

Inventory of organisms interfering with transmission of a marine trematode

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*It has increasingly been recognized that organisms can interfere with parasitic free-living stages, preventing them from infecting their specified host and thus reducing infection levels. This common phenomenon in freshwater and terrestrial systems has been termed the ‘dilution effect’ and, so far, is poorly studied in marine systems. Ten common intertidal organisms found in the Dutch Wadden Sea (North Sea) were tested to establish their effects on the free-living cercarial stages of the trematode parasite *Himasthla elongata*. Most species tested resulted in a significant reduction in cercariae over a 3 hr time period. The amphipod *Gammarus marinus* removed 100% of the cercariae, while other effective diluters were *Crangon crangon* (93%), *Sargassum muticum* (87%), *Semibalanus balanoides* (71%), *Crassostrea gigas* (67%), *Hemigrapsus takanoi* (>54%), *Crassostrea gigas* shells (44%) and *Idotea balthica* (24%). In contrast, mixed shells (*Cerastoderma edule*, *Mytilus edulis*, *Ensis americanus* and *Littorina littorea*) and *Fucus vesiculosus* had no significant effect. These results suggest that dilution effects are widespread in the trematode of *H. elongata*, with potentially strong effects on its population dynamics.*

Keywords: dilution effect, parasite ecology, trematode, *Himasthla elongata*, marine parasitology

Submitted 15 August 2013; accepted 23 December 2013; first published online 11 March 2014

INTRODUCTION

Parasites are ubiquitous in marine ecosystems and can have effects on individual hosts, host populations, communities and entire ecosystems (Mouritsen & Poulin, 2002). For example, they can manipulate the behaviour of their host (Swennen, 1969; Edelaar *et al.*, 2003; Thomas *et al.*, 2005; Bates *et al.*, 2011), reduce fecundity and reproduction (Lafferty, 1993; Mouritsen & Poulin, 2002; Fredensborg *et al.*, 2005) and contribute to mortality (Jensen & Mouritsen, 1992; Mouritsen & Jensen, 1997), which can subsequently alter competition and predation interactions among hosts. Furthermore, parasites can play a vital role in marine ecosystems by significantly contributing to biomass (Kuris *et al.*, 2008), as well as affecting the topology and stability of food webs (Lafferty *et al.*, 2008; Dunne *et al.*, 2013). However, more recently it has been shown that parasites can also act as a resource themselves; thus, local community composition of consumers of parasites can strongly influence parasite dynamics by interfering with transmission pathways (Johnson *et al.*, 2010). This interference, which removes parasites from the system and prevents successful host infection, has been termed the ‘dilution effect’, and can lead to a reduction in disease risk (Keesing *et al.*, 2006; Johnson & Thieltges, 2010).

Dilution effects on macroparasite transmission are widespread and have been particularly well studied in trematode

parasites from freshwater ecosystems (for review see Thieltges *et al.*, 2008a). Trematodes have complex lifecycles, with vertebrates being used as definitive hosts. Miracidia, a free-living stage released from the definitive host, infect the first intermediate host (a mollusc) from which a second free-living stage (termed cercariae) is released. These cercariae have a short lifespan (usually <1 d) and infect a second intermediate host (invertebrates or fish, depending on the parasite species). When the second intermediate host is consumed by a definitive host the cycle is closed allowing the parasite to sexually reproduce and for the cycle to start again (Galaktionov & Dobrovolskij, 2003). Recent studies have shown that the free-living stages of trematodes are also subjected to dilution effects in marine ecosystems (Mouritsen & Poulin, 2003; Hopper *et al.*, 2008; Thieltges *et al.*, 2008b; Kaplan *et al.*, 2009; Prinz *et al.*, 2009; Studer *et al.*, 2013). A particularly strong factor seems to be predation by non-host species, either through active or passive predation. For example, it is postulated that some marine predators such as shrimps, may actively seek and consume free-living stages, whilst others, such as filter feeders, may not selectively but accidentally ingest cercariae. Another mechanism can be physical obstacles, e.g. algae, which may obstruct the cercariae from reaching the host. These examples illustrate how ambient fauna and flora may interfere with free-living trematode cercariae, thus removing infective stages from the system and reducing the disease risk for down-stream hosts in marine ecosystems.

For most marine parasite species and localities we lack an inventory of potential diluting organisms, making it difficult to evaluate the generality of dilution effects. This is also true for trematodes in the Wadden Sea, an extensive area of marine-to-estuarine intertidal mudflats and sub-tidal gullies

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along the Danish, German and Dutch coast, dominated by benthic molluscs and polychaetes (van der Graaf *et al.* 2009). The high production of invertebrates is used by a plethora of fish and birds, making it an ideal habitat for trematodes due to the presence of the necessary sequential hosts (Thieltges *et al.*, 2012). Dilution effects have only been studied in one of the dominant local trematodes, *Himasthla elongata*. This trematode uses the periwinkle *Littorina littorea* as first and mussels and cockles as second intermediate and birds as definitive hosts (Werding, 1969). In a field experiment also conducted in the Wadden Sea, dilution effects by Pacific oysters (*Crassostrea gigas*) could be observed which reduced infection levels of *H. elongata* in blue mussels (*Mytilus edulis*) more than three-fold (Thieltges *et al.*, 2009). Laboratory experiments conducted at the Limfjord, a large brackish water system in the north of Denmark, suggest that other species also interfere with transmission of cercariae of *H. elongata*, namely the filter-feeding gastropod *Crepidula fornicata*, the crustacean predators *Crangon crangon* and *Carcinus maenas* and the bivalve *Mya arenaria* (Thieltges *et al.*, 2008b). However, it remains unclear if the experimental results from the study in the Limfjord can be transferred to different ecosystems, and what other species might cause dilution effects in this trematode species.

Here, our aim is to experimentally test the dilution potential of various common species from the Wadden Sea with regard to *Himasthla elongata* cercariae. In addition, from our results and other examples from the literature, we compile an extended inventory of diluters of *H. elongata* in different marine ecosystems.

MATERIALS AND METHODS

Selection of diluters

A range of potential diluters (predators, filter feeders, live biotic obstacles and dead biotic obstacles) were collected from the Wadden Sea, along the eastern coast of Texel, The Netherlands (Table 1). The organisms were chosen due to their observed presence and abundance in intertidal areas of

Table 1. Organisms used in the different treatments, their expected dilution mechanism and densities or fresh weights used in the experiments per 1.7 l tanks containing 1.5 l filtered seawater.

Treatment	Mechanism	Density/wet weight/volume
<i>Sargassum muticum</i>	Live biotic obstacle	25 g (\pm 1 g)
<i>Fucus vesiculosus</i>	Live obstacle	25 g (\pm 1 g)
<i>Crassostrea gigas</i> shells	Dead biotic obstacle	40 g (\pm 10 g)
Mixed shells (<i>Cerastoderma edule</i> , <i>Mytilus edulis</i> , <i>Ensis americanus</i> , <i>Littorina littorea</i>)	Dead biotic obstacle	85 g (\pm 1 g)
<i>Gammarus marinus</i>	Predator	3 (10 mm each)
<i>Idotea balthica</i>	Predator	4 (12 mm each)
<i>Crangon crangon</i>	Predator	6 (30 mm each)
<i>Hemigrapsus takanoi</i>	Predator	3 (30 mm each)
<i>Semibalanus balanoides</i>	Filter feeder	2.5 cm ²
<i>Crassostrea gigas</i>	Filter feeder	1 (40 ml \pm 10 ml vol. each)

the Wadden Sea where all *Himasthla elongata* hosts can be found (primarily on hard bottom structures like dykes, mussel and oyster beds). Densities used in the experiments were kept at levels observed in the field. Upon returning to the laboratory, all organisms and physical objects were cleaned and any epibionts carefully removed. The organisms were then stored in tanks (60 × 30 × 30 cm) filled with filtered and aerated seawater within a climate controlled room at 15°C (based on air temperatures at time of collection).

Source of cercariae

Periwinkles (*Littorina littorea*) collected from the field were screened for the presence of *H. elongata* infections by keeping them in an incubator at 25°C for several hours and checking for shed cercariae. Infected snails were then separated and kept in aquaria. To obtain cercariae for the experiments, 150–200 infected snails were incubated in 1.8 l of filtered seawater at 27°C and under light for 3 h to encourage the release of cercariae. The water and cercariae (hereafter termed 'broth') was drained into a 2 l beaker. The broth was then gently stirred (anticlockwise three times and then clockwise three times using a plastic spoon), and immediately after 50 ml of broth was scooped out of the beaker using a small measuring jug and added to experimental containers (1.7 l), resulting in a uniform infection dose. Eight samples of 50 ml were also taken to get an average of the number of parasites added to each container.

Experimental set-up

Five experiments were carried out, each testing two different diluters versus a control. For each experiment eight replicates were used for each of the three treatments. Each replicate consisted of a 1.7 l container with 1.5 l of filtered seawater randomly placed in a single climate controlled room at 18.5°C (\pm 0.2°C), a typical water temperature which occurs during the summer transmission period.

All organisms were starved and kept in the experimental containers for 24 h prior to adding the cercariae for acclimation. At the start of the experiment, 50 ml of cercariae broth was added to each container (see above). After 3 h large diluters were quickly removed by forceps and discarded; smaller diluters were fixed along with the cercariae. Experiments were run for 3 h as survival of cercariae usually starts to decrease after about 10 h (Thieltges & Rick, 2006), to avoid the decrease in cercarial survival confounding the effects of diluters (maximum age of cercariae in our experiments was 6 h, including the 3 h incubation period). At the end of the experiment, all water from the containers was filtered through a 25 µm sieve to retain any remaining cercariae. Containers were then rinsed and filtered twice. Subsequently, cercariae were washed from the sieve with 50–100 ml filtered seawater and fixed using 10 ml 96% ethanol and stained using rose Bengal. Cercariae were then counted in Petri dishes under a light microscope.

Statistical analysis

The effect of a potential diluter versus the respective control was tested using an ANOVA followed by Dunnett's family error *post-hoc* test. All ANOVAs were conducted on raw, untransformed data as all experiments proved to be normally

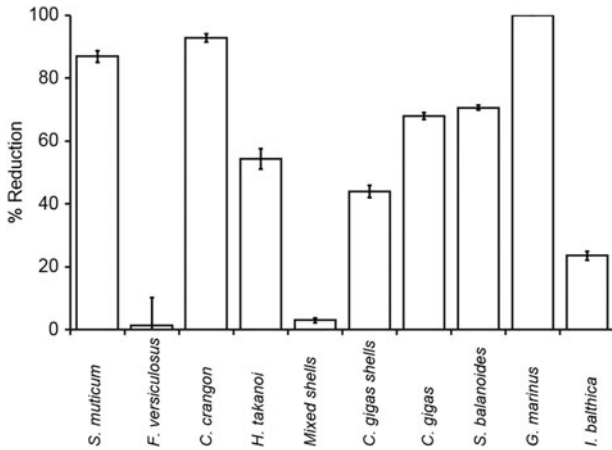


Fig. 1. Percentage (\pm standard error) of cercariae of *Himathla elongata* removed from the experimental containers by the different potential diluters compared to the relevant control.

distributed after checking for normality and homoscedasticity. Finally, the percentage of cercariae removed by each potential diluter (versus the mean of the respective control) was calculated. All results shown are means \pm standard error.

RESULTS

Overall, most species tested resulted in a reduction of cercariae of more than 40% (Figure 1; Table 2). The grazer *G. marinus*, the predator *C. crangon* and the live biotic obstacles *S. muticum* removed the most amount of cercariae (87–100%). Other species, *H. takanoi*, *C. gigas shells*, *C. gigas*, *S. balanoides* and *I. balthica*, removed between 24% and 71% (Figure 1; Table 2). However, *F. vesiculosus* and mixed

bivalve shells did not show a significant reduction (Figure 1; Table 2).

The average number of cercariae recovered from controls decreased with each experiment (Table 2). Whilst an average of 116 cercariae were recovered in the controls of the first experiment (testing *S. muticum* and *F. vesiculosus*), an average of only 11 was observed in the final experiment (which tested *G. marinus* and *I. balthica*). This reduction was also shown in the sub-samples (decreasing from 128 ± 6 to 38 ± 4 , 23 ± 2 , 17 ± 2 and 14 ± 1 , respectively) and was presumably due to depletion of the cercarial production by the pool of snails during the repeated shedding procedures. However, as we only compare the respective controls with the treatments within each experiment, such a reduction in production does not affect the overall results.

DISCUSSION

The experiments showed that most of the potential diluters tested significantly reduced the number of cercariae, with all significant diluters resulting in a $>24\%$ reduction. Only two of the 10 tested organisms did not show any dilution effect. This suggests that dilution effects on the trematode *H. elongata* are widespread, with the potential to cause strong effects on the parasite’s population dynamics.

The results shown here are in line with previous findings from a study conducted in the brackish Limfjord in Denmark, where the shrimp *Crangon crangon* and the bivalve *Crassostrea gigas* also strongly reduced the numbers of cercariae of *H. elongata* (Thieltges *et al.*, 2008b; Table 3). This suggests that dilution effects on this trematode species may be more widespread than shown previously and are not confined to specific habitats or ecosystems. The Limfjord study also found additional diluters not tested in this study

Table 2. Mean number of cercariae (\pm standard error) recovered from tanks containing different potential diluting organisms in five different experiments. Controls contained no diluting organism. Also shown are the *P* values from Dunnett’s family error *post-hoc* tests, which compared controls with the respective treatments and the overall ANOVA results for each experiment; *N* = 8 replicates per treatment. Degrees of freedom for all *F* values = 23. SE, standard error; *P*, significance level of test.

Experiment	Treatment	Mean No. of cercariae	SE	<i>P</i>
1	Control	115.75	20.56	–
	<i>Sargassum muticum</i>	15.25	1.89	<0.001
	<i>Fucus vesiculosus</i>	114.13	8.77	0.944
ANOVA: <i>F</i> = 19.75				
2	Control	31.13	2.75	–
	<i>Crangon crangon</i>	2.25	1.24	<0.001
	<i>Hemigrapsus takanoi</i>	14.25	3.27	0.002
ANOVA: <i>F</i> = 31.91				
3	Control	21.38	1.03	–
	<i>Crassostrea gigas shells</i>	12.00	0.84	0.005
	Mixed shells (<i>Cerastoderma edule</i> , <i>Mytilus edulis</i> , <i>Ensis americanus</i> and <i>Littorina littorea</i>)	20.75	1.93	0.647
ANOVA: <i>F</i> = 15.02				
4	Control	14.00	1.39	–
	<i>C. gigas</i>	4.50	1.05	<0.001
	<i>Semibalanus balanoides</i>	4.13	0.72	<0.001
ANOVA: <i>F</i> = 26.46				
5	Control	10.63	1.85	–
	<i>Gammarus marinus</i>	0.00	0.00	<0.001
	<i>Idotea balthica</i>	8.13	1.38	<0.001
ANOVA: <i>F</i> = 17.35				

Table 3. Organisms shown to reduce free living *Himasthla elongata* cercariae in the Limfjord, Denmark (Thieltges *et al.*, 2008b, 2009) and in the Wadden Sea (this study); ns, no significant effect.

Taxon	Species	Reduction (%) of infections or cercariae	
		Limfjord	Wadden Sea (this study)
Amphipod	<i>Gammarus marinus</i>	Not tested	100%
Barnacle	<i>Semibalanus balanoides</i>	Not tested	71%
Bivalve	<i>Crassostrea gigas</i>	95–99%	67%
Bivalve	<i>Macoma balthica</i>	ns	Not tested
Bivalve	<i>Mya arenaria</i>	64%	Not tested
Decapod	<i>Carcinus maenas</i>	65%	Not tested
Decapod	<i>Crangon crangon</i>	78%	93%
Decapod	<i>Hemigrapsus takanoi</i>	Not tested	54%
Gastropod	<i>Crepidula fornicata</i>	93–99%	Not tested
Isopod	<i>Idothea balthica</i>	Not tested	24%
Macroalgae	<i>Fucus vesiculosus</i>	Not tested	ns
Macroalgae	<i>Sargassum muticum</i>	Not tested	87%
Shells	Mixed (non-oyster) shells	Not tested	ns
Shells	Oyster shells	Not tested	44%

(Table 3). Our study now adds several new diluting species, including filter feeders and dead and live biotic obstacles, to the list of known diluters of *H. elongata*. While filter feeder ingest cercariae via their filtration current, the effect of biotic obstacles is different. The debris from organisms such as shells probably act as a physical barrier: cercariae become entangled in the structure preventing the free-living parasite stages from getting to their hosts within their short lifespan. The convoluted structure of oyster shells may explain why dilution effects were observed in the oysters shell (44%) but not in the mixed shell treatment (consisting of species with much smoother shell surfaces). Similarly, differences in structural complexity probably also explain the observed differences between the two algae species (see detailed discussion below). However, other mechanisms such as olfactorial cues or other exudates cannot completely be ruled out and deserve further study.

The resulting inventory of diluters shows that a wide range of organisms interferes with the transmission of *H. elongata* cercariae (Table 3). In general, we observed relatively high dilution rates and it can be questioned whether this reflects actual dilution rates under natural conditions in the field. In addition to the biotic dilution effect there are also various abiotic factors (e.g. temperature and salinity) known to affect the transmission stages of trematodes (Pietroock & Marcogliese, 2003; Thieltges & Rick, 2006; Studer & Poulin, 2013). This suggests that the observed dilution effects may actually be simply compensatory rather than additive, since mortality of cercariae is very high in the field anyway. However, there is evidence from a field experiment that dilution effects can be additive and comparably high in more natural settings: treatments with Pacific oysters showed a more than three-fold reduction in infection levels of target hosts with *H. elongata* compared to controls without oysters, despite the presumed high natural background mortalities of cercariae (Thieltges *et al.*, 2009).

The interference caused by diluters on cercarial transmission may potentially have strong effects on trematode population dynamics, and thus, have consequences for the hosts. Metacercarial infections (resulting from penetrating cercariae)

of trematodes have been shown to have a range of detrimental effects on their invertebrate intermediate hosts, including reducing survival and growth (Jensen *et al.*, 1998; Desclaux *et al.*, 2004; Fredensborg *et al.*, 2005; Thieltges, 2006). Since such effects are usually density-dependent (Fredensborg *et al.*, 2005; Thieltges, 2006), any reductions of parasite loads will relieve the hosts from the negative effects of infections. Here we looked at single species effects on dilution rates. However, in natural settings, cercariae will encounter many different identities and densities of diluters with the potential for a multitude of compensatory, additive or synergistic effects on dilution rates resulting in an interesting avenue for further research.

In general, the strength of the dilution effect seems to depend more on the diluter identity than on the respective dilution mechanism. Direct comparison among different diluters and mechanisms is difficult because the strength of the dilution effect is related to the density of diluters (Thieltges *et al.*, 2009). However, for two diluters a direct comparison is possible, since the same weight was used for the two algae species, *S. muticum* and *F. vesiculosus*. While *S. muticum* significantly reduced cercariae by 87%, *F. vesiculosus* did not. The difference between the two species probably results from the fact that *S. muticum* has a very fine branching habitus, leading to the entanglement of cercariae, while *F. vesiculosus* has broad blades which probably do not trap cercariae. Interestingly, *S. muticum* is classed as an invasive species in the Wadden Sea, and this draws attention to potential 'positive' impacts that non-native organisms may have on their new habitat (Buschbaum *et al.*, 2006; Thieltges *et al.*, 2006). Here, it was shown that *S. muticum* may alleviate native host organisms from parasites more than the native *F. vesiculosus* which is found to grow in the same locality. Dilution effects on cercariae of *H. elongata* were also caused by other invasive species, e.g. the Pacific oyster *Crassostrea gigas* and the American slipper limpet *Crepidula fornicata* (this study; Thieltges *et al.*, 2009). Similar effects have also been observed in other trematode species. For example, the invasive barnacle *Austrominius modestus* interferes with the transmission of cercariae of *Echinostephillia patellae* and *Parorchis acanthus* (Prinz *et al.*, 2009). Likewise, the invasive algae *Caulerpa taxifolia* interfere with the transmission of various trematode species, presumably due to toxic exudates (Bartoli & Boudouresque, 1997). These examples point to the potential importance of invasive species in mediating parasite–host interactions with potential 'positive' effects on native hosts in contrast to the usually perceived 'negative' effects (Harvell *et al.*, 2004; Kopp & Jokela, 2007; Kelly *et al.*, 2009; Keesing *et al.*, 2010).

In conclusion, the experiments in this study have produced an extended inventory of diluters, and indicate that there are many non-host species that interfere with the transmission of cercarial stages of *H. elongata*. Together with published data from other marine parasites and systems this suggests that trematode transmission can be interfered with by a multitude of organisms. However, despite increasing evidence of biotic factors interfering with parasite transmission pathways, there is still much that is unknown about how and under what conditions organisms remove the most parasites. Therefore, the future challenge is to determine the mechanisms which result in a reduction in disease risk caused by diluting organisms, and to understand the effects of whole communities on pathogen transmission pathways.

ACKNOWLEDGEMENTS

We would like to thank Hans Witte, Siem Gieles, Rob Dekker, Anouk Goedknegt and Andreas Wasser for their assistance with collecting the organisms. We also thank the two referees for their comments.

REFERENCES

- Bartoli P. and Boudouresque C.** (1997) Transmission failure of parasites (Digenea) in sites colonized by the recently introduced invasive alga *Caulerpa taxifolia*. *Marine Ecology Progress Series* 154, 253–260.
- Bates A.E., Leiterer F., Wiedeback M.L. and Poulin R.** (2011) Parasitized snails take the heat: a case of host manipulation? *Oecologia* 167, 613–621.
- Buschbaum C., Chapman A.S. and Saier B.** (2006) How an introduced seaweed can affect epibiota diversity in different coastal systems. *Marine Biology* 148, 743–754.
- Desclaux C., de Montaudouin X. and Bachelet G.** (2004) Cockle *Cerastoderma edule* population mortality: role of the digenean parasite *Himasthla quissetensis*. *Marine Ecology Progress Series* 279, 141–150.
- Dunne J.A., Lafferty K.D., Dobson A.P., Hechinger R.F., Kuris A.M., Martinez N.D., McLaughlin J.P., Mouritsen K.N., Poulin R., Reise R., Stouffer D.B., Thieltges D.W., Williams R.J. and Zander C.D.** (2013) Parasites affect food web structure primarily through increased diversity and complexity. *PLoS Biology* 11, e1001579. doi:10.1371/journal.pbio.1001579.
- Edelaar P., Drent J. and de Goeij P.** (2003) A double test of the parasite manipulation hypothesis in a burrowing bivalve. *Oecologia* 134, 66–71.
- Fredensborg B., Mouritsen K. and Poulin R.** (2005) Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. *Marine Ecology Progress Series* 290, 109–117.
- Galaktionov K.V. and Dobrovolskij A.A.** (2003) *The biology and evolution of trematodes: an essay on the biology, morphology, life cycles, transmission, and evolution of digenetic trematodes*. Dordrecht: Kluwer Academic Publishers.
- van der Graaf S., de Vlas J., Herlyn M., Voss J., Karin H. and Drent J.** (2009) Macrozoobenthos. Thematic Report No. 10. Wadden Sea Ecosystem No. 25. In Marencic H. and Vlas J. de (eds) *Quality Status Report 2009. Wadden Sea Ecosystem No. 25*. Wilhelmshav: Common Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group.
- Harvell D., Aronson R., Baron N., Connell J., Dobson A., Ellner S., Gerber L., Kim K., Kuris A., McCullum H., Lafferty K., McKay B., Porter J., Pascual M., Smith G., Sutherland K. and Ward J.** (2004) The rising tide of ocean diseases: unsolved problems and research priorities. *Frontiers in Ecology and the Environment* 2, 375–382.
- Hopper J.V., Poulin R. and Thieltges D.W.** (2008) Buffering role of the intertidal anemone *Anthopleura aureoradiata* in cercarial transmission from snails to crabs. *Journal of Experimental Marine Biology and Ecology* 367, 153–156.
- Jensen K.T. and Mouritsen K.N.** (1992) Mass mortality in two common soft-bottom invertebrates, *Hydrobia ulvae* and *Corophium volutator*—the possible role of trematodes. *Helgoländer Meeresuntersuchungen* 46, 329–339.
- Jensen T., Jensen K.T. and Mouritsen K.N.** (1998) The influence of the trematode *Microphallus claviformis* on two congeneric intermediate host species (*Corophium*): infection characteristics and host survival. *Journal of Experimental Marine Biology and Ecology* 227, 35–48.
- Johnson P.T.J., Dobson A., Lafferty K.D., Marcogliese D.J., Memmott J., Orlofske S.A., Poulin R., and Thieltges D.W.** (2010) When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends in Ecology and Evolution* 25, 362–371.
- Johnson P.T.J. and Thieltges D.W.** (2010) Diversity, decoys and the dilution effect: how ecological communities affect disease risk. *Journal of Experimental Biology* 213, 961–970.
- Kaplan A.T., Rebhal S., Lafferty K.D. and Kuris A.M.** (2009) Small estuarine fishes feed on large trematode cercariae: lab and field investigations. *Journal of Parasitology* 95, 477–480.
- Keesing F., Belden L.K., Daszak P., Dobson A., Harvell C.D., Holt R.D., Hudso P., Jolles A., Jones K.E., Mitchell C.E., Myers S.S., Bogich T., and Ostfeld R.S.** (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 468, 647–652.
- Keesing F., Holt R.D. and Ostfeld R.S.** (2006) Effects of species diversity on disease risk. *Ecology Letters* 9, 485–498.
- Kelly D.W., Paterson R.A., Townsend C.R., Poulin R. and Tompkins D.** (2009) Parasite spillback: a neglected concept in invasion ecology? *Ecology* 90, 2047–2056.
- Kopp K. and Jokela J.** (2007) Resistant invaders can convey benefits to native species. *Oikos* 116, 295–301.
- Kuris A.M., Hechinger R.F., Shaw J.C., Whitney K.L., Aguirre-Macedo L., Boch C.A., Dobson A.P., Dunham E.J., Fredensborg B.L., Huspeni T.C., Lorda J., Mababal L., Mancini F.T., Mora A.B., Pickering M., Talhouk N.L., Torchin M.E. and Lafferty K.D.** (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* 454, 515–518.
- Lafferty K.** (1993) Effects of parasitic castration on growth reproduction and population dynamics of the marine snail *Cerithidea californica*. *Marine Ecology Progress Series* 96, 229–237.
- Lafferty K.D., Allesina S., Arim M., Briggs C.J., De Leo G., Dobson A.P., Dunne J.A., Johnson P.T., Kuris A.M., Marcogliese D.J., Martinez N.D., Memmott J., Marquet P.A., McLaughlin J.P., Mordecai E.A., Pascual M., Poulin R. and Thieltges D.W.** (2008) Parasites in food webs: the ultimate missing links. *Ecology Letters* 11, 533–546.
- Mouritsen K.N. and Jensen K.T.** (1997) Parasite transmission between soft-bottom invertebrates: temperature mediated infection rates and mortality in *Corophium volutator*. *Marine Ecology Progress Series* 151, 123–134.
- Mouritsen K.N. and Poulin R.** (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* 124, S101–S117.
- Mouritsen K.N. and Poulin R.** (2003) The mud flat anemone–cockle association: mutualism in the intertidal zone? *Oecologia* 135, 131–137.
- Pietroock M. and Marcogliese D.J.** (2003) Free-living endohelminth stages: at the mercy of environmental conditions. *Trends in Parasitology* 19, 293–299.
- Prinz K., Kelly T.C., O’Riordan R.M. and Culloy S.C.** (2009) Non-host organisms affect transmission processes in two common trematode parasites of rocky shores. *Marine Biology* 156, 2303–2311.
- Studer A., Kremer L., Nelles J., Poulin R. and Thieltges D.W.** (2013) Biotic interference in parasite transmission: can the feeding of anemones counteract an increased risk of parasitism in amphipods at higher temperature? *Journal of Experimental Marine Biology and Ecology* 445, 116–119.
- Studer A. and Poulin R.** (2013) Cercarial survival in an intertidal trematode: a multifactorial experiment with temperature, salinity and ultraviolet radiation. *Parasitological Research* 112, 243–249.
- Sweenen C.** (1969) Crawling-tracks of trematode infected *Macoma baltica* (L.) *Netherlands Journal of Sea Research* 4, 376–379.

- Thieltges D.** (2006) Effect of infection by the metacercarial trematodes *Renicola roscovita* on growth in intertidal blue mussel *Mytilus edulis*. *Marine Ecology Progress Series* 319, 129–134.
- Thieltges D.W., Bordalo M.D., Hernández A.C., Prinz K. and Jensen K.T.** (2008a) Ambient fauna impairs parasite transmission in a marine parasite-host system. *Parasitology* 135, 1111–1116.
- Thieltges D.W., Engelsma M.Y., Wendling C.C. and Wegner K.M.** (2012) Parasites in the Wadden Sea food web. *Journal of Sea Research* 82, 122–133.
- Thieltges D.W., Jensen K.T. and Poulin R.** (2008b) The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology* 135, 407–26.
- Thieltges D.W., Reise K., Prinz K. and Jensen K.T.** (2009) Invaders interfere with native parasite–host interactions. *Biological Invasions* 11, 1421–1429.
- Thieltges D.W. and Rick J.** (2006) Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode *Renicola roscovita* (Digenea: Rencolidae). *Diseases of Aquatic Organisms* 73, 63–68.
- Thieltges D.W., Strasser M. and Reise K.** (2006) How bad are invaders in coastal waters? The case of the American slipper limpet *Crepidula fornicata* in western Europe. *Biological Invasions* 8, 1673–1680.
- Thomas F., Adamo S. and Moore J.** (2005) Parasitic manipulation: where are we and where should we go? *Behavioural Processes* 68, 185–199.
- and
- Werdning B.** (1969) Morphologie, Entwicklung und Ökologie digener Trematoden-Larven der Strandschnecke *Littorina littorea*. *Marine Biology* 3, 306–333.

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