Filtering out parasites: Sand crabs (*Lepidopa benedicti*) are infected by more parasites than sympatric mole crabs (*Emerita benedicti*)

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Two digging decapod crustaceans, the sand crab species *Lepidopa benedicti* and the mole crab species *Emerita benedicti*, both live in the swash zone of fine sand beaches. They were examined for two parasites that infect decapod crustaceans in the region, an unidentified nematode previously shown to infect *L. benedicti*, and cestode tapeworm larvae, *Polypocephalus* sp., previously shown to infect shrimp (*Litopenaeus setiferus*). *Lepidopa benedicti* were almost always infected with both parasite species, while *E. benedicti* were rarely infected with either parasite species. This difference in infection pattern suggests that tapeworms are ingested during sediment feeding in *L. benedicti*, which *E. benedicti* avoid by filter feeding. Larger *L. benedicti* had more *Polypocephalus* sp. larvae. The thoracic ganglia, which make up the largest proportion of neural tissue, contained the largest numbers of *Polypocephalus* sp. larvae. Intensity of *Polypocephalus* sp. infection was not correlated with how long *L. benedicti* remained above sand in behavioural tests, suggesting that *Polypocephalus* sp. do not manipulate the sand crabs in a way that facilitates trophic transmission of the parasite. *L. setiferus* may be a primary host for *Polypocephalus* sp., and *L. benedict* may be a secondary, auxiliary host.

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2 than sympatric mole crabs (*Emerita benedicti*)

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11   **INTRODUCTION**

12   Parasites can generalize and infect many host species, or specialize to infect

13   only a small number of host species (only one host species in extreme cases). A benefit

14   of being a specialist may be increased adaptation to a host species. Specialization

15   should be favoured in endoparasites that manipulate host behaviour (Adamson & Caira

16   2011; Fredensborg 2014), because the nervous systems generating behaviour are

17   probably anatomically and physiologically more variable than other types of tissue

18   (Bullock 1993; Bullock 2004; Bullock 2006).

19   Sand crabs (*Lepidopa benedicti*) and mole crabs (*Emerita benedicti*) are digging

20   anomuran crustaceans in the same superfamily (Hippoidea), which are both found in

21   the swash zones of sandy beaches in the Gulf of Mexico. Given that they are related

22   and found in the same habitat, it is reasonable to predict that they might have similar

23   parasites to each other. *Lepidopa benedicti* is often infected by an unidentified

24   nematode species that does not appear to manipulate host behaviour (Joseph &

25   Faulkes 2014), which might suggest the nematode is a generalist that would also infect

26   *E. benedicti*.

27   Other parasites that infect decapod crustaceans where these two species live are

28   larval cestode tapeworms in the genus *Polypocephalus*, which infect white shrimp

29   (*Litopenaeus setiferus*) (Carreon, Faulkes & Fredensborg 2011; Carreon & Faulkes

30   2014). Although the life cycle of species in this genus is not completely worked out, it

31   seems likely that it has a two part life cycle (Figure 1): crustaceans (Villella, Iversen &

32   Sindermann 1970; Owens 1985; Shields 1992; Hudson & Lester 1994; Brockerhoff &

33   Jones 1995; Payne 2010) and other invertebrates (Cake 1979) for the larval stage, and

34 elasmobranch fishes (e.g., skates and rays) as the definitive hosts for adults (Butler

35 1987; Call 2007; Koch 2009). *Polypocephalus* spp. larvae infect multiple species from at

36 least two phyla (Cake 1979; Owens 1985; Brockerhoff & Jones 1995), suggesting that

37 species in this genus are generalists. *Polypocephalus* sp. inhabit the nervous system of

38 crustaceans, and appear to manipulate behaviour in *L. setiferus* (Carreon, Faulkes &

39 Fredensborg 2011), which are factors that suggest species in this genus are specialists.

40   *Polypocephalus* sp. is also a candidate for studying the manipulation of host

41 behavior, because the larval stage infects the neural tissue of their decapod crustacean

42 hosts. Being in or near the nervous system would seem to make such manipulation

43 easier for parasites. In white shrimp, increased infection was correlated with increased

44 activity of the host (Carreon, Faulkes & Fredensborg 2011), which was hypothesized to

45 be a case of parasite-induced trophic transmission (PITT). A trophically transmitted

46 parasite in a digging crustacean might be excepted to change the behaviour of its host

47 so it spends more time above sand (Joseph & Faulkes 2014). *Litopenaeus setiferus* do

48 dig into sand (Eldred et al. 1961; Fuss 1964; Pinn & Ansell 1993), and their increased

49 activity with infection would be consistent with the prediction above.

50   This paper compares the patterns of infection in *L. benedicti* and *E. benedicti* for

51 both nematode and cestode parasites, and tests whether *Polypocephalus* sp.

52 manipulates the behaviour of either of these species as they do with shrimp (Carreon,

53 Faulkes & Fredensborg 2011).

54   **METHODS**

55   Sand crabs (*Lepidopa benedicti*) and mole crabs (*Emerita benedicti*) were

56 collected from the beaches of South Padre Island, Texas. Individuals were sexed by

57 examining pleopod size (longer in females) and the carapace length was measure with

58 digital calipers. Different individuals were used to study nematodes and *Polypocephalus*

59 sp.

60   Individuals were anaesthetised by chilling for ~20 minutes on crushed ice,

61 dissected in sea water, and the nerve cord was removed. The nerve cord was cut into

62 smaller sections, which were pinned in dishes lined with Sylgard (Dow Corning). The

63 nerve cords were dehydrated in a progressive ethanol series (70% ethanol for 5

64 minutes, 90% ethanol for 5 minutes, 100% ethanol for 5 minutes, then 100% ethanol

65 again for 10 minutes), cleared in methyl salicylate on a depression slide, viewed under a

66 compound microscope (Olympus CX41), and photographed (Olympus C-5050 Zoom

67 digital camera). In some cases, consecutive images at different focal points in the Z axis

68 were compiled into a single image using Helicon Focus v. 6.7.1 Lite (Helicon Soft Ltd.).

69   Initial observations of 10 individuals of each species indicated that variation in

70 numbers of parasites infecting *L. benedicti* was sufficient to test whether there was a

71 correlation between infection and host behaviour. Because *E. benedicti* had very little

72 variation in the number of parasites, their behaviour was not examined.

73   Behavioural tests were similar to those described in Joseph & Faulkes (2014).

74 Individuals were video-recorded digging in a tank 300 mm wide × 150 mm deep × 200

75 mm high, filled with ~75 mm of sand from South Padre Island covered by ~75mm of

76 seawater. Video was recorded with an iPad 3 using Coach My Video v. 4.4

77 (http://www.coachmyvideo.mobi). Individuals were released at the top of the tank, and

78 was filmed until the carapace was covered by sand. The total time was calculated by

79 subtracting the submergence times from release time (rounded down to whole

80   seconds). Individual made three digging trials, each separated by a 5 minute rest period

81   when the animal was not disturbed to minimize habituation. The average of the three

82   trials was used for analysis.

83   The behaviour of crabs fell into three basic categories. An individual could (1)

84   immediately dig into sand ("direct"), (2) stay above sand by tailflipping and rowing its

85   legs (Faulkes & Paul 1997) before digging ("swim"), or; (3) remain on the top of the

86   sand, immobile, before digging ("sit"). "Swim" and "sit" are not mutually exclusive. An

87   individual could do both in one trial, in either order. For simplicity of analyses,

88   individuals that both swam and "sat" in their three trials were omitted from analyses that

89   examined individuals (i.e., Figure 8).

90   Parametric statistical analyses and graphs were made in Origin 2017 (OriginLab

91   Corporation). Nonparametric tests were used for infection intensity and behavioural

92   analysis because of nonhomogenous variation in data distribution. Nonparametric

93   statistical analyses were performed in SPSS v. 23 (IBM).

94   **RESULTS**

95   The previously reported prevalence of nematodes in *L. benedicti* (87.0%, n = 46)

96   (Joseph & Faulkes 2014) was higher than in *E. benedicti* (0.0%, n = 22) (Figure 2A).

97   Similarly, the prevalence of *Polypocephalus* sp. infection in *Lepidopa benedicti* (98.0%,

98   n = 50) was higher than in *E. benedicti* (18.2%, n = 22) (Figure 2B). The mean intensity

99   of *Polypocephalus* sp. infection (Figures 2B, 3) was significantly greater in *L. benedicti*

100   (mean = 34.5, SD = 33.0, n = 49) than *E. benedicti* (mean = 1.5, SD = 1.0, n = 4). These

101   differences are not because of the overall size of individuals examined: the average size

102   of *L. benedicti* was smaller than *E. benedicti* (Table 1) in both cases. Because there

103   were so few parasites of either species in *E. benedicti*, all further analyses concern only

104   *L. benedicti*.

105   *Polypocephalus* sp. larvae were closely associated specifically with neural tissue,

106   including peripheral nerves to appendages (Figure 3). The larvae often appeared on the

107   surface of ganglia and could sometimes be seen on the dissected nerve cord using a

108   stereomicroscope. In *L. setiferus*, larvae appeared to be more deeply embedded in

109   neural tissue and were rarely visible until the nerve cord was either squashed or

110   cleared.

111   There is a significant correlation ($r = 0.31$, $n = 49$, $p = 0.03$) between *L. benedicti*

112   size and mean intensity of *Polypocephalus* sp. infection (Figure 4).

113   Like other anomurans, *L. benedicti* have shorter abdomens than familiar

114   decapods like shrimp and crayfish. Because *L. benedicti* are specialized for digging an

115   swimming with thoracic legs 1 through 4, the legs are proportionately more robust.

116   Thoracic leg 5 is very small and used for grooming. These anatomical features are

117   reflected in the relative sizes of the ganglia in *L. benedicti* compared to other decapod

118   crustaceans. The thoracic ganglia associated with thoracic legs 1-4 are substantially

119   larger than the abdominal ganglia. The fourth and fifth thoracic ganglia and the first

120   abdominal ganglion are fused. The number of larvae in the ganglia differed significantly

121   across the nervous system (Kruskal Wallis = 16.71, df = 6, $p = 0.01$), with thoracic

122   ganglia containing the most larvae, particularly in highly infected individuals (Figure 5).

123   Contrary to the prediction that more heavily infected animals would spend more

124   time above sand, the mean intensity of *Polypocephalus* sp. infection was not

125   significantly correlated ($r = -0.25$, $p = 0.13$, $n = 38$) with overall digging time (Figure 6).

126   The three main behaviours of *L. benedicti* (directly digging into sand, swimming,

127   or remaining stationary, or "sitting") were significantly different (Kruskal-Wallis 70.76, df

128   = 2, p < 0.01) in how long individuals remained above sand (Figure 7). Swimming above

129   sand and remaining stationary on top of it did not differ significantly in the duration of

130   exposure for sand crabs, although "sitting" times had greater variation, resulted in the

131   longest times that sand crabs were exposed.

132   Individuals showing different behaviour patterns had significantly different mean

133   intensities of infection (Kruskal Wallis = 8.72, df = 2, p= 0.013): animals that "sat" at

134   least once had lower infection intensities than those that swam at least once or always

135   dug directly into sand (Figure 8).

DISCUSSION

136

137   Two parasite species, an unidentified nematode (Joseph & Faulkes 2014) and

138   *Polypocephalus* sp., infect *Lepidopa benedicti* with much higher prevalence and

139   intensity than in *Emerita benedicti*. In the case of *Polypocephalus* sp., a high prevalence

140   and intensity also occurs in white shrimp (*L. setiferus*) which also dig into sand (Eldred

141   et al. 1961; Fuss 1964; Pinn & Ansell 1993) What distinguishes *E. benedicti* from both

142   *L. benedicti* and *L. setiferus* is the feeding mode. *Emerita* species are filter feeders

143   (Efford 1966), which *L. benedicti* and *L. setiferus* are not. *Lepidopa* species are

144   probably sediment feeders (Boyko 2002). This suggests that ingestion is a common

145   route of *Polypocephalus* sp. infection for *L. benedicti* and *L. setiferus*.

146   The lack of parasites in *E. benedicti* in this population is usual not only because

147   the sympatric *L. benedicti* is infected, but because other populations of *Emerita* species

148   are infected with other parasites (Smith 2007; Oliva et al. 2008; Kolluru et al. 2011;

149   Violante-Gonzalez et al. 2015; Violante-González et al. 2016).

150   In *L. setiferus*, the greatest number *Polypocephalus* sp. larvae is in the

151   abdominal ganglia (Carreon, Faulkes & Fredensborg 2011), but in *L. benedicti*, the

152   greatest number is in the thoracic ganglia. This probably reflects which region has the

153   proportionately greater mass of neural tissue available in the two species, although

154   neural mass does not entirely explain distribution patterns across the nervous system

155   (Carreon & Faulkes 2014).

156   *Polypocephalus* sp. does not seem to manipulate *L. benedicti* in a way that would

157   facilitate trophic transmission. Intuitively, one would predict that if *Polypocephalus* sp.

158   were manipulating sand crabs to make them vulnerable to predators, animals with more

159   *Polypocephalus* sp. would be more likely to swim or remain immobile on the top of the

160   sane. In anything, the evidence points towards more heavily infected individuals being

161   more likely to dig into sand immediately. Nevertheless, this result can be viewed as

162   consistent with the results in *L. setiferus*, where higher levels of infection increased

163   activity (Carreon, Faulkes & Fredensborg 2011). Digging directly into sand and

164   swimming could both be considered higher activity by *L. benedicti*.

165   The apparent difference in parasite-induced behavioural manipulation in *L.*

166   *setiferus* and *L. benedicti* has several potential explanations. First, the *Polypocephalus*

167   species infecting *L. setiferus* may not be the same species as the one infecting *L.*

168   *benedicti*. Genetic testing will eventually be able to determine of there is one cestode

169   species or mutliple. Second, *L. setiferus* may be the preferred primary host for

170   *Polypocephalus* sp. (perhaps along with other shrimp species), and *L. benedicti* is a

171   non-preferred auxiliary host. The intensity of *Polypocephalus* sp. larvae in *L. setiferus*

172   (mean = 97.7, SD = 102.6; maximum 397; n = 53; (Carreon, Faulkes & Fredensborg

173   2011) is approximately triple that of *L. benedicti* (mean = 34.5, SD = 33.0; maximum

174   170; n = 49). *Litopenaeus setiferus* may be more abundant than *L. benedicti*.

175   *Litopenaeus setiferus* is commercially fished, and annual catches from trawling in the

176   Texas waters of Gulf of Mexico average 7 million pounds per year (Texas Parks and

177   Wildlife 2002). In contrast, 10 m transects of beach often yields less than 10 *L. benedicti*

178   individuals (Faulkes ; Murph & Faulkes 2013). *L. benedicti* populations have only been

179   sampled in the swash zone (Faulkes ; Faulkes 2014) and its abundance in deeper

180   waters is unknown (it has been recorded up to 60 m depth; (Boyko 2002). Nevertheless,

181   it seems plausible that the biomass for *L. benedicti*, and thus its potential as host for

182   *Polypocephalus* sp., is lower than *L. setiferus*. Thus, there may be greater selection

183   pressure for *Polypocephalus* sp. to manipulate its primary host but not secondary ones.

184   **ACKNOWLEDGEMENTS**

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186   images from the Noun Project (https://thenounproject.com): shrimp by Jeffrey Qua, crab

187   by Mallory Hawes, scallop by B Barrett , and skate by Örn Smári Gíslason.

188

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290

Size of animals used in study.

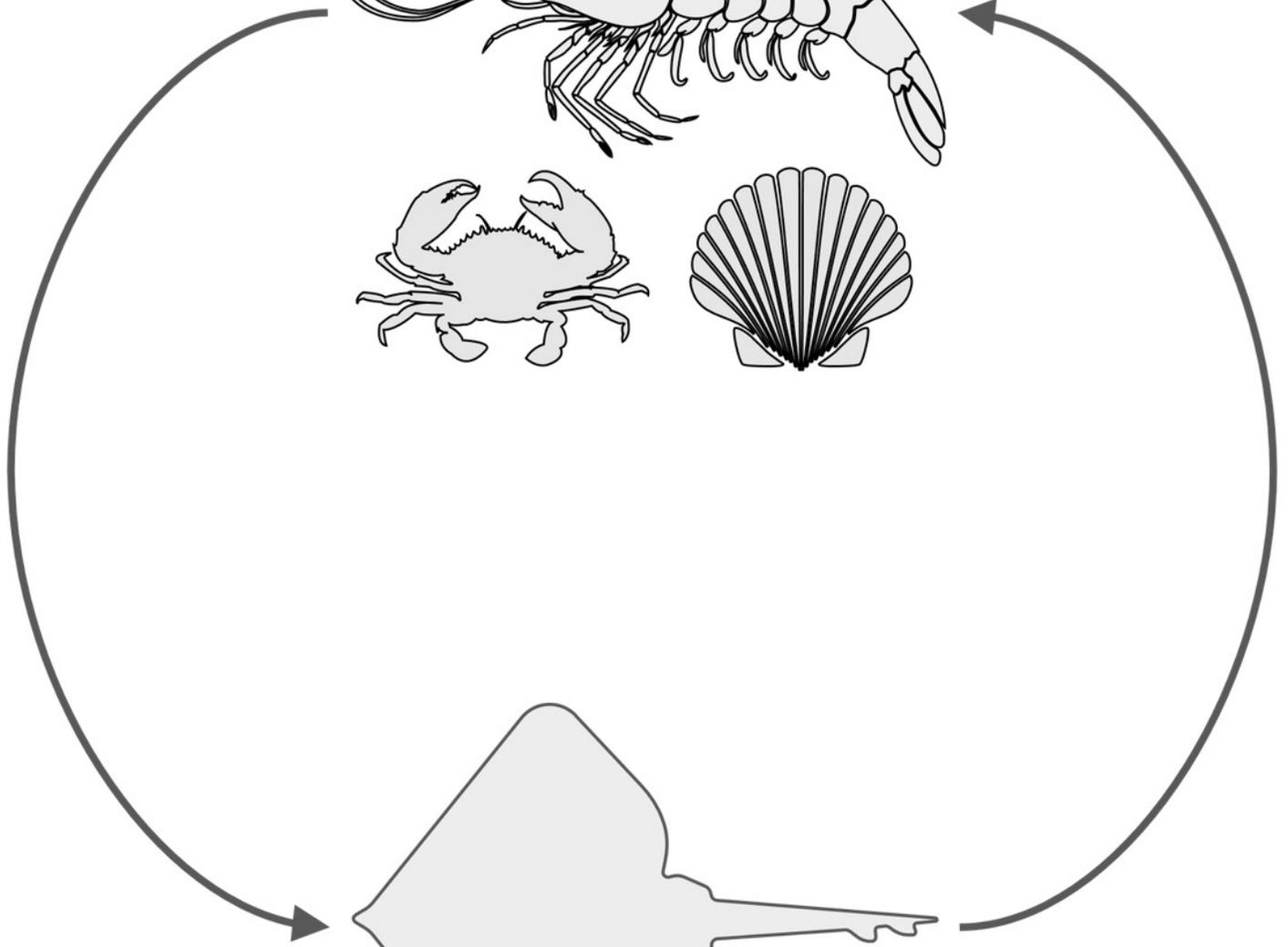
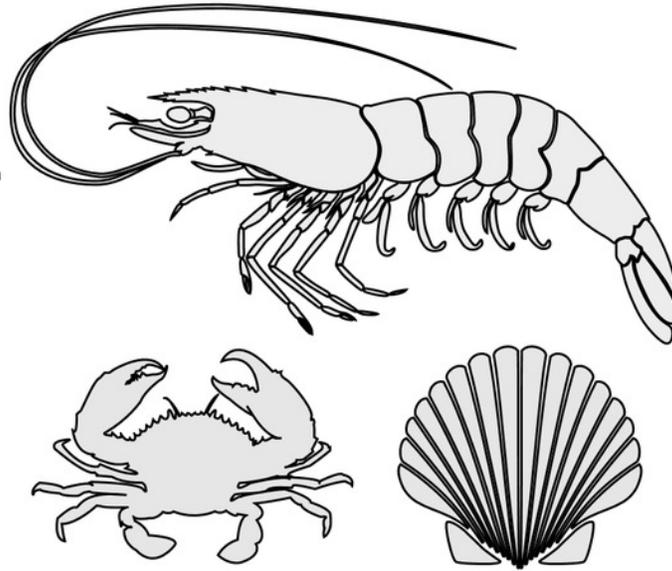
1   Table 1. Size of animals used in study.

Parasite	Lepidopa benedicti			Emerita benedicti		
	Mean carapace length	SD	n	Mean carapace length	SD	n
Nematode sp.	11.44 mm	2.83	46	13.44 mm	5.45	22
Polypocephalus sp.	9.95 mm	1.72	49	18.19 mm	3.65	21

Figure 1

Hypothesized life cycle of *Polypocephalus* sp.

Larval stages of cestode tapeworms in the *Polypocephalus* infect crustaceans and other invertebrates. These intermediate hosts are presumably ingested by skates and rays, which are expected to excrete eggs.

Intermediate hosts

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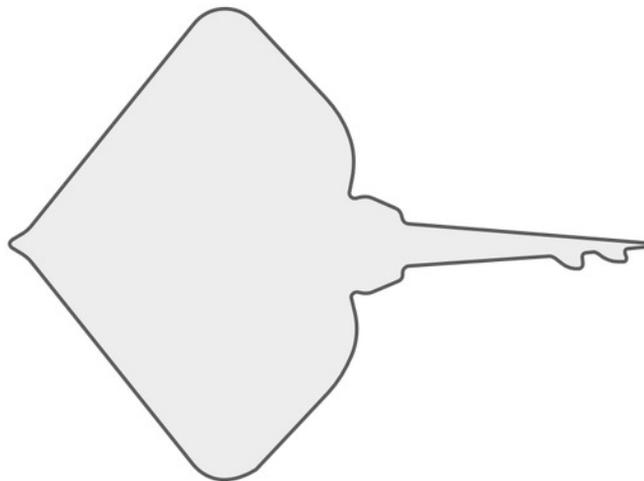
Figure 2

Infection patterns of sand crabs and mole crabs by parasites.

A) Infection of crabs by unidentified nematode species. *Lepidopa benedicti* data redrawn from Joseph & Faulkes (2014). B) Infection pattern of crabs by *Polypocephalus* sp. larvae. Summary statistics shown on left: square = mean; line dividing box = median; box = 50% of data; whiskers = 95% of data; triangles = minimum and maximum. Raw data shown on right
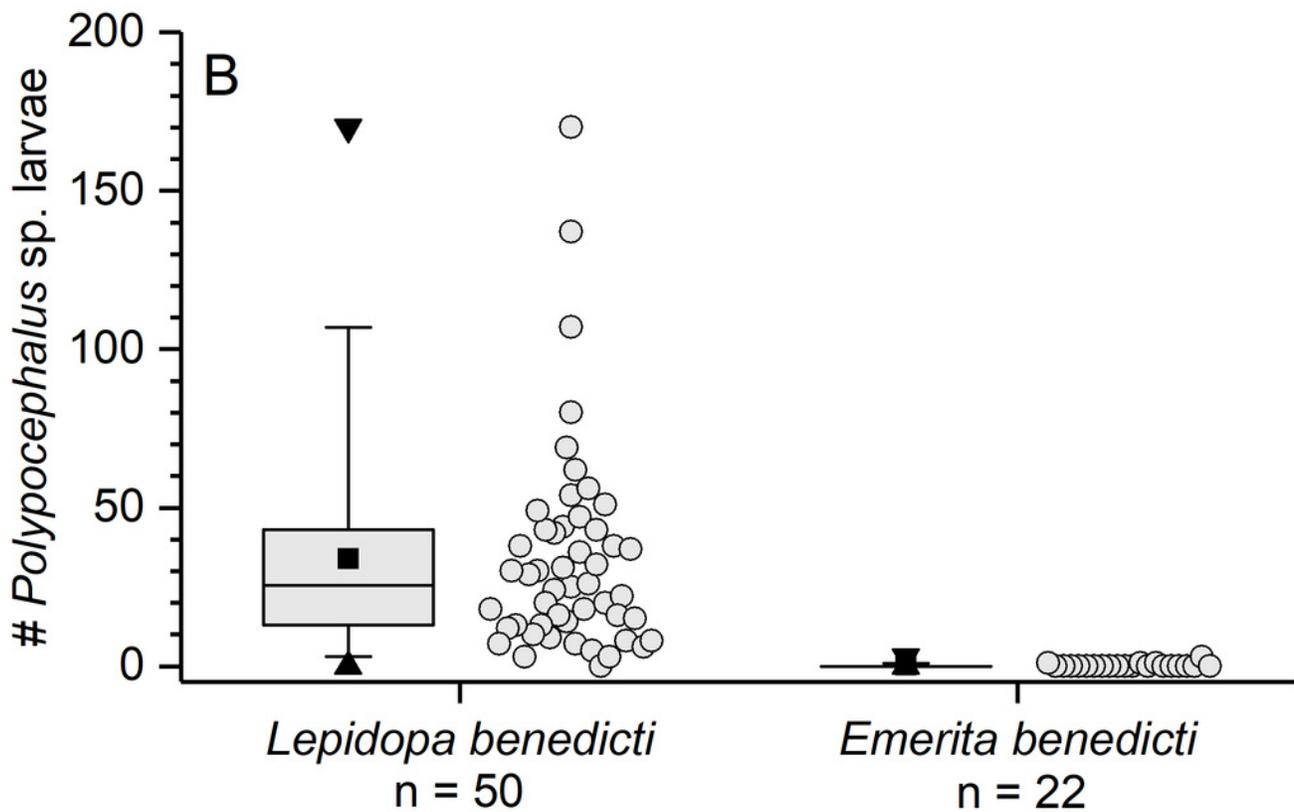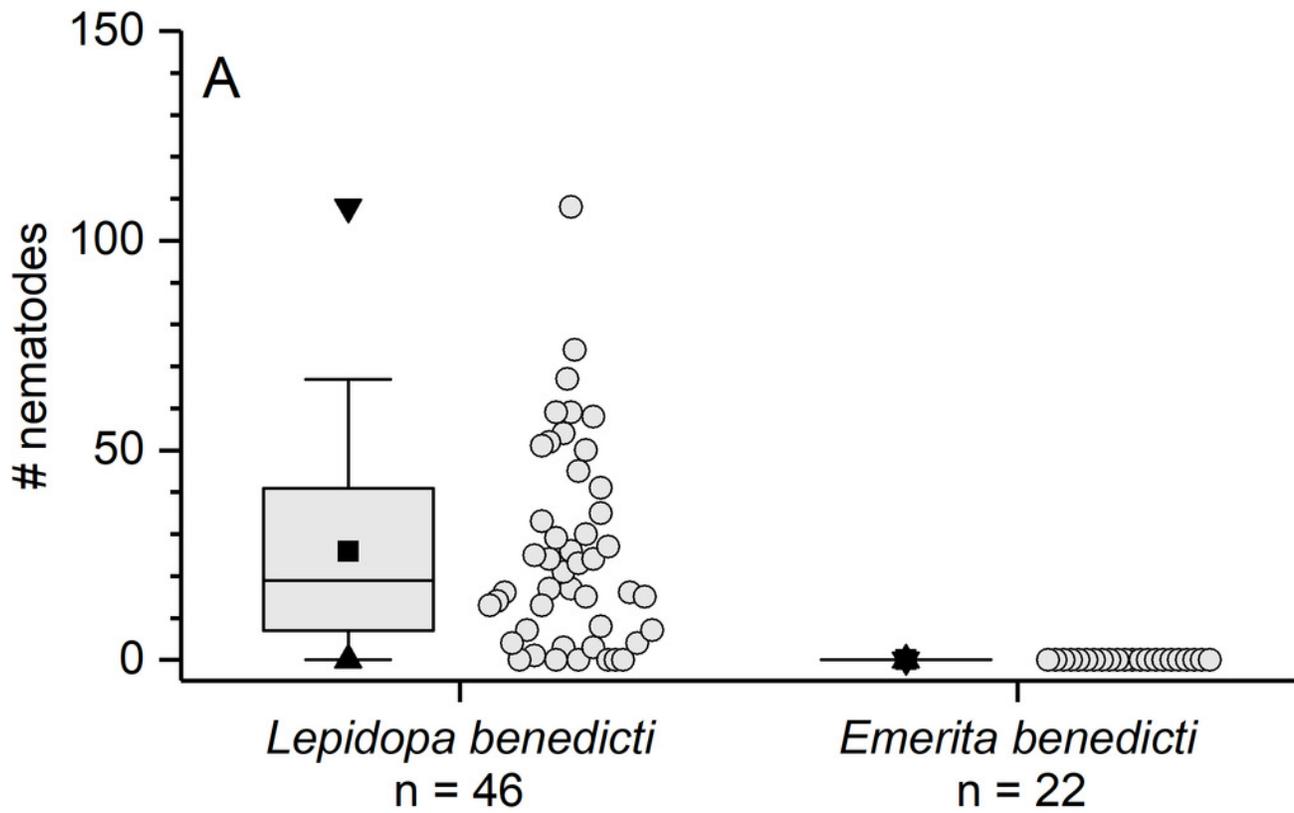
Figure 3

Micrographs of *Polypocephalus* sp. in nervous tissue.

A) *Lepidopa benedicti* thoracic ganglion 2. B) *Lepidopa benedicti* thoracic ganglia 3, and fused ganglion consisting of thoracic ganglia 4, 5, and abdominal ganglion 1. Different individual than A. C) *Emerita benedicti* brain. Arrow indicates single *Polypocephalus* larvae. D. *Emerita benedicti* thoracic ganglion 1. No *Polypocephalus* sp. larvae in this individual. Different individual than C. Anterior towards top in A, and towards left in B-D.
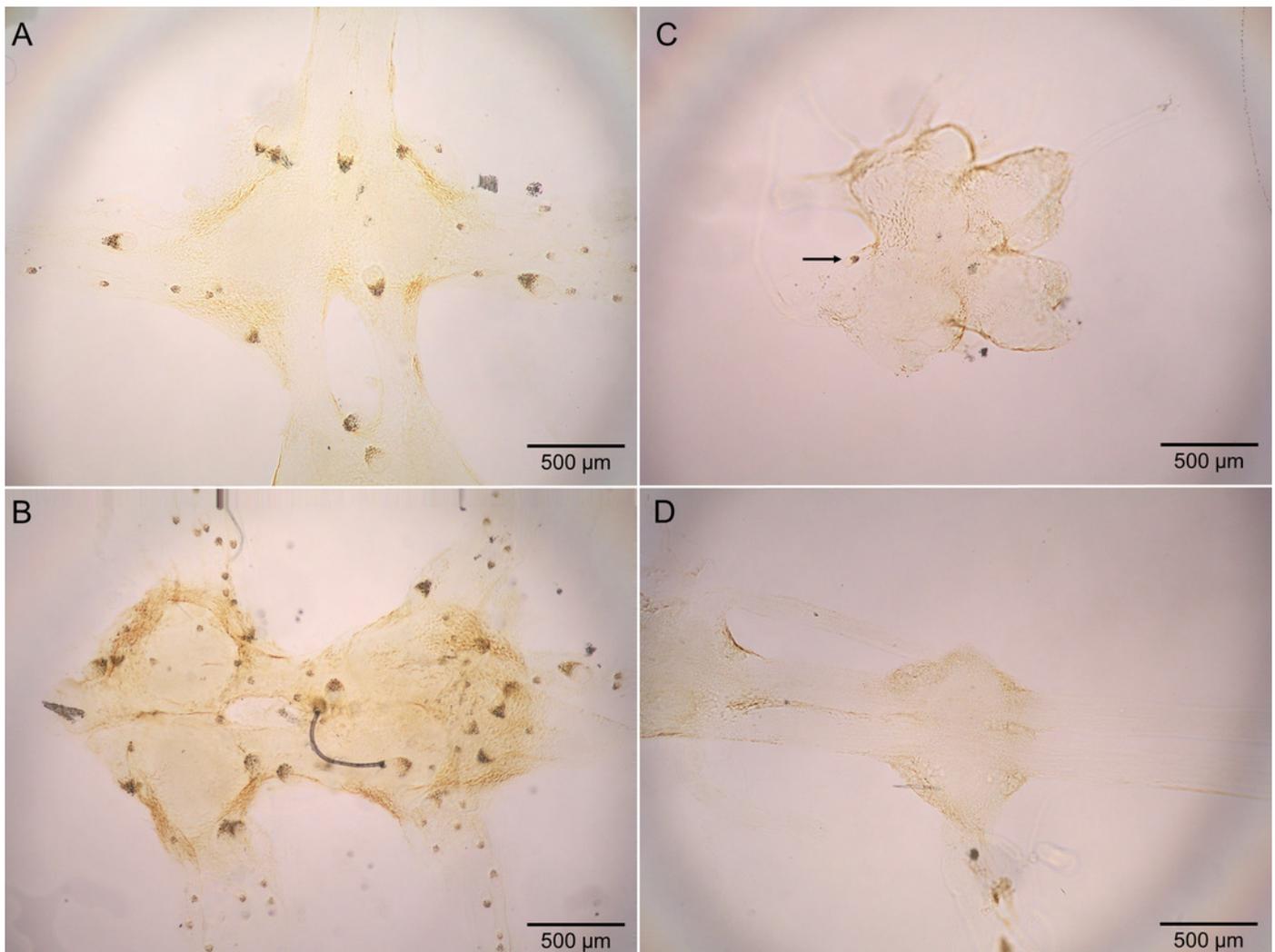
Figure 4

Relationship between size of *L. benedicti* and intensity of *Polypocephalus* sp. infection.

Dashed line = linear regression; shaded area = 95% confidence intervals.
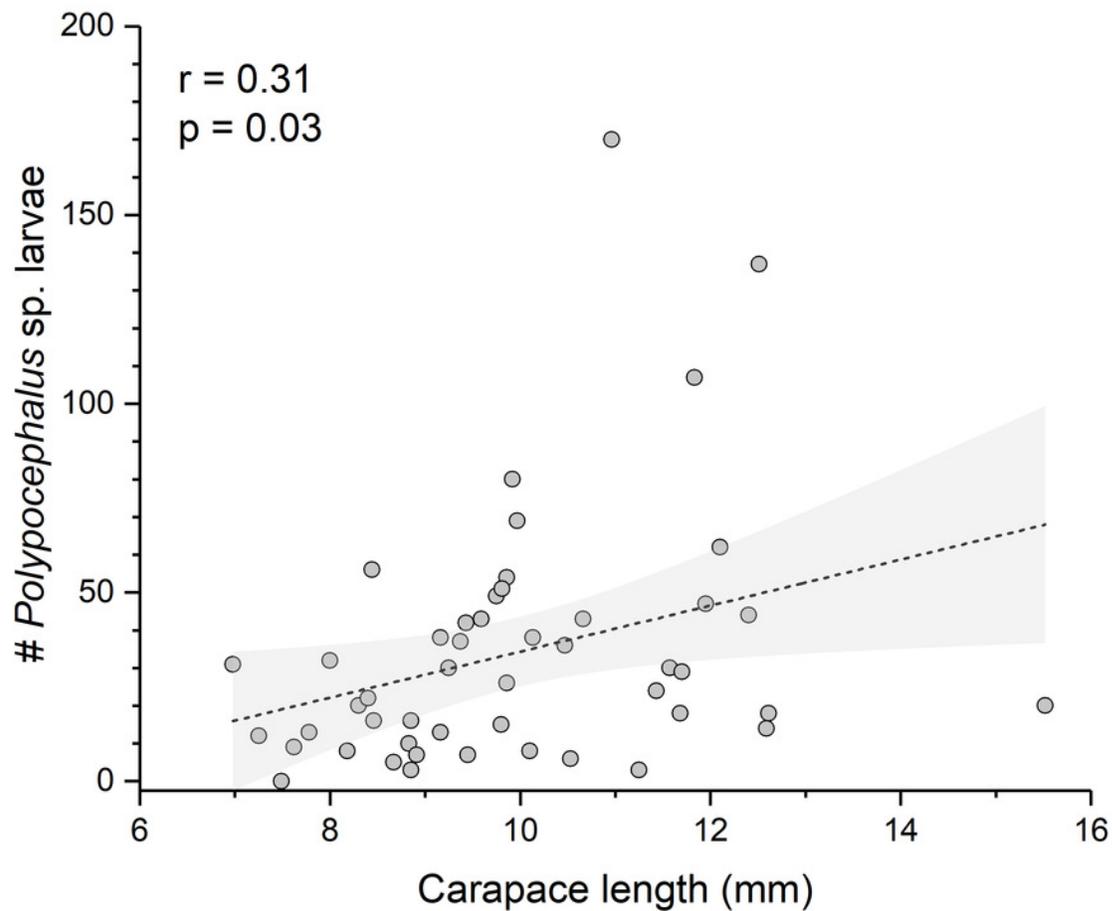
Figure 5

Number of *Polypocephalus* sp. larvae in different regions of the nerve cord in *L. benedicti*.

Summary statistics shown on left: square = mean; line dividing box = median; box = 50% of data; whiskers = 95% of data; triangles = minimum and maximum. Raw data shown on right. Groups sharing a letter do not differ significantly from each other. Sample sizes vary because some ganglia were damaged during dissection.
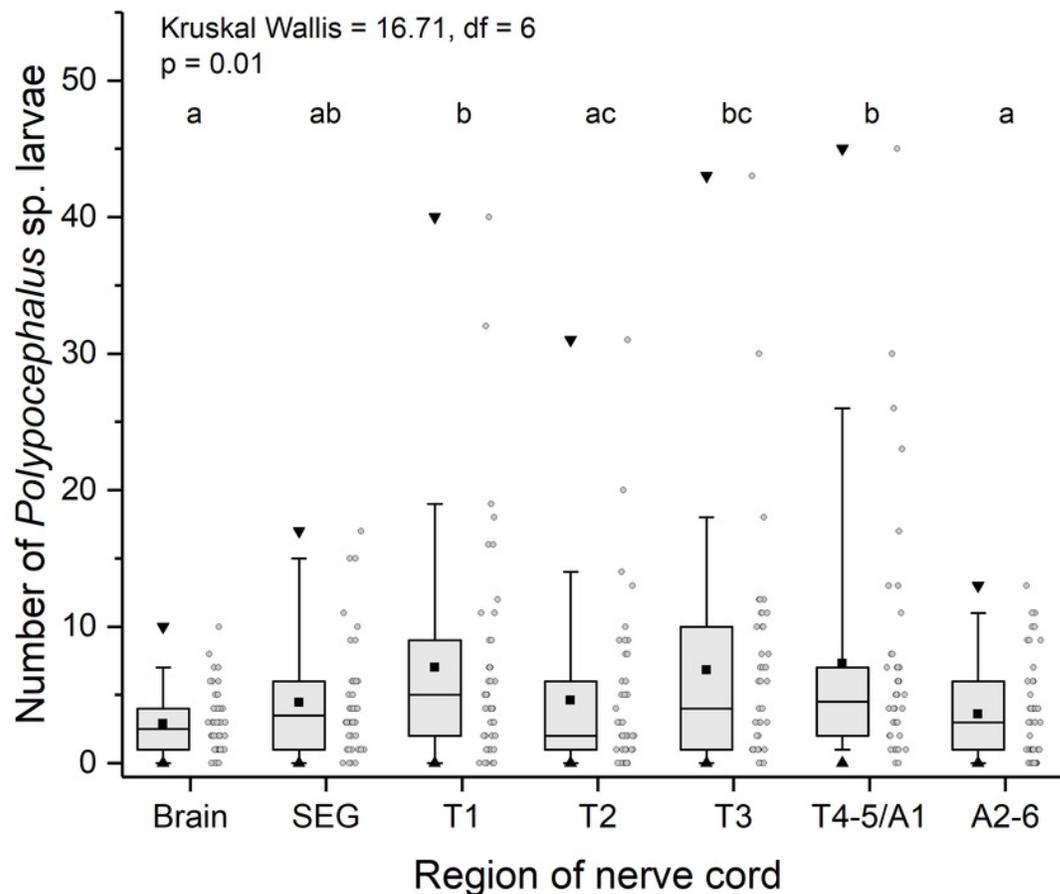
Figure 6

Relationship between intensity of *Polypocephalus* sp. infection and digging time in *L. benedicti*.

Dashed line = linear regression; shaded area = 95% confidence intervals.
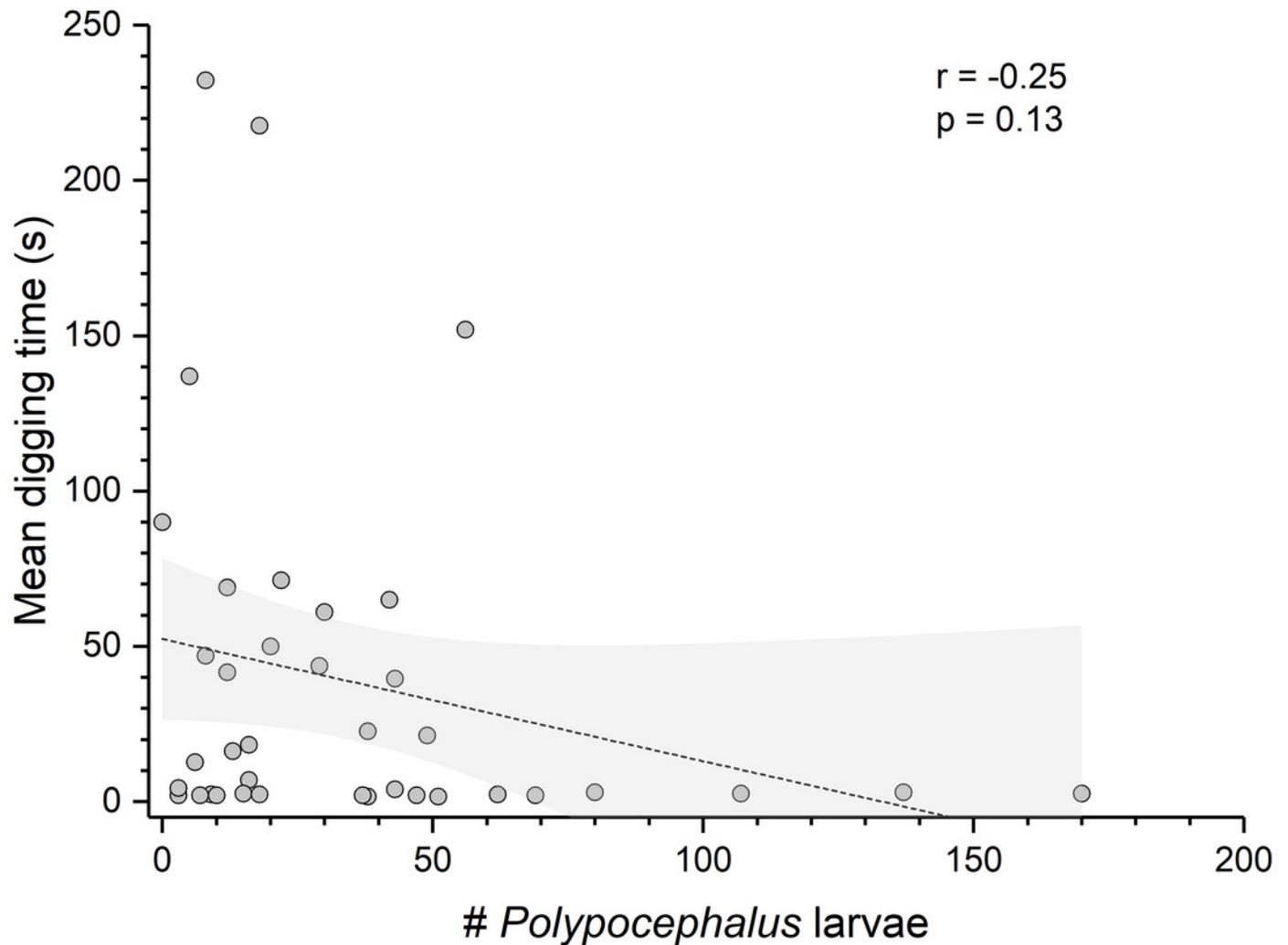
Figure 7

Time above sand for different behaviours by *L. benedicti*.

Duration of individual behaviour trials, grouped by different behaviours. N = 110 trials. Four trials involved combinations of sitting and swimming, and are not shown due to their rarity. Summary statistics shown on left: square = mean; line dividing box = median; box = 50% of data; whiskers = 95% of data; triangles = minimum and maximum. Raw data shown on right. Groups sharing a letter do not differ significantly from each other.
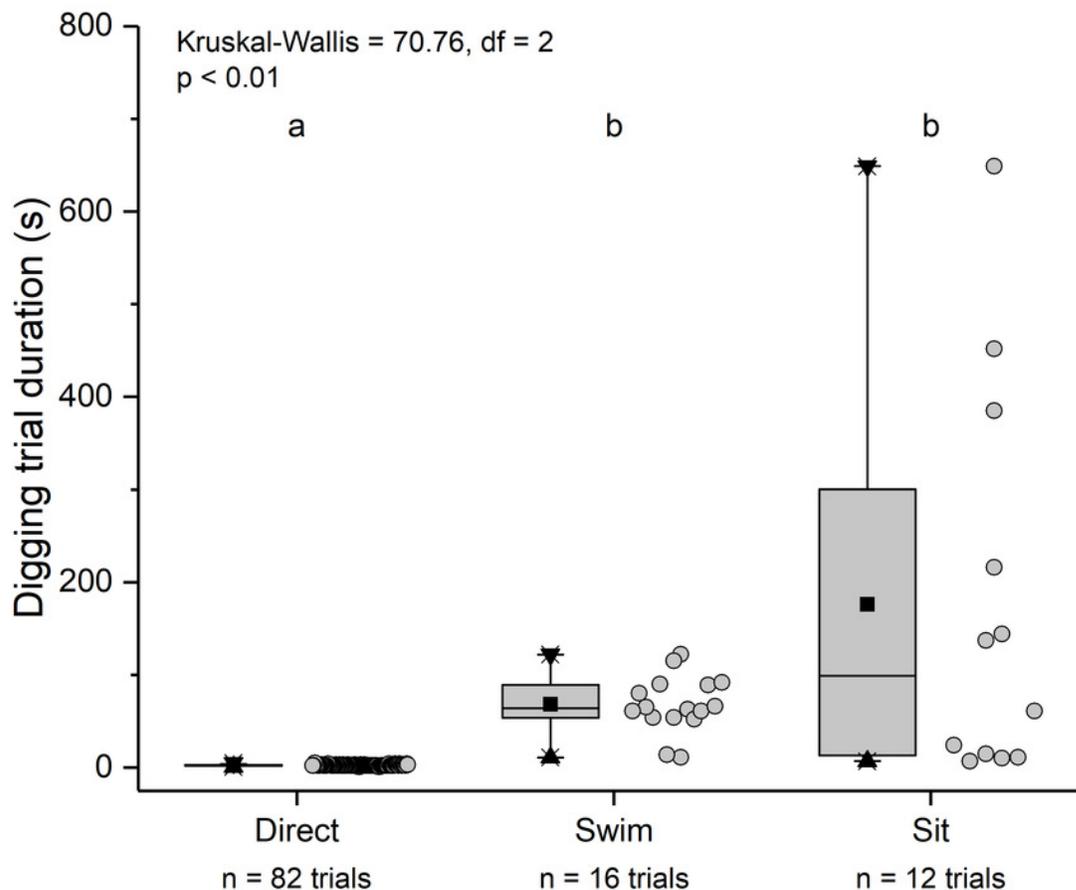
Figure 8

Infection intensity of *L. benedicti* individuals showing different behaviours.

Individuals categorized into three groups: those that always dug directly; those that swim at least once, but never sat; those that "sat" at least once (i.e., remining immobile on the surface), but never swam. Summary statistics shown on left: square = mean; line dividing box = median; box = 50% of data; whiskers = 95% of data; triangles = minimum and maximum. Raw data shown on right. Groups sharing a letter do not differ significantly from each other.