allopatric host-parasite combination. In addition, we found that the decrease in parasite spore numbers, caused by the regain of reproduction, was much smaller for the sympatric host. The opposite relationship was found to be true with respect to the percentage of host offspring produced after the regain of reproduction, compared to lifetime reproduction. The fact that the regain of reproduction had a lower impact on host and parasite fitness proxies, could explain why we found a negative correlation between host and parasite lifetime reproduction for the allopatric combination, but not for the sympatric combination. Finally, the parasite spore numbers were much higher in the sympatric host.

These findings indicate that *P. ramosa* gained greater control of the host-parasite interaction when it infected a sympatric *D. magna*. We also found further data in support of this conclusion, when comparing the prevalence of the parasite in the sympatric and allopatric hosts in paper III. These findings are in accordance with what one would expect based upon theory on host-parasite interactions and local adaptation (Lively 1989, Ebert & Hamilton 1996, Gandon *et al.* 1996, Gandon & Van Zandt 1998, Kaltz & Shykoff 1998). Data from other studies also support the existence of parasite local adaptation in our study system (Ebert *et al.* 1998, Ben-Ami *et al.* Unpubl. data in Ebert 2008). Local adaptation seems to be a general phenomenon with *Daphnia*-parasite interactions, as studies on other parasites also support its existence (Ebert 1994, Altermatt *et al.* 2007, Refardt and Ebert 2007).

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## Concluding remarks

This thesis shows that the environment can greatly affect the life histories of hosts and their parasites. We found that maintaining *P. ramosa* infected *D. magna* in growth mediums based upon lake water resulted in the regain of reproduction among many of the animals. Such a substantial regain of reproduction has never been found in previous experiments using an artificial growth medium. We therefore suggest that the regain of reproduction occurred due to the use of lake water based growth mediums and question whether permanent castration is the norm in this system. Based upon these findings, I suggest that further investigation into the life histories of the host and parasite under natural conditions is needed.

Our data also show that the increased resources in straw water, compared to lake water, were of great benefit to infected *D. magna*, but of no or little benefit to *P. ramosa*. This finding contradicts previous findings from the system which have shown that both the host and the parasite benefit greatly from increased resources. Based upon the findings from the system, it seems that different resources may impact host-parasite interactions in different ways. I therefore suggest that further investigation into the field of stoichiometry and life histories of the host and parasite is needed.

The findings from our studies support host castration being adaptive only to the parasite, even when hosts can regain reproduction, in the *D. magna-P. ramosa* system. The conclusion from our studies and previous studies with permanent castration are therefore the same. This suggests that host castration generally can be viewed as adaptive to the parasite. However, no one has investigated whether the host is permanently infected and/or castrated in nature. If the host can clear the parasite it could change the understanding of the adaptive value of host castration in this system. Investigations into the nature of host infection and castration under natural conditions would therefore be beneficial.

Based upon our data, we question whether gigantism always is adaptive to the parasite in the *D. magna-P. ramosa* system. We found that the hosts which regained reproduction were the largest and contained the fewest spores. This is the opposite of what we would expect if gigantism benefits the parasite. I therefore suggest that further investigation into the adaptive value of gigantism is necessary in this system.

Finally, the fact that *D. magna* can regain reproduction also opens up for the possibility that *P. ramosa* could be transmitted vertically. If this was found to be true, it would alter our

understanding of host-parasite interactions in this system. Among other things it would alter our understanding of host castration and gigantism. It is therefore important to investigate whether or not vertical transmission occurs when the host can regain reproduction.

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