

# In situ growth of juvenile cockles, *Cerastoderma edule*, experimentally infected with larval trematodes (*Himasthla interrupta*)

Anne Margrethe Wegeberg, K. Thomas Jensen\*

Department of Marine Ecology, Institute of Biological Sciences, University of Aarhus, Finlandsgade 14, DK-8200 Aarhus N, Denmark

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## Abstract

To examine the influence of larval trematodes (metacercariae) on survivorship and growth of bivalves, juvenile cockles were experimentally infected with *Himasthla interrupta* and afterwards deployed in enclosures on an intertidal flat at two different tidal levels for one month. The hypothesis is that the impact of such metacercariae varies with food availability and environmental conditions. The experimental cockles exhibited significantly different growth rates at the two study sites, but there was no discernible impact of the metacercariae on survivorship and shell-growth. Body condition, however, differed among infected and non-infected cockles at the site with the shortest immersion time. Both the experimentally infected and non-infected cockles lost body condition during their incubation in the laboratory because of starvation. Infected cockles, however, lost more flesh weight than the non-infected, and this difference was maintained for high-shore cockles during the in situ experiment, whereas both infected and non-infected low-shore cockles (living under more optimal food conditions) had regained their pre-experimental body condition. In conclusion, it seems that *Himasthla interrupta* metacercariae are non-exploitative and relatively harmless under moderate infection intensities and normal environmental conditions.

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## 1. Introduction

Bivalves from marine shallow water ecosystems are hosts to several digenean trematode species that are potentially pathogenic and thus may influence the population dynamics of their hosts (Lauckner, 1983; Sousa, 1991). In particular, digeneans using bivalves as first intermediate host have a severe impact on hosts as

their offspring production is reduced or they become castrated by reproducing trematode larvae located in the gonads (Lauckner, 1983; Campbell, 1985; Kabat, 1986; Jonsson and André, 1992; Jokela et al., 1993; Holopainen et al., 1997; Taskinen, 1998). In contrast, trematodes that use their bivalve host as a transport organism (paratenic), linking them to the definitive host through prey-predator relationships, are supposed to inflict less harm. The larval stage (metacercaria) of these parasites is often enclosed in a cyst located in more or less species-specific regions of the host such as connective tissue of the foot region, in the mantle,

\* Corresponding author.

E-mail address: kthomas@biology.au.dk (K.T. Jensen).

siphons, gills or palps (Lauckner, 1983; Wegeberg et al., 1999), where they remain relatively inactive and serve as resistant hypobiotic stages (Chappell, 1993). As they do not reproduce inside the bivalves their exploitation of host resources is expected to be modest. Nonetheless, parasites using their bivalve host as second intermediate host have also been reported to cause severe harm. Reported effects induced by the presence of metacercariae in bivalves include: reduced ability to burrow (Bowers et al., 1996; Thomas and Poulin, 1998; Jensen et al., 1999), reduced byssal thread production (Lauckner, 1983), reduced tolerance of anoxia (Wegeberg and Jensen, 1999), impaired growth (Calvo-Ugarteburu and McQuaid, 1998) and possibly enhanced mortality (Goater, 1993; De Montaudouin et al., 2000). Except in cases where parasites induce altered host behaviour to promote their transmission to the final host, it appears maladaptive to reduce the fitness of a host significantly. In any case, following an infection event, bivalve hosts have to repair penetration holes and damaged tissue. Host response also includes the accumulation of haemocytes and fibrous tissue around the parasite cyst (Lauckner, 1983; Jensen et al., 1999). The energetic cost of the host response is unknown and probably depends on the intensity of the infections. Consequently, the effect of such parasites can be expected to interact with the availability of food, host condition and environmental factors influencing the energy budget of bivalves.

As many bivalve populations exhibit high frequencies of trematode infections, knowledge of host effects of such trematodes is important. Field observations of the prevalence patterns of trematodes in bivalves indicate a dominance of species utilising bivalves as their second intermediate hosts (De Montaudouin et al., 2000).

Demonstration of their effects under laboratory conditions may not be sufficient for extrapolating to natural conditions fluctuating in environmental conditions and food availability (Thomas et al., 2000). Field experiments with infected organisms may therefore be an important methodological approach to assess the significance of trematodes to wild populations. In the present study a field experiment was performed in the Wadden Sea with juvenile cockles, *Cerastoderma edule* L., as host organism. The cockle is important in the Wadden Sea ecosystem and occurs in densities of up to several thousand adult individuals per m<sup>2</sup>

(Dankers and Beukema, 1981; Jensen, 1992). The cockle is host to a dozen trematode species in shallow water ecosystems (Lauckner, 1971; De Montaudouin et al., 2000). Among these, *Himasthla* species may be prevalent (De Montaudouin et al., 2000). Cercariae produced by prosobranch gastropods (*Littorina*, *Hydrobia*) penetrate cockles primarily in the foot and visceral region of the foot after being inhaled through the inhalent siphon (Wegeberg et al., 1999) where they remain as metacercariae until the cockle is eaten by a bird. We investigate the effect of metacercariae of the trematode *H. interrupta* Loos-Frank, 1967 (first intermediate host: *Hydrobia*) on growth, body condition and survival of juvenile *C. edule*. Juvenile cockles were chosen as experimental animals rather than adults because: (1) they can show a measurable growth response within a short period, (2) *H. interrupta* has the highest infection efficiency in small cockles (Wegeberg et al., 1999), and (3) small cockles are much easier to examine for parasites and will generally have fewer natural infections than adults (age dependent). Experimentally infected cockles were deployed in cages at two sites along an intertidal gradient reflecting different levels of environmental stress and food supply.

## 2. Materials and methods

### 2.1. Study site

The field experiment was performed at Emmerlev Klev (near Højer) in the Danish Wadden Sea (54°59'N, 8°39'E). The sediment type was fine sand with a median grain size within the range of 125–170 µm, and a fauna belonging to the *Macoma baltica* community. The mean tidal amplitude is about 1.8 m in this area.

Juvenile cockles were collected on 8 August 1996 in the intertidal zone at Skallingen (northern part of the Danish Wadden Sea). They were stored in running seawater (filtered) (20 °C, 27‰) and fed with algae caught with a plankton net until establishment in the field.

### 2.2. Natural infections

*Hydrobia ulvae* specimens collected in the Danish Wadden Sea were screened for *Himasthla* infections

by incubating the snails individually in Petri-dishes under light (20–25 °C) to induce shedding of cercariae from infected snails. *H. interrupta* cercariae were identified by counting flame cells in viable specimens from each infected snail (Loss-Frank, 1967). To assess the intensity of natural infections in the experimental cockles, 63 individuals of the cockles collected from Skallingen were dissected and examined for metacercariae. The soft parts of the cockles were squeezed in between two microscope slides and observed under a dissecting microscope. Metacercariae from the *Himasthla* species from the Wadden Sea can be identified according to their sizes (Lauckner, 1983; De Montaudouin et al., 2000).

### 2.3. Experimental infections

Viable and infective cercariae were obtained from a stock of 55 *H. interrupta*-infected *H. ulvae* incubated in water (27‰ S) under light at 24 °C. Twenty-five cercariae emitted within one hour were collected with a pipette and transferred to Petri-dishes containing 10 cm<sup>3</sup> of seawater (27‰ S). Cockles with a shell-length of 3.5–4.5 mm were added individually to each Petri-dish and left for infection 24 h at 20 °C under an artificial light source. Control cockles were prepared at the same time by incubating specimens individually in Petri-dishes without cercariae and kept for 24 h under identical conditions. Infected and control cockles were then transferred to separate aquariums with identical conditions of food supply and running water. Within 2 w a total of 350 controls and 350 infected cockles were prepared. The field experiment was established immediately hereafter.

### 2.4. Field experiment

To study the influence of parasites on growth and survival of juvenile cockles a factorial experiment was conducted in which infection level (infected cockles vs. control cockles) and immersion time (high shore site: HSS vs. low shore site: LSS) were the two fixed factors. Five replicates were used for each combination of immersion time and infection level. The experimental units were cages made of a PVC tube (d = 16 cm, l = 30 cm) each containing 35 cockles (corresponding to a density of 1740 individuals m<sup>-2</sup>). The upper part of the

tube had 8 holes, each 3 cm in diameter, covered with 1000 µm nylon mesh to ensure water flow through the cage. Likewise, a 1000 µm nylon mesh was fastened as a lid on top of the PVC-tube.

The field experiment started on 3 September 1996. The bottoms of the cages were pushed 20 cm into the sediment at low tide leaving the mesh-covered holes above the sediment surface. Enclosed sand was removed to a depth of 20 cm and each cage was refilled with ambient sediment sieved through a 500 µm mesh to avoid the presence of macrozoobenthic organisms before cockles were incubated in the cages. The water depth at the LSS was about 1 m when the tide reached the HSS and the immersion time differed by about 3 h per tidal cycle between the two experimental sites. The cages were inspected every 10 d to avoid clogging of the mesh.

One month after establishment, the cockles from the cages were collected, measured and dissected. Infection intensity and position of metacercariae in all cockles were determined.

Seventy-five individuals (15 from each cage) from each site (HSS, LSS) and treatment (infected, non-infected) were used for determination of site and treatment specific length-weight regressions. Flesh from the

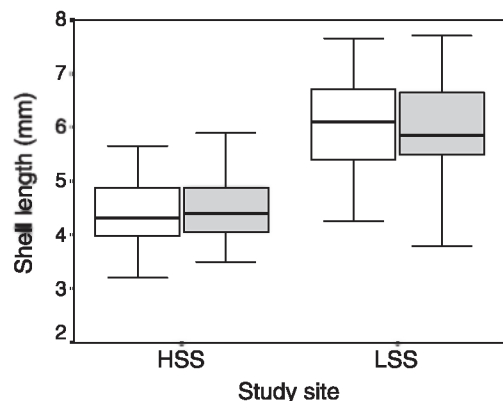


Fig. 1. Boxplot (median, 25% and 75% quartiles) of the size distribution of uninfected (control) cockles and cockles infected with *H. interrupta* from experimental cages from the two study sites (HSS and LSS) after one month on the intertidal flat near Emmerlev Klev (Danish Wadden Sea) (white fill: non-infected cockles; grey fill: experimentally infected cockles). A line is drawn from the top of the box (75% quartile) to the largest cockle observed and from the bottom of the box (25% quartile) to the smallest cockle observed.

Table 1

Nested analysis of growth data from the two sites along the tidal gradient (LSS and HSS)

Source of variation	SS	df	MS	F	p
<i>Low-shore site</i>					
Treatment	1.10	1	1.10	1.57	p>0.05
Between cages	5.59	8	0.70		
Within cages	226.70	300	0.76		
<i>High-shore site</i>					
Treatment	0.77	1	0.77	1.48	p>0.05
Between cages	3.15	6	0.52		
Within cages	60.31	240	0.25		

The two data sets were analysed separately because of significant heteroscedasticity caused by more variance in growth rates at the HSS than at the LSS. The treatment factor - (infected vs. non-infected) is a fixed factor whereas cage is a random factor. The effect of treatment is tested against the between-cages (within infection level) factor (Underwood, 1997). The data matrix was balanced by randomly removing data from each cage until all contained 31 specimens. For the HSS-station only 4 cages for each treatment were applied as one cage failed (SS: sums of squares; df: degrees of freedom; MS: mean squares).

individual cockles was carefully collected from the cover glass after microscopical inspection and transferred to a crucible and oven-dried at 105 °C for 24 h. Afterwards the crucibles were incinerated at 500 °C for 24 h. Ash-free-dry-weight (AFDW) of the soft-parts was determined and length-AFDW regressions estimated.

Daily length-specific growth rates ( $r$ ) of cockles were determined by the formula:  $l_t = l_0 \exp^{rt}$  ( $l_0$  = mean length of cockles at start of the experiment;  $l_t$  = mean length of cockles after one month;  $t$  = time in days) (Kautsky, 1982).

Statistical analyses were performed using the statistical package SPSS 10.0 for Windows. For details of the analyses see Results section.

### 3. Results

Cockles collected for the field experiment accommodated only very few infections. The average intensity was 0.15 metacercariae per cockle and only 15% of the cockles were infected. To produce infected cockles for the experiment, individual specimens were exposed to viable *H. interrupta* cercariae shed by infected mud snails (*H. ulvae*). In total, 99% of the

cockles exposed to the cercariae became successfully infected. On average, they acquired 12 metacercariae each and 90% of these were located in the foot.

The survival rate of cockles during the 30-d experimental period was generally high: 96.7% (S.D. = 3.8), except in one cage with infected cockles at the HSS where an unintended enclosure of one *Nereis diversicolor* specimen caused a low survival rate (54%). Neither parasites nor study site influenced mortality rate in our field experiment (Kruskal-Wallis test,  $p>0.1$ ).

Cockle growth was higher at the low water site (LSS) than at the high water site (HSS). There was no difference in size of infected and non-infected cockles from either experimental site at the end of the experiment (Fig. 1 and Table 1). Therefore, a site-specific growth rate can be calculated from the data. During the 30-d experiment, caged cockles from the LSS showed an increase in mean shell length from 3.9 mm (S.D. = 0.4) to 5.9 mm (S.D. = 0.9) corresponding to a specific growth rate of  $0.0138 \text{ d}^{-1}$ . At the HSS the mean shell length of the cockles increased only from 3.9 mm (S.D. = 0.4) to 4.5 mm (S.D. = 0.5) corresponding to a specific growth rate of  $0.0048 \text{ d}^{-1}$ .

There was a significant difference between the two sites with respect to the fraction of cockles that had passed the upper size (4.5 mm) used in the experiment. At the HSS, only 43.8% ( $n=322$ ) had passed the size threshold of 4.5 mm, whereas 94.5% ( $n=343$ )

Table 2

Summary of the ANCOVA results

Source of variation	df	Mean squares	F-ratio	P
<i>Low-shore site</i>				
Treatment	1	0.004	0.390	0.533
Length	1	3.604	346.683	0.000
Residual	144	0.010		
<i>High-shore site</i>				
Treatment	1	0.123	9.58	0.002
Length	1	4.842	378.15	0.000
Residual	147	0.013		

Impact of treatment (infected vs. non-infected) and length ( $\log_{10}$ ) (covariate) from the two experimental plots (HSS vs. LSS) on body weight ( $\log_{10}$  AFDW) of the experimental cockles. Prior to these analyses, variance homogeneity among the residuals was verified ( $p > .05$ ) using separate length-weight regressions for each treatment level for predicting the body weight.



at the LSS exceeded 4.5 mm at the end of the experiment ( $\chi^2 = 202.79$ ,  $p < 0.01$ ). There was no difference between infected and non-infected cockles in this respect.

It was also examined whether body-mass-index (BMI) [= weight/length<sup>3</sup>] differed among cockles below and above 4.5 mm in October (at the end of the experiment) for infected and non-infected specimens at the HSS. There was no difference in BMI between cockles below and above 4.5 mm (ANOVA,  $F_{1,146} = 1.528$ ,  $p > 0.05$ ) but infected cockles had 12% lower BMI than non-infected (ANOVA,  $F_{1,146} = 9.276$ ,  $p < 0.01$ ).

The potential for cockle growth at the upper site during the experimental period is indicated by the growth of the non-manipulated cockles collected from the experimental plot outside the enclosures. They grew significantly from a mean shell length of 5.1 mm (S.D. = 1.5) to 8.0 mm (S.D. = 2.1) (t-test,  $t = -7.038$ ,  $df = 74$ ,  $p < 0.001$ ), corresponding to a specific growth rate of  $0.01494 \text{ d}^{-1}$ .

To examine the possible impact of parasites on condition, weights ( $\log_{10}$  AFDW) of cockles from the various treatments were compared by using ANCOVA's with length ( $\log_{10}$ ) as covariate (Table 2). Data from the two sites were analysed separately because of the limited overlap in shell lengths of cockles. Whereas there was no effect of metacercariae on cockle weight at the LSS, infected and non-infected cockles differed significantly at the HSS (Table 2). In

conclusion, a common length-weight regression line can be used to predict the weight of cockles from the LSS, whereas separate length-weight regressions are needed for infected and non-infected cockles from the HSS (Table 3). As a result, the weight of a 5 mm infected cockle from the HSS was 83% of the weight of an uninfected cockle from the same site and only 74% of the weight of a LSS-cockle (Table 3).

There was no significant correlation between parasite intensity and shell length, or parasite intensity and ash-free dry weight (regression analysis,  $r^2 \leq 0.07$ ;  $p > 0.05$ ).

## 4. Discussion

### 4.1. In situ growth

As cockles at the LSS experienced a longer immersion time and thus a higher supply of food than cockles at the HSS, the observed site difference in growth rate of cockles was expected. Several authors (Kreger, 1940; Reise, 1985; Jensen, 1992; De Montaudouin, 1996) have reported increased shell growth of cockles with immersion time. However, the growth rates of the enclosed cockles were lower than expected for the experimental period (Jensen, 1992). Extant juvenile cockles from the upper-shore site (HSS) exhibited a substantial increment in shell length in contrast to the caged cockles at this site during the experimental period (1 month), indicating a positive balance between food supply and metabolic demand of cockles at this tidal level. The cages were hardly the cause of the poor growth of experimental cockles at the HSS because enclosed cockles from the LSS showed close to normal increment in shell length. So, what is then the explanation of the slow growth among caged cockles at the HSS? The experimental cockles had been kept in the laboratory one month prior to the experiment to obtain the infections. During this period they were not fed optimally and growth ceased as evidenced by growth marks on the shells. As a result, body condition may have declined prior to the deposition in the field. Food consumed during the in situ experiment was probably allocated to the re-conditioning of cockles at the HSS, whereas the supply of food to the LSS-cockles, because of the longer submersion time, was sufficient for both reconditioning and shell increment.

Table 3

The relationship between shell-length (mm) and flesh AFDW ( $\mu\text{g}$ ) for the experimental specimens of *Cerastoderma edule* after finishing the field experiment at Emmerlev Klev according to the equation,  $\log_{10} \text{ weight} = b \log_{10} \text{ length} + \log_{10} a$ , with correlation coefficient ( $r^2$ )

Site	Intercept (log a)	Slope (b)	$r^2$	Weight ( $\mu\text{g}$ ) (5 mm cockle)
<i>Low-shore site</i>				
All	1.104	2.4	0.70	604.7
<i>High-shore site</i>				
Non-infected	0.129	3.724	0.79	539.5
Infected	0.312	3.349	0.65	449.6

Separate regression equations are provided for infected and non-infected cockles from the HSS, whereas a common regression equation describes the LSS - data in accordance with the ANCOVA results (see Table 1).

A rough estimate of the specific growth rate (based on weight) of a normal cockle growing at the HSS can be estimated by using the growth rate based on length of the extant cockles and the length-weight regression of the caged cockles at the LSS (Table 2). Using these data the following growth formula can be prepared:  $w_t = w_0 \exp^{0.03586 \cdot t}$  [ $w_t$ : AFDW in  $\mu\text{g}$  at time  $t$ ;  $w_0$ : AFDW in  $\mu\text{g}$  at start;  $t$ : time in days]. From this, the time needed to reach the weight of a 5 mm cockle from the LSS can be estimated. So, non-infected cockles ( $w = 539.5 \mu\text{g}$  at the end of the experiment) needed 3.2 d to reach the final weight, whereas infected cockles needed 8.3 d.

#### 4.2. Impact of parasite cysts

The present experiment indicates that a moderate intensity of cysts of *H. interrupta* does not inflict much damage to the cockles, and that the effect will be untraceable after a relatively short period with natural food supplies. We were able to detect an effect of parasites on body condition apparently only because the cockles were starved unintentionally in the laboratory prior to the field experiment. Cockles established at the HSS were not able to regain their former condition within the experimental period in contrast to those from the LSS. As a result, the cockles experimentally infected with *H. interrupta* still had lower body condition than non-infected cockles after 30 d in the field at the HSS corresponding to a delay of about 5 d of growth at the HSS. Nevertheless, this impact, even if reversible, shows that *Himasthla* cysts do impose some kind of energetic demand on their host. Penetrating cercariae produce holes in the epidermis of the foot, which enables exudates to leak from the cockle tissue (Lauckner, 1983) and necessitates repair of penetration ducts. Histological studies have indicated a host-produced tissue layer surrounding *Himasthla* cysts located in the connective tissue of the foot (Jensen et al., 1999). Furthermore the infection could induce some other sort of immune responses in the host during and after the infection.

Cockles have a high plasticity towards hunger and they may lose a considerable fraction of their flesh content (AFDW) without dying (Thomsen, 1991). It is evident that apart from impaired shell growth, the costs of losing weight will include reduced reproductive output until they have regained their standard

condition. Infections may also reduce the tolerance threshold of the host organism towards adverse environmental conditions such as oxygen depletion, salinity fluctuations and extreme temperatures (Pascoe and Woodworth, 1980). As an example, *H. elongata* metacercariae have been shown to decrease survival rates of infected cockles exposed to oxygen deficiency (Wegeberg and Jensen, 1999).

The intensity of the parasite infection may likewise be important for the impact of parasites on their host organism. In the present experiment no significant correlation between parasite intensity and shell length, or parasite intensity and ash-free dry weight was found. This lack of correlation could be due to the moderate infection intensity and the narrow range of parasite intensities studied. The discrepancy between the effects reported here and the effects of *H. elongata* reported by Lauckner (1983, 1984) could be due to differences in parasite intensity or environmental conditions of the cockles studied. Furthermore, cercariae and cysts of *H. elongata* are larger than those of *H. interrupta*, and thus may inflict more tissue damage. Reproducing stages of trematodes are often pathogenic to their hosts, whereas quiescent stages such as metacercariae that use their second intermediate host only as a paratenic host are considered more or less harmless.

However, this is not necessarily the case even if the pathological effects of metacercariae are benign. As many parasites capitalise on established prey-predator relationships, there has been an evolutionary impetus for the parasites to alter host behaviour to promote their transmission to the final host. Such induced behavioural modifications of intermediate hosts have been reported from a variety of taxonomical groups including bivalves. A typical bivalve response on parasites is a reduced ability to burrow (Lauckner, 1983; Bowers et al., 1996; Thomas and Poulin, 1998; Jensen et al., 1999; Mouritsen, 2002). From New Zealand, evidence of enhanced consumption of such surfacing bivalves infected with metacercariae has been provided from a parasite-host system similar to the system here reported (Thomas and Poulin, 1998). It is possible that *H. interrupta*-infected cockles also remain longer on the sediment surface and thus become more accessible to water birds. Nonetheless, *H. interrupta* cysts themselves do not appear to impose a significant energetic drain on their bivalve intermediate host.

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