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The gastropod parasitic nematode

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***Phasmarhabditis hermaphrodita* does not affect**

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non-target freshwater snails *Lymnaea stagnalis*,

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***Bithynia tentaculata* and *Planorbarius corneus*.**

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19 **Abstract**

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21 *Phasmarhabditis hermaphrodita* is a lethal parasite of several slug and
22 snail species that has been formulated into a biological control agent. However,
23 the complete host range of this nematode is poorly understood, in particular its
24 potential to affect non-target aquatic snail species. Here we exposed three
25 species of juvenile and adult freshwater snail (*Lymnaea stagnalis*, *Planorbium*
26 *corneus* and *Bithynia tentaculata*) to 30 and 150 *P. hermaphrodita* per cm² and
27 assessed survival, as well as differences in weight for 66 days. We show that *P.*
28 *hermaphrodita* has no effect on the survival of *L. stagnalis*, *P. corneus* and *B.*
29 *tentaculata* after 66 days of exposure. In summary, we found little evidence of *P.*
30 *hermaphrodita* causing mortality to three freshwater snail species at two
31 different life stages and believe that *P. hermaphrodita* would have little effect on
32 non-target snail species in the wild.

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34 **Keywords**

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36 Slugs, aquatic snails, parasites, non-target organisms.

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42 The gastropod parasitic nematode *Phasmarhabditis hermaphrodita* is a
43 lethal parasite of several pest slugs and snails including *Deroceras reticulatum*

44 and *Arion ater* (Wilson et al., 1993; 2000) and has been formulated into a
45 biological control agent (Nemaslug®) for farmers and gardeners in Northern
46 Europe available from BASF-Becker Underwood (Rae et al., 2007). Once applied
47 nematodes seek out slugs and snails, responding to mucus and faeces, then
48 penetrate through the mantle and kill the host in between 4 and 21 days (Rae et
49 al., 2006; 2009a; Wilson et al., 1993; Tan and Grewal, 2001). *P. hermaphrodita*
50 has been used to protect many crops from slug damage including Chinese
51 cabbage (Rae et al., 2009b), winter wheat (Wilson et al., 1994) and oilseed rape
52 (Wilson et al., 1995).

53 The complete host range of *P. hermaphrodita* is poorly understood and
54 many slug and snail species have never been tested for their susceptibility
55 towards this nematode. One group of molluscs that have been neglected are
56 freshwater snails. There are only two studies that have focused on investigating
57 the effects of *P. hermaphrodita* on aquatic snails, which showed that under lab
58 conditions *P. hermaphrodita* can kill the non-target snail *Lymnaea stagnalis* but
59 not *Physa fontalis* (Wilson et al., 1993; Morley and Morrill, 2006). Here we
60 decided to investigate whether *P. hermaphrodita* could kill three common non-
61 target species of freshwater snail including the Great Pond snail (*L. stagnalis*),
62 the Great Ram's-horn snail (*Planorbis corneus*) and *Bithynia tentaculata*,
63 which are common, widely distributed snails which live in slow moving and
64 large ponds (Beedham, 1972). We also decided to examine whether the
65 susceptibility of snails to *P. hermaphrodita* could be due to differences in size as
66 previously it has been shown that *P. hermaphrodita* can kill juveniles of the snail
67 *Helix aspersa*, and the slugs *A. ater* and *A. lusitanicus* but adults remain resistant
68 (Glen et al., 1996; Grimm, 2002).

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70 **Materials and Methods**

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72 **Source of invertebrates**

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74 *P. hermaphrodita* was purchased from BASF-Becker Underwood and was
75 stored at 10°C prior to use. Freshwater snails (*L. stagnalis*, *B. tentaculata* and *P.*
76 *corneus*) were supplied by Sciento, U.K. and collected from ponds at Calderstones
77 Park, Liverpool. Snails were kept in fresh water at 10°C prior to use.

78

79 **Infection assay with freshwater snails exposed to *P. hermaphrodita***

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81 *P. hermaphrodita* were mixed with tap water and numbers of nematodes per 100 μ l
82 were quantified. Non-airtight plastic boxes (10 x 9 x 6 cm) were filled with 120 ml of
83 fresh water. Evaporation of water was monitored by weighing boxes every 5 days
84 and adding fresh pond water if necessary to maintain approximately the same
85 volume throughout the experiment. To three boxes the recommended rate of *P.*
86 *hermaphrodita* was applied (30 nematodes per cm^2) and to another three boxes five
87 times the recommended rate was applied (150 per cm^2). Three boxes received no
88 nematodes and acted as the controls. For the first experiment, ten juvenile *L.*
89 *stagnalis* (mean weight = 0.48 ± 0.03 g, n = 90) were added to each box. To
90 investigate the difference in weight of snails when infected with *P. hermaphrodita*
91 we also exposed adult *L. stagnalis* to a high dose of 150 *P. hermaphrodita* per cm^2
92 (mean weight = 4.03 ± 0.13 g, n = 60). This experimental set up was also repeated for

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93 both sizes of *P. corneus* (juvenile mean weight = 0.118 ± 0.004 , n = 90; adult mean
94 weight = 2.14 ± 0.12 , n = 60) and only one size of *B. tentaculata* (mean weight =
95 0.302 ± 0.006 , n = 90) was exposed to 0, 30 and 150 *P. hermaphrodita* per cm². All
96 species of snails were weighed before and after the experiment to determine if the
97 nematode caused any effect on weight gain and food consumption which has been
98 documented in other molluscan species (Glen et al., 2000). Snails were provided
99 with food including pond weed and cabbage *ad libitum*.

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100 Survival was monitored every 3-4 days for 66 days. Any dead snails were
101 dissected to examine nematode penetrance.

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103 **Data analysis**

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105 Survival of snails was analysed using the log rank test carried out in
106 OASIS (Yang et al., 2011) and the weight of snails before and after nematode
107 treatment was compared using a Student t-test.

108

109 **Results**

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111 **The effect of *P. hermaphrodita* on the survival of juvenile and adult *L.***
112 ***stagnalis*, *P. corneus* and *B. tentaculata*.**

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115 *P. hermaphrodita* applied at both 30 and 150 nematodes per cm² had no
116 significant effect on the survival of juvenile or adult *L. stagnalis* after 66 days
117 exposure ($P > 0.05$) (Fig 1a, b). Similarly, *P. hermaphrodita* had no effect on the

118 survival of both juvenile and adult *P. corneus* at both doses (30 and 150
119 nematodes per cm²) ($P>0.05$) (Fig 2a, b). Also adult *B. tentaculata* were resistant
120 to both doses of *P. hermaphrodita* as there were no significant differences in
121 survival over 66 days ($P>0.05$) (Fig 3). Therefore, *P. hermaphrodita* had no effect
122 on the survival of three species of aquatic snails when applied at two different
123 doses for 66 days.

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125 **The effect of *P. hermaphrodita* on the weight of juvenile and adult *P.*** 126 ***corneus* and adult *B. tentaculata***

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128 There was no significant difference between the weight of juvenile or
129 adult *P. corneus* on day 0 and day 66 when exposed to no nematodes, 30 and 150
130 *P. hermaphrodita* per cm² ($P>0.05$) (Fig 4a, b). However, there was a significant
131 difference between the weight of *B. tentaculata* on day 0 and day 66 ($P<0.001$)
132 (Fig 4c), but this was the case for the untreated and both doses of *P.*
133 *hermaphrodita*, hence these snails lost weight in general throughout the
134 experiment regardless of treatment. Therefore, *P. hermaphrodita* has no effect on
135 the weight gain of aquatic snails.

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137 **Discussion**

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139 Previous studies have shown that *P. hermaphrodita* may affect non-target
140 aquatic molluscs including *L. stagnalis* (Wilson et al., 1993; Morley and Morritt,
141 2006). However, in our studies we have shown that *P. hermaphrodita* is unable
142 to kill a selection of non-target freshwater snails including *L. stagnalis*, *B.*

143 *tentaculata* and *P. corneus* at two different doses of *P. hermaphrodita* (30 and
144 150 nematodes per cm²) after 66 days exposure. Ultimately, this study shows
145 that *P. hermaphrodita* poses little risk to non-target fresh water snails.

146 The host range of *P. hermaphrodita* is best characterized in terrestrial slugs and
147 snails. Pestiferous slugs such as *D. reticulatum* and *D. panormitanum* are highly
148 susceptible to *P. hermaphrodita* (Wilson et al., 1993) but other species such as
149 *Limax maximus* and *L. pseudoflavus* (Rae et al., 2008; Grewal et al., 2003) are
150 resistant. Resistance in other species is dependent on size as adult *A. lusitanicus*
151 and *A. ater* are resistant to *P. hermaphrodita* but juveniles are susceptible (Glen
152 et al., 1996; Grimm, 2002). Similarly, in terrestrial snails, some species of
153 terrestrial snails are resistant to *P. hermaphrodita* including *Cepaea nermoralis*,
154 *Oxychilus helveticus*, *Pnentina ponentina*, *Discus rotundatus* and *Clausilia*
155 *bidentata* (Wilson et al., 2000; Coupland, 1995; Iglesias et al., 2003). It is
156 unknown why there are these differences in susceptibility to *P. hermaphrodita*
157 but some terrestrial snails, such as the Giant African snail (*Achatina fulica*) have
158 the ability to encapsulate and kill invading nematodes in their shell (Williams
159 and Rae, 2015), which has also been shown in slugs (Rae et al., 2008). However,
160 upon dissection of dead snails no encapsulated nematodes were observed so
161 perhaps this defensive ability is only in terrestrial molluscs. Similarly, we rarely
162 found *P. hermaphrodita* inside the snails, but this is not uncommon when *P.*
163 *hermaphrodita* is exposed to other snails e.g. *H. aspersa* (Rae et al., 2009a). Either
164 it is harder for *P. hermaphrodita* to penetrate into snails than slugs or our
165 experimental assay is suppressive to nematode infection. However, this seems
166 unlikely as two studies (Morley and Morrirt, 2006 and Wilson et al., 1993)
167 showed that *P. hermaphrodita* can kill *L. stagnalis* under similar conditions. One

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168 important factor maybe the way snails were reared. Morley and Morritt (2006)
169 showed that laboratory reared *L. stagnalis* were susceptible to *P. hermaphrodita*
170 In our study we collected snails from the wild, which have been exposed to an
171 array of naturally occurring parasites and may potential have a stronger immune
172 system and are able to cope with *P. hermaphrodita*. Perhaps laboratory reared *L.*
173 *stagnalis* used in Morley and Morritt (2006) may potentially have unchallenged
174 and impaired immune systems, which made them more susceptible to *P.*
175 *hermaphrodita*?

176 In conclusion we have shown that *P. hermaphrodita* has little
177 pathogenicity towards wild caught freshwater snails and therefore poses little
178 threat to non-target aquatic snails.

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