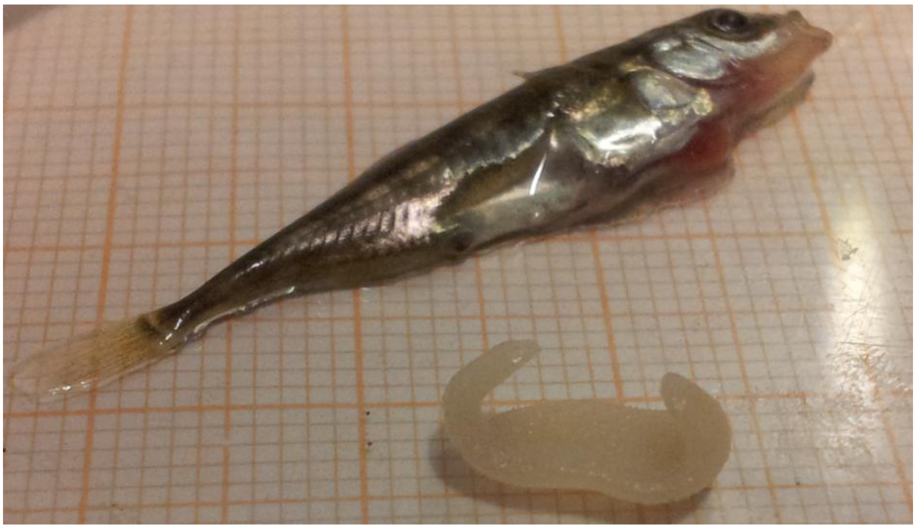
Morph-based resistance against *Schistocephalus solidus* in three-spined sticklebacks (*Gasterosteus aculeatus*) selected under different infection- and predation regimes



Master of Science in Biology

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Abstract

The morphology of a host may have an effect on its resistance against parasites. Two geographically separated and morphologically divergent populations of three-spined sticklebacks (*Gasterosteus aculeatus*, Linnaeus, 1758) were experimentally infected with *Schistocephalus solidus* (Müller, 1776) through well documented methods. Extremely low prevalence of the parasite in both single-infection and mass-infection experiments, not previously documented, leave us with no satisfying conclusions on the effect of morphology on resistance, however compatibility and strong local adaptation are some points that are discussed and suggestions for future research are included.

1. Introduction

If parasites or hosts were perfectly adapted to their local environment, no parasite would be able to establish in its host, nor would any host be able to defend itself against the parasite. As parasites are not perfectly adapted, hosts are able to counter threats of parasite-induced fitness loss. Parasites depend on interaction with hosts for their survival and hosts are selected by their ability to decrease fitness loss caused by the parasite. Thus each part in a host-parasite system responds to the selective forces imposed by the other (Dawkins & Krebs, 1979) and genes are changed in order to adapt. Such co-evolution (Woolhouse *et al.*, 2002) can lead to a continuous cycle of adaptations and counter-adaptations in host-parasite systems in changing environments where "it takes all the running you can do, to keep in the same place" (the "Red Queen hypothesis", van Valen, 1973). This suggests that both hosts and parasites need to develop new combinations of genes to cope with what is currently dominating.

Co-evolution depends on the strength of selection and the genetic material available (Gandon, 2002). Selection pressures are asymmetric in host-parasite systems where offensive adaptations by one part, in this case the parasite, is countered by the defensive adaptations by the host. Parasites may gain more in fitness due to successful infection compared to their hosts who lose from it and the other way around. In an "attack-defense" arms race such as the host-parasite system (Dawkins & Krebs, 1979) relatively fewer genes may be involved in parasite resistance rather than those involved in parasite virulence. Parasite selection may also be faster than that on the host (Frank, 1996). The shorter life cycles of most parasites also suggest that pathogen adaptation outpaces evolutionary responses on the part of the host (Ebert, 1994). However, parasite resistance evolution is driven by the frequency of host defense genes (Hamilton, 1980; Hamilton *et al.*, 1990) which may give rare host resistance genes a selective advantage (also known as negative frequency dependent selection = NFDS, Ebert, 2008). One source of rare resistance genes is the major histocompability complex (MHC), known for its extreme genetic diversity (Potts & Slev, 1995; Lenz *et al.*, 2013). Additionally, mate choice may give a twofold benefit for resistance in each generation (Milinski, 2006). Variability and recombination of host genes then accelerates their evolution, making hosts able to stay in the arms race. Dawkins and Krebs (1979) list different outcomes for how the arms races may end. The most relevant in existing host-parasite systems is that one side reaches a definable optimum, thus preventing the other side from reaching its optimum or that both sides may reach a mutual local optimum. Most empirical works narrow their focus to either of these, and two separate literatures coexist (Van Baalen, 1998; Restif *et* *al.,* 2001; Restif & Koella, 2003; Grech *et al.,* 2006). These views are not mutually exclusive due to differences in individual compatibilities between hosts and parasites.

In studies where parasite and host evolution have been examined together, infectivity and virulence have been found to depend on the interaction of particular host and parasite genotypes (Carius *et al.,* 2001; Kaltz & Shykoff, 2002; Webster *et al.,* 2004; Lambrechts *et al.,* 2006). Consequently, these traits are controlled by both the host and the parasite and this opens up for a set of novel predictions. Gandon (2002) predicted that parasites should evolve higher virulence, if resistance of their host is allowed to evolve in his models. When the host controls parasite growth, the parasite is predicted to become more exploitative in response (Gandon & Mickalakis, 2000). Restif & Koella (2003) developed this further and showed theoretically that when both host and parasites are controlling transmission and virulence, it can have qualitative effects on evolutionary predictions. Grech *et al.* (2006) conducted one of the first empirical tests, evaluating the relative impact of parasites and hosts, using four clones of *Plasmodium chabaudi* in combination with four mouse strains in a cross infection experiment. Although there was a clear host effect, they found that most of the effect could be explained by the parasite. The parasites and hosts used, had however, no evolutionary prehistory and the hosts were not the natural host and came from four inbred strains. Hence, as the authors confirm, their model system is not a natural one. An important aspect is also that the interaction between the two antagonistic partners is tightly coupled and thus also tightly reciprocal (Lajeunesse & Forbes, 2002). Extensive work on *Daphnia magna* and its parasites have given insight into the extent of parasite-mediated local adaptation in natural populations (Ebert, 2008). Altermatt *et al.* (2006) found in a common garden experiment of sympatric and allopatric hosts that fitness reduction was greatest in their local hosts. Moreover, immigrated hosts were found to have a higher fitness when local hosts were heavily infected by a local parasite (Altermatt *et al.*, 2006). This facilitation of immigration of *Daphnia magna* from other populations forces then the parasite to adapt to new genotypes.

For this study the Diphyllobothriidean (Kuchta *et al.,* 2008) cestode *Schistocephalus solidus* and the intermediate three-spined stickleback host (*Gasterosteus aculeatus*) were used to test host-parasite interactions (see Appendix 6.1 for life cycle). Experimental infection studies and field studies have previously shown a great variation in infectivity among different populations of sticklebacks, even when age, sex and size has been considered (Barber & Scharsack, 2010). It has been hypothesized that such variation may come as a result of different morphological characteristics of the host. External features and body form show great variation in sticklebacks, to the point where we talk about a *Gasterosteus* species complex (Bell & Foster, 1994). Much research has been focused on the variation in the 'armor complex' consisting of bilateral pelvic structures, dorsal spines and the lateral plates in the plesiomorphic state (Bell & Foster, 1994). From marine environments three-spined sticklebacks have spread widely to postglacial freshwater environments in the northern hemisphere where armor reduction has evolved several times independently (Bell, 1987; Bell *et al.*, 1993). Several theories for reduction in three-spined stickleback pelvic structure have been proposed. Absence of local predatory fish (Reimchen, 1980; Bell *et al.*, 1993), low calcium availability (Giles, 1983a) and predation by piscivorous insects (Reimchen, 1980; Reist, 1980) have all been suggested to lead to armor reduction in sticklebacks; however these hypotheses do not necessarily exclude each other.

Support for the 'predatory fish theory' comes from Reimchen (2000) who showed that when gape-limited predators preyed on fully armed sticklebacks, longer handling time and more incidents of prey escaping the predator were observed compared to when the predator was preying on partially armed sticklebacks. Indirect support for the anti-predator effectiveness of fully armored sticklebacks has been found from field samples from North American lakes (Vamosi & Schluter, 2004). It is thereby reasonable to suggest that where fully armed sticklebacks are found, piscivorous fish that prey on sticklebacks may be found as well. Consequently, lakes with partially armed sticklebacks suggest a lower degree of predatory fish influence. This creates an interesting scenario when parasitism of *Schistocephalus solidus* is introduced. In lakes of fully armed sticklebacks, tri-trophic interactions may occur where the parasite may end up in a fish instead of the final host, a piscivorous bird. Previous field and experimental studies suggest that parasitized sticklebacks are at a great disadvantage in dealing with piscivorous predators (Jakobsen *et al.*, 1988) and may be more easily preyed upon due to manipulation of the host (Giles, 1983b; Tierney *et al.*, 1993; Ness & Foster, 1999; Barber *et al.*, 2004). In lakes of less armored sticklebacks however, it is expected that such tri-trophic interactions are less common. Divergent morphologies may therefore lead to different strengths of selection against parasites. Reduced armor is hypothesized to lead to greater resistance against parasites, as more energy can be traded off to the immune system compared to fully armed sticklebacks (Lee *et al.*, 2011).

This study tests offspring from two morphologically divergent three-spined stickleback populations from the same lake system to see if there is a significant difference in parasite resistance against offspring from six foreign families of *S. solidus*. In the Vigdarvassdraget lake system in Sveio, Norway two lakes separated by another lake and an altitude barrier were used for sampling adult sticklebacks. Sticklebacks from Lake Vigdarvatnet are “normal morphs” with complete pelvic girdle and three dorsal spines similar to their marine counterparts (Austad, 2011). Sticklebacks from Lake Nesavatnet however have reduced pelvis and only two dorsal spines (Austad, 2011). Offspring from these populations were infected with offspring from a total of six *S. solidus* families from Lake Skogseidvatnet in Fusa, Norway. Two separate experiments were carried out: In the first sticklebacks were infected with a single copepod infected with a single *S. solidus* procercoid. However, this may be confounded by other factors such as behavioral resistance or ecological differences between the two morphs due to for example differences in distribution between benthic, sheltered or open habitats. In the other experiment, the morphs were therefore mixed together and mass-infected in large tanks provided with shelter to evaluate other potential differences.

With this background the following hypothesis was investigated:

Sticklebacks with reduced armor (from Lake Nesavatnet) are expected to show a lower infection rate of the *S. solidus* parasite compared to sticklebacks with full armor in a common garden situation (from Lake Vigdarvatnet) as low armor sticklebacks are assumed to allocate more of their resources to immune defense in order to challenge the higher selective pressure from parasites.

2. Material and methods

2.1 Study areas

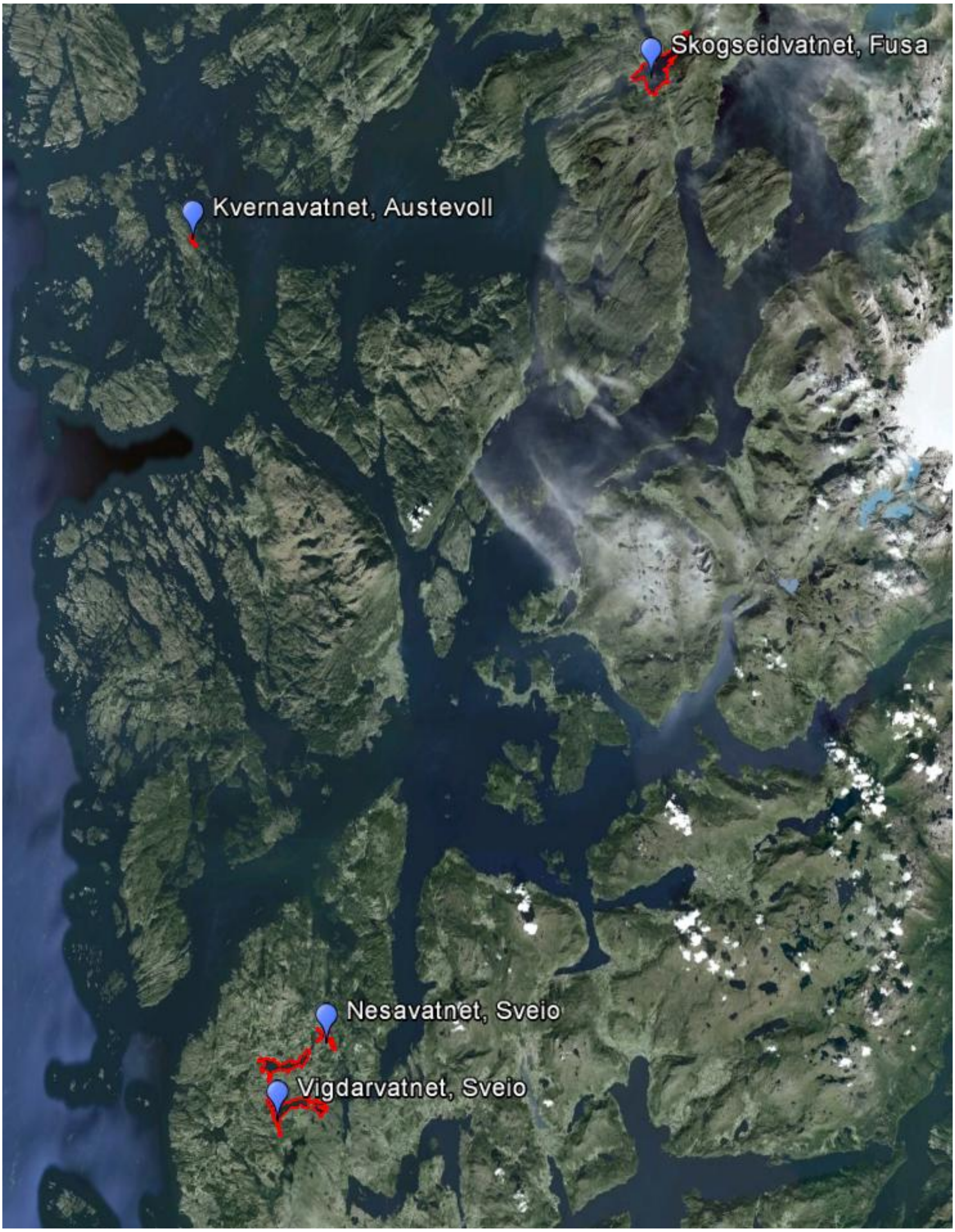
In total four lakes were used for the experiment (Figure 1). Sticklebacks used to breed offspring (first generation - F1) were caught by Tom Klepaker in two closely located lakes in Vigdarvassdraget, a freshwater lake system located on the west coast of Norway in the municipality of Sveio. *Schistocephalus solidus* adults were collected from sticklebacks in Lake Skogseidvatnet in Fusa, western Norway, located approximately 77 km northeast from Vigdarvassdraget (Google Earth, 2013). This distance is within feeding range of non-breeding birds (van der Veen & Kurtz, 2002). Plexiglas fry traps were used to collect three-spined sticklebacks as described by Breder (1960). Copepods used to develop a new generation for infection were sampled in Lake Kvernavatnet on Huftarøy Island in Austevoll, western Norway. Copepods were collected with a 180 µm plankton net towed slowly by a boat at 0.5 meters depth.

2.1.1 Lakes used for stickleback sampling

Lake Vigdarvatnet (59° 30’34.02’’N, 5° 25’54.37’’E) situated 10 m above sea level is the largest lake of the Vigdarvassdraget system with a surface area of 6.91 km^2. It is connected to several smaller lakes by a river in the area. Relevant to this study is the northeast 50 m river outlet from Lake Liavatnet, a lake that further upstream connects with Lake Nesavatnet, the other lake used for collecting sticklebacks. Fish fauna in Lake Vigdarvatnet consists of arctic char (*Salvelinus alpinus*), brown trout (*Salmo trutta*), European eel (*Anguilla Anguilla*) and three-spined sticklebacks. To the southeast is a river outlet flowing into Ålfjorden where salmon *(Salmo salar*) and sea trout (*Salmo trutta trutta*) can ascend from. Grey heron (*Ardea cinerea*) and red-breasted merganser (*Mergus serrator*) are piscivorous birds regularly observed in Vigdarvassdraget. Occasionally the great cormorant (*Phalacrocorax carbo*) and the common tern (*Sterna hirundo*) are spotted.

Lake Nesavatnet (59° 33’08.88’’N, 5° 26’19.23’’E) situated at 16 m above sea level has an altitude barrier of 6 m to Lake Liavatnet which blocks introgression of sticklebacks between the two lakes and introgression between Lake Nesavatnet and Lake Vigdarvatnet which share no direct connection. Lake Nesavatnet has a 0.62 km^2 surface area and no lakes connected upstream. The shortest distance between Lake Nesavatnet and Lake Vigdarvatnet is approximately 700 m by air. Previous field samples indicate a low amount of predatory fish in Lake Nesavatnet (Austad, 2011).

Figure 1: Location of all lakes used for the experiment (Google Earth, 2013).



2.1.2 Lake used for *S. solidus* sampling

Lake Skogseidvatnet (60^{o}13’6.48’’N, 5^{o}52’40.68’’E) situated at 13 meters above sea level is the largest lake (5.33 km^{2} surface area) in the Sævarreidvassdraget lake system which consists of two other lakes and connecting rivers. Fish and bird fauna is similar to what is found in the Vigdarvassdraget lake system.

2.1.3 Lake used for copepod sampling

Lake Kvernavatnet (60^{o} 5’46.03’’N, 5^{o}14’27.62’’E) is a small artificially dammed up lake with a 0.14 km^{2} surface area situated at 10 m above sea level. Fish fauna consists of rainbow trout (*Oncorhynchus mykiss*), landlocked salmon, European eel and three-spined sticklebacks. Grey heron is regularly observed in the area. Although the parasite has been present earlier (Jakobsen *et al.,* 1988), a parasite screening from 2011 showed that *S. solidus* was absent in the lake (Kalbe, unpublished data)

2.2 Culturing of *S. solidus* eggs

Initially the experiments were designed to use offspring from size-matched plerocercoids from Vigdarvatnet and Nesavatnet. 19 attempts were made at developing such offspring by *in vitro* methods as described by Smyth (1946; 1954) and further developed by Wedekind (1997). However none of the crossings produced enough coracidia for the copepod infection phase. It is at present unclear why the hatching success was low. Producing offspring from plerocercoids *in vitro* from the Vigdarvatnet lake system have never been attempted prior to this study.

As a replacement eggs from size-matched plerocercoids from wild infected sticklebacks collected from Lake Skogseidvatnet in November 2011 were used. These had been cultured at the Max Planck Institute in Plön, Germany. Skogseidvatnet *S. solidus* was chosen due to the long history of its use in related experiments (Benesh, 2010; Benesh & Hafer, 2012). Plerocercoid sizes in families used were between 93 and 149 mg and therefore larger than the 50 mg threshold size of maturation (Tierney & Crompton, 1992). Offspring from 6 randomly chosen families, named “Family 1” to “Family 6”, were used for the final experiments (Table 1, 2). The eggs had been refrigerated until three weeks prior to hatching when they were placed at 20 ^{o}C in the dark. On the day before infection of copepods, eggs were moved to Petri dishes with tap water where a light source was placed over them to stimulate hatching (Smyth 1963; Dubinina, 1980).

2.3 Infection of copepods

Acanthocyclops sp. offspring (first generation – F1) were reared as described by van der Veen & Kurtz (2002). These were used for single infections as well as mass infections and controls (uninfected copepods used for sham-infecting sticklebacks). *Acanthocyclops* sp. were selected due to their abundance in samples from the source lake (Kvernavatnet, Austevoll) and their similarity to the commonly used *Macrocyclops albilus*, which has been used for *S. solidus* infections with a large degree of success (Orr & Hopkins, 1969; Dubinina, 1980; Wedekind, 1997; Hammerschmidt & Kurtz, 2007; Hammerschmidt & Kurtz, 2009; Benesh & Hafer, 2012). Size differences between copepods and worms used is expected to have a low influence and recent research has indicated that copepod size and thus *S. solidus* size has no effect on the fitness of the parasite, however procercoid size relative to host size is considered important (Benesh & Hafer, 2012).

After two weeks at room temperature C4 and older *Acanthocyclops* sp., without egg sacs, were selected for individual infections. They were isolated in a 24-well ELISA plate (Sarstedt, Germany) in 2 ml of water. Here each copepod (1782 in total) was kept at 20^{o} C in accordance with other authors (Wedekind, 1997; Christen & Milinski, 2003; Hammerschmidt, 2006) and fed one *Artemia salina* nauplii every second day until two days prior to infection.

For the single infections, a single *S. solidus* coracidium was transferred to each well in the ELISA plates under a microscope (Hammerschmidt & Kurtz, 2005; Scharsack *et al.*, 2007). Infection success was determined 14 days post-exposure (dpe) (Dubinina, 1980; Wedekind & Jakobsen, 1998; Hammerschmidt & Kurtz, 2009) using an inverted phase contrast microscope with 20X and 40X long working distance objectives. Copepods from ELISA plate chambers with single developed procercoids, noted by the cigar-shaped body and the cercomer with its six hooks on one end (Dubinina, 1980), were used for the single infection experiment (Table 2). Carbonated water was used to anesthetize copepods for a short time which facilitates observing infection status *in vivo* (Benesh, 2010; Benesh & Hafer, 2012).

Copepods for mass infections were kept in five small boxes and fed *Artemia salina* at the same frequencies. To each box, containing approximately 400 to 5000 copepods (approximately 16400 in total), coracidia from *S. solidus* families 2-6 were added (Table 3).

Subsamples of 20 copepods from each mass infection group were checked to estimate infection success 14 dpe (Table 2).

2.6 Production of uninfected F1 fish for infection

Prior to infection first generation (F1) stickleback offspring were bred in two tanks of 7 m diameter with 1.3 m water depth. Here boxes of sand, gravel and green threads were placed together with artificial plant cover to facilitate reproduction. Water added to the tanks was first UV-filtered and then went through 180 µm plankton net. 30 wild-caught adult stickleback males from each population (Vigdarvatnet and Nesavatnet) were placed separately in the two tanks and were given time to establish nests. Then wild-caught female sticklebacks, 60 from each population, were introduced 11 days later. During the whole experiment all sticklebacks were fed equal amounts of frozen chironomids five times a week.

2.7 Infection of sticklebacks in single infection experiment

The resulting F1 offspring were then transferred to 3.5 m diameter tanks with 1.2 m water depth in which they were fed until four days pre-infection. Four days prior to infection 5 l boxes were filled with 2 l of UV-filtered water and kept at 18 °C (Hammerschmidt & Kurtz, 2005). Two days later sticklebacks were moved to one box each and placed randomly (Hammerschmidt & Kurtz, 2005). On the day of infection (22 dpe of copepods) either an uninfected copepod (sham infection control) or an infected one was placed in each box containing a live stickleback. Two days post-infection all boxes were sieved to check for presence of the copepod. Fish from boxes where the copepod had not been eaten (10) were not used in the experiment. Fish from boxes where the copepod was no longer present (i.e. eaten) were moved to 85L aquariums with 17 °C running water (Jãger & Schjørring, 2006; Macnab *et al.*, 2009) and an artificial plant in each. Here 8 groups of 33 fish (total of 264 fish) were made so that sticklebacks infected from families 1-3 were kept separate as well as separating the Vigdarvatnet and Nesavatnet sticklebacks and the sham-infected. Another aquarium was set up with uninfected replacement sticklebacks (17 Vigdarvatnet and 17 Nesavatnet sticklebacks) in case densities decreased in any aquarium during the 88 days they were kept here, however no sticklebacks died post-infection.

2.8 Mass infection of sticklebacks

In addition to single infections of sticklebacks a mass-infection experiment was carried out. This was done in order to control for confounding effects such as differences in the distribution and behavioral resistance. F1 sticklebacks came from the same breeding tanks as the individual infection experiment. We used four 7m in diameter tanks with a 0.6 m high UV-filtered water column. Each tank had a standardized amount and placement of artificial plant structures, including two crab traps, to act as cover. To each tank 100 sticklebacks of each morph were added. Thus a total of 800 sticklebacks were used. Two tanks acted as sham controls while the other two were given a mix of copepods infected with families 2-6 of *S. solidus* (Table 2). Over 5000 infected copepods were added to each tank used for infection (Table 2). These were added over a five day period as not all families were hatched on the same day. Water temperature in all tanks was stable at 11 ^{o}C during the infection phase. Sticklebacks were starved in these tanks four days prior to adding of copepods. Four days after the last infection, groups were given equal amount of frozen chironomids regularly in the center of each tank. As there was no temperature control or circulation in these tanks, temperature varied with the seasons and was at one period at freezing point. A low average water temperature of 3, 5 ^{o}C during this experiment led to a longer post-infection phase that lasted for 153 days. Longer post-exposure phase was applied to facilitate finding plerocercoids during dissection, as low temperatures may lead to slower growth (Macnab & Barber, 2011).

2.9 Dissection and growth measurements

Sticklebacks were euthanized by an overdose of tricaine methane-sulfonate (MS-222, Sigma Aldrich, Germany) and dissected immediately. Fork length (to the nearest 0.1 mm, from snout tip to fork of the tail, Tierney *et al.*, 1996) of all the fish, used in the single infection experiment, was determined on the day of transfer to boxes (4 days pre-exposure) and on the day of dissection (88 days post-exposure). Pre-exposure measurements were considered as subsamples for the fish used for mass infection as they all came from the same tanks. To treat the fish gently, each individual was photographed in a small container of water with a millimeter paper in the background and measurements of fork length were made via digital files using ImageJ version 1.46r (Rasband, 2013). In order to account for the accuracy of these measurements, a subsample was also measured using a ruler after dissection. As the measurements using these two methods did not significantly differ, length all fish found after the mass-infection experiment was measured with a ruler.

Additionally weight of each stickleback (to the nearest 0.01 g) was measured right after euthanization. Sticklebacks were dried on tissue paper on both lateral sides of the body before they were put on the scale. Stickleback morphology was analyzed through scoring of the pelvic structure as described by Bell *et al.* (1993) (Figure 2, 3) and counting of dorsal spines. Each lateral side of the stickleback is scored from 0-4 depending on the stickleback having a complete or reduced pelvis. A complete pelvic structure on both sides gives the Combined Pelvic Score of 8 (CPS= 8, figure 2). Reduced pelvic structure may be symmetrical (CPS = 0, figure 3) or asymmetrical (CPS = 1).

Figure 2: Lake Vigdarvatnet stickleback with complete pelvic structure (CPS = 8).

Figure 3: Lake Nesavatnet stickleback with no pelvic structure (CPS = 0).

Each fish was cut open with a fine scissor starting behind the gills and down towards the anus opening with the sharp end of the scissor blade always pointing away from the body cavity to avoid damaging plerocercoids. The body cavity was then rinsed out with 0.9% NaCl and thoroughly checked for infections (Hammerschmidt & Kurtz, 2005). Plerocercoids found were dried off on lens paper and measured thrice on a scale (Sartorius Supermicro S4) to the nearest 0.01 mg and average weight was assessed. Sticklebacks were placed in individual bags and stored in a freezer while plerocercoids were kept on 70% ethanol in dark at room temperature.

2.10 Statistical methods

All statistical tests were executed with R version 2.31 (www.r-project.org). Significant p-values were set at <0.05. The same test methods for significance were carried out for both the single- and mass infections.

As fish were grouped together in numerous aquariums (single infections) or tanks (mass infections), testing for significance of weight and length between Nesavatnet and Vigdarvatnet sticklebacks relied on linear mixed effects models (lme). These models take clusters into account that appear when groups are made where targets are not individually marked and followed throughout the experiment. Tanks or aquariums were considered random effect factors. In one incidence significance had to be tested with separation of aquarium groups in mind (groups as categorical predictor with several levels) and in this case one way ANOVA test and Tukey HSD test for contrasts were carried out. Note that weight and length at the end of the experiments, which are compared here, does not give an indication of the growth rate of sticklebacks groups.

Welch two sample t-tests were made in order to test for significance between the morphs for combined pelvic score (CPS, Figure 2, 3) and number of dorsal spines.

3. Results

3.1 Single infections

Of the 198 singly infected sticklebacks in this experiment, only 7 *S. solidus* plerocercoids were found. 3 (3%) of these came from Vigdarvatnet sticklebacks and the other 4 (4%) came from Nesavatnet sticklebacks. Average plerocercoid weight after 88 days was 121, 6 mg (Appendix 6.4). Prevalence (%) for the copepod single infection phase was also calculated for this experiment which is summarized in Table 1.

Table 1: Infection success of Skogseidvatnet *S. solidus* in singly infected *Acanthocyclops* sp.

Family No.	Size of parents (mg)	No. checked copepods	No. infected	% prevalence
1	131 x 122	432	79	18.3
2	140 x 149	312	79	25.3
3	141 x 144	408	79	19.4

Pelvic scoring showed that the morphology of the two stickleback populations differed significantly, CPS averaging at ~7.95 in Vigdarvatnet and ~0.78 in Nesavatnet (Welch Two Sample t-test, p-value < 2.2×10^6). This is consistent with previous field data (Austad, 2011). Number of dorsal spines also varied significantly between the two populations and averaged at ~2.98 in the Vigdarvatnet population and ~2.26 in Nesavatnet (Welch Two Sample t-test, p-value < 2.2×10^6). Loss of the second dorsal spine was most commonly observed.

Fork length 4 days pre-exposure did not vary between sham-infected and infected sticklebacks (Appendix 6.2, linear mixed-effect models, p-value > 0.11) or between morphs (Appendix 6.3, linear mixed-effects models, p-value > 0.08). Fork length did not vary significantly between infected and sham-infected fish 88 days post-exposure (Figure 4, linear mixed effect models, p-value > 0.16), nor between the morphs (Figure 5, linear mixed-effect models, p-value > 0.45). Weight 88 days post-exposure did not vary between infected sticklebacks and sham-infected ones (Figure 6, linear mixed-effect models, p-value > 0.73), however weight varied significantly between the morphs (Figure 7, linear mixed-effect models, p-value < 0.05). A closer look tells us that weight did not vary significantly between the exposure groups (Figure 8), however Vigdarvatnet sticklebacks infected with *S. solidus* families 2 and 3 as well as sham-infected fish had a significantly higher mean weight compared to sham-infected Nesavatnet sticklebacks (one-way ANOVA and Tukey contrasts, p-values > 0.02, 0.01 & 0.01 respectively.

Figure 4: Box plots comparing fork length (mm) of sticklebacks infected (inf) with *S. solidus* and sham-infected (sham) 88 days post-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.

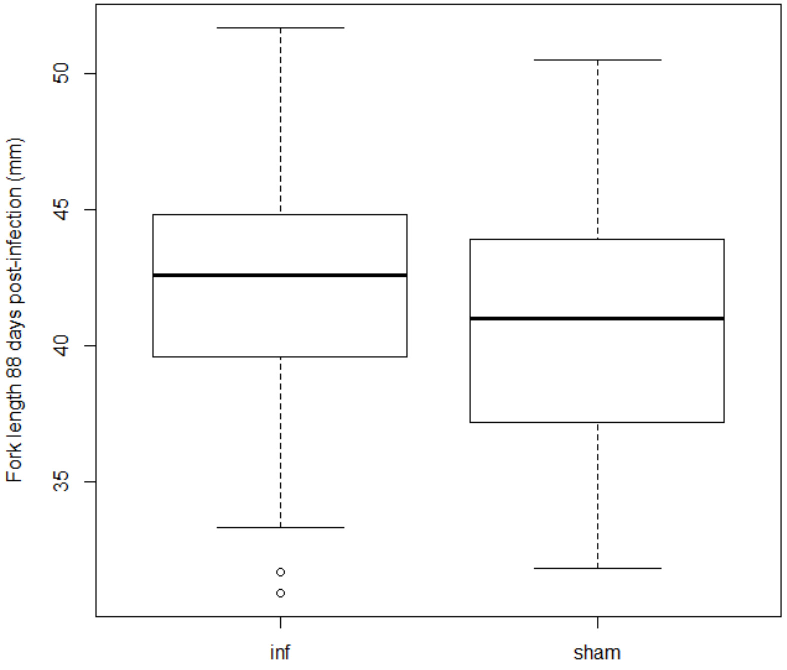


Figure 5: Fork length (mm) comparison of Nesavatnet sticklebacks (nesa) and Vigdarvatnet sticklebacks (vigdar) from the single infection experiment 88 days post-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.

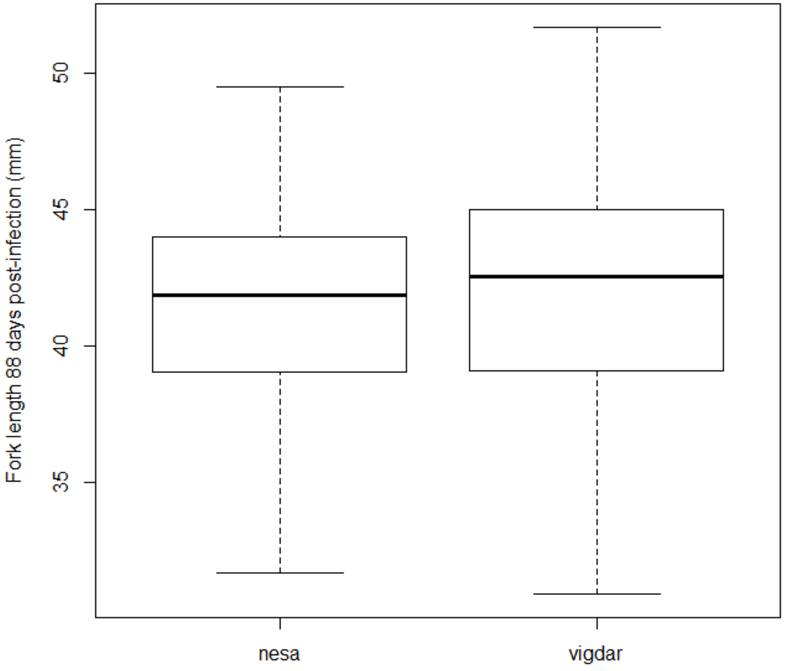


Figure 6: Weight (g) comparison of *S. solidus* single infected sticklebacks (inf) and sham-infected (sham) 88 days post-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.

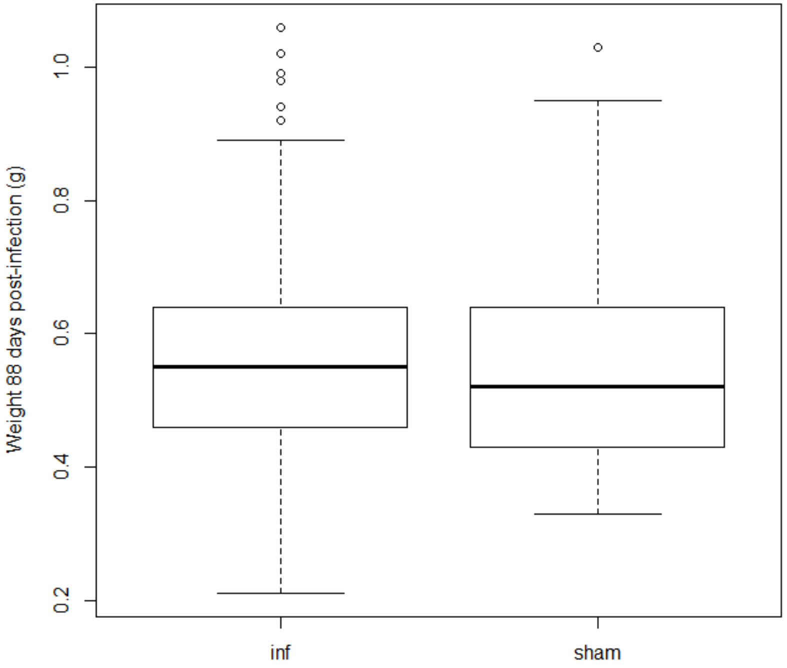


Figure 7: Weight (g) comparison of Nesavatnet sticklebacks (nesa) and Vigdarvatnet sticklebacks (vigdar) from the single infection experiment 88 days post-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.

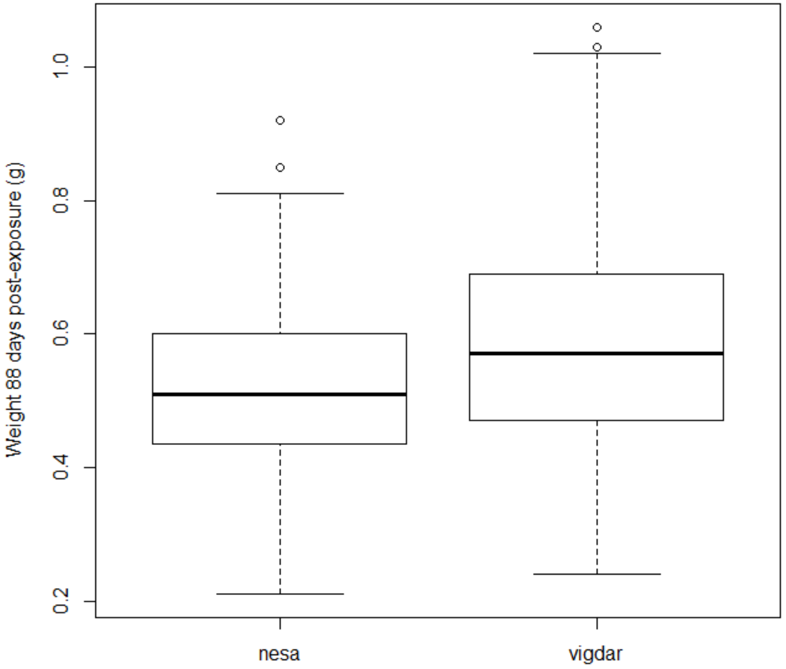
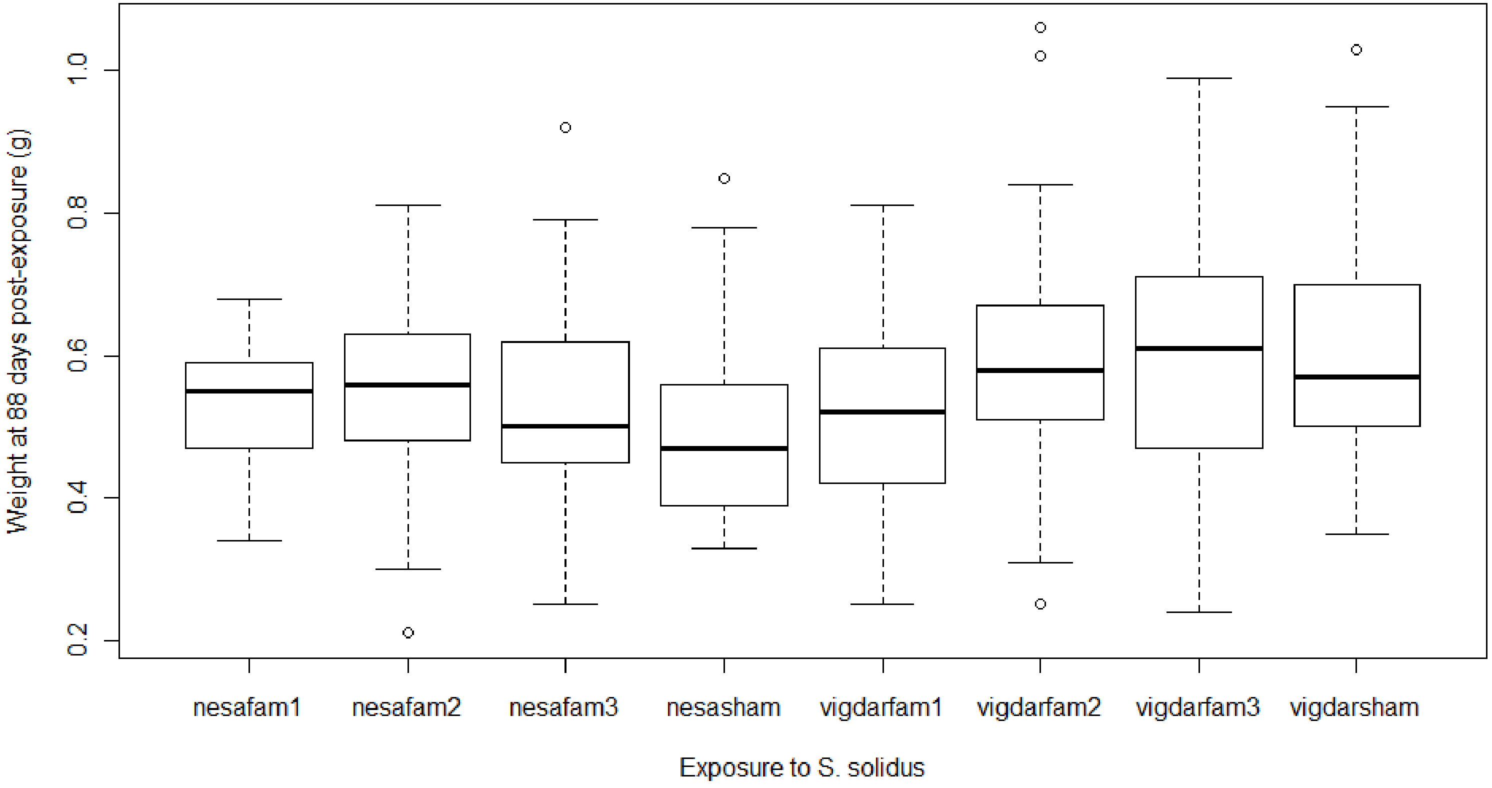


Figure 8: Weight (g) comparison of all exposure groups in the single-infection experiment 88 days post-exposure. Vigdarvatnet sticklebacks (vigdar) infected with *S. solidus* families 2 (vigdarfam2) and 3 (vigdarfam3) as well as sham-infected (vigdarsham) had a significantly higher mean weight compared to sham-infected Nesavatnet sticklebacks (nesasham, one-way ANOVA and Tukey contrasts, p-values > 0.02, 0.01 & 0.01 respectively). Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.



3.2 Mass infections

Not a single plerocercoid was found in the 244 exposed sticklebacks that were controlled 153 days post-exposure. The rest of the sticklebacks in the infection groups (n = 156) are assumed dead. A total of 241, of the original 400, sham-infected sticklebacks were found alive after 153 days. It is estimated that over 5000 infected copepods from different *S. solidus* families were added to each infection tank (Table 2).

Table 2: Mass infection of copepods by Skogseidvatnet *S. solidus*

Family No.	Plerocercoid parent size (mg)	Subsample infection success	Estimated % infection success	Estimated no. infected copepods added per tank
2	140 x 149	20/20	100	200
3	141 x 144	7/20	35	375
4	102 x 104	13/20	65	1625
5	95 x 115	16/20	80	2000
6	93 x 112	10/20	50	1250

Length of the fish 153 days post exposure did not vary significantly between the infected and sham-infected groups (Figure 9, linear mixed effect models, $p > 0.61$) or between the morphs (Figure 10, $p > 0.61$). As Vigdarvatnet and Nesavatnet sticklebacks were clustered together in the tanks for this experiment I used the results from pelvic scoring for the single infection experiment as a background to determine which population each fish belonged to here. We observed a very clear difference in the pelvic scores in line with the single infection experiment with most specimens having a CPS score of either 0 or 8. Sticklebacks with high CPS score (CPS = 6 and higher) were considered Vigdarvatnet sticklebacks while fish with a low CPS score (CPS = 5 and lower) were considered as Nesavatnet sticklebacks (see Appendix 6.5). Weight 153 days post-exposure did not vary significantly between infected and sham-infected groups (Figure 11, linear mixed effect models, $p > 0.13$), nor between the morphs (Figure 12, linear mixed effect models, $p > 0.47$).

Figure 9: Fork length (mm) comparison of *S. solidus* mass-infected sticklebacks (inf) and sham-infected (sham) 153 days post-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.

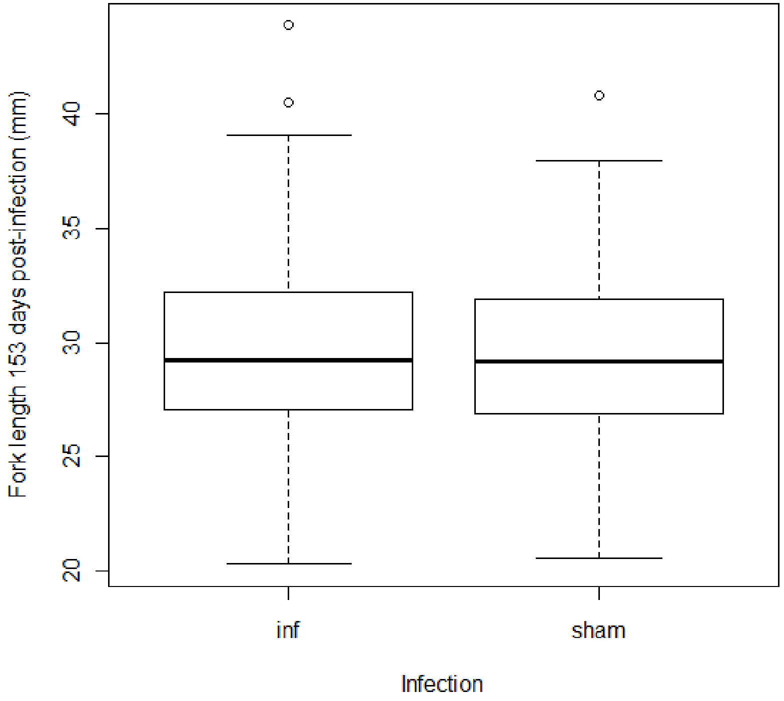


Figure 10: Fork length (mm) comparison of Nesavatnet sticklebacks (n) and Vigdarvatnet sticklebacks (v) from the mass infection experiment 153 days post-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.

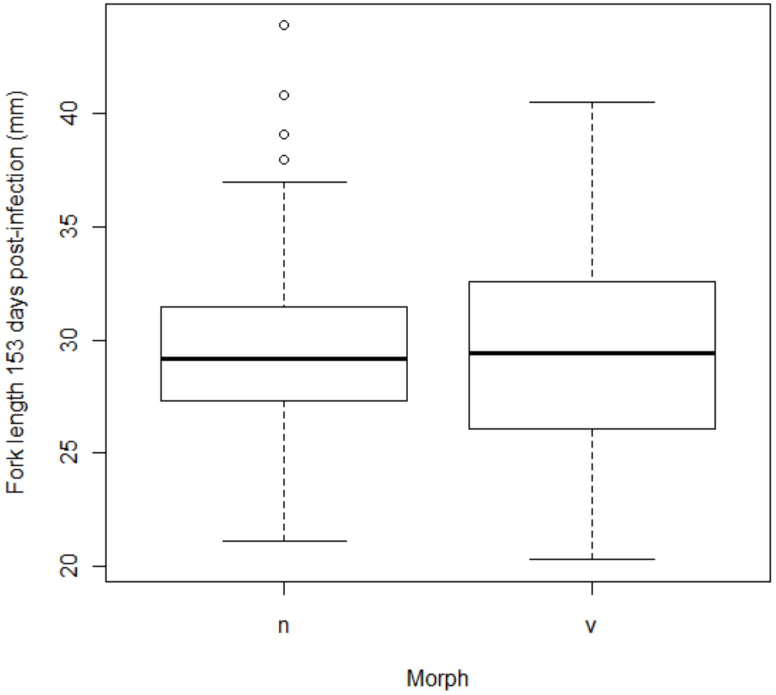


Figure 11: Weight (g) comparison of *S. solidus* mass-infected sticklebacks (inf) and sham-infected (sham) 153 days post-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.

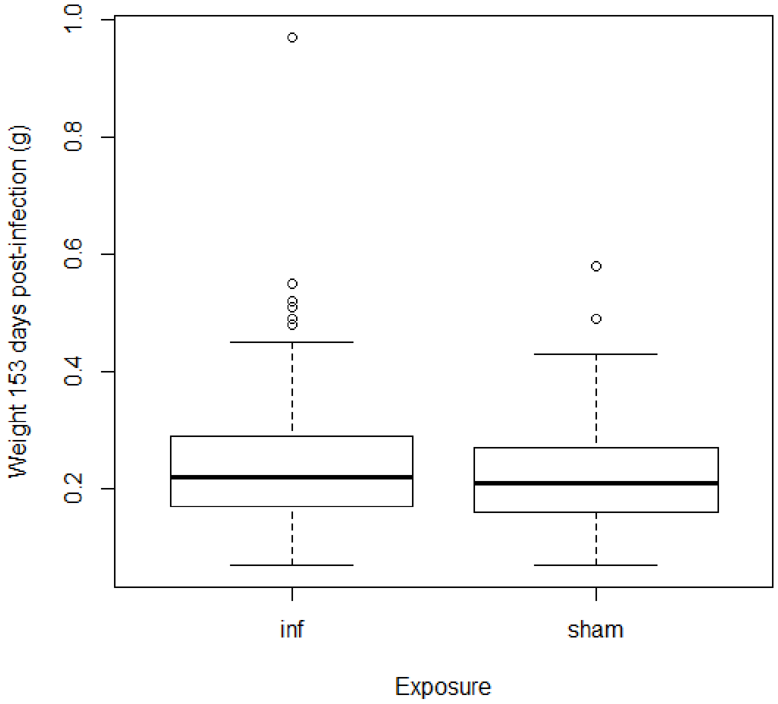
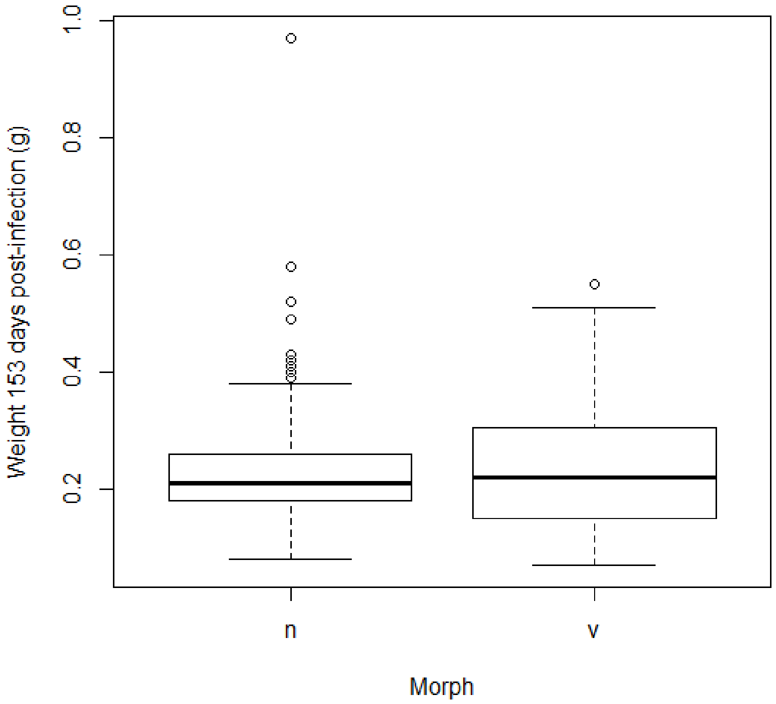


Figure 12: Weight (g) comparison of Nesavatnet sticklebacks (n) and Vigdarvatnet sticklebacks (v) from the mass infection experiment 153 days post-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.



4. Discussion

In our experiment we observed a surprisingly low prevalence of *S. solidus* infections in both stickleback morphs (3% for Vigdarvatnet and 4% for Nesavatnet sticklebacks). With these extreme results our initial hypothesis of one morph performing better than the other with regard to *S. solidus* infection was not answered. We propose that the low prevalence is a result of a strong local adaptation in the two fish populations examined and not due to the methods used.

S. solidus was one of the first tapeworms to be bred successfully in the laboratory by Smyth (1954) and due to this it is one of our main model species for understanding host – parasite interactions (Barber & Scharsack, 2010). Breeding methods have been improved and the breeding procedures are standardized in most studies. One of the most widely used populations is the Skogseidvatnet *S. Solidus* (Benesh & Hafer, 2012; Henrich *et al.,* 2013, Scharsack, personal communication), the same as used in this study. Hence this gives us an opportunity to compare our results with other infection experiments. A higher prevalence (%) of *S. solidus* infections was observed in all experiments in comparison to these results (Table 3 shows the summarized prevalence (%) of various infection experiments including ours).

Skogseidvatnet *S. solidus* populations have been used by several authors in infection experiments using either the sympatric or the allopatric host populations. Scharsack (personal communication) exposed sympatric host fish populations to copepods infected with 3 procercoids. He investigated different abiotic factors and achieved a higher prevalence compared to our results. In another experiment Henrich *et al.* (2013) out-crossed two *Schistocephalus* species and tested their infection success in two German stickleback populations (*G. aculeatus* and nine-spined sticklebacks, *Pungitius pungitius*). In *S. solidus / G. aculeatus* pairings they achieved a prevalence of 44.7 % despite using an allopatric host population. These and other studies using Skogseidvatnet *S. solidus* show that the population is not likely to have a low compatibility with its hosts (Benesh & Hafer, 2012, Henrich *et al*, 2013). In this study the infection success of allopatric host-parasite combination of the Skogseidsvatnet population of *S. solidus* is much lower than other allopatric infections with German sticklebacks. The fact that field studies have shown a high prevalence of sympatric *S. solidus* from Nesavatnet (Austad, 2011) and a notable prevalence of the same parasite in field samples of Vigdarvatnet sticklebacks (Tom Klepaker, personal communication), suggests that the compatibility between Skogseidsvatnet tapeworms and Vigdarvatnet system sticklebacks are low. Conclusions such as this rely however on careful consideration of a number of factors in the experimental design.

One important consideration in infection studies is to make sure that the parasites are given adequate time to develop. Benesh & Hafer (2012) found that the developmental success varied for procercoids 11 dpe but most were morphologically mature at 17 dpe. In this experiment we checked the infection success and maturity of procercoids 14 dpe (Dubinina, 1980; Wedekind & Jakobsen, 1998; Hammerschmidt & Kurtz, 2009) and then gave infected copepods to sticklebacks 22 dpe. This is in accordance with Benesh & Hafer’s (2012) results, and we believe that our procerocids were developmentally mature and infective 22 dpe.

Plerocercoid performance in its host may rely on different factors and this has been investigated in several studies. Scharsack *et al.* (2007) checked the prevalence of infection (%) at seven different time points, 7 – 67 dpe of sticklebacks. Prevalence (%) was highest at 7 dpe (64.5%) and 17 dpe (63.3%) and lowest at 27, 47 and 67 dpe had a prevalence of 45.9% (Scharsack *et al.*, 2007). Growth rate of plerocercoids peaked at 17 dpe and gradually decreased in the following time points while respiratory burst activity was elevated starting from 47 dpe. Neither growth rate nor respiratory burst activity was studied in our experiments that lasted for 88 and 153 days. Scharsack *et al.* (2007) suggested that a late but strong immune response could harm but not destroy the parasite. However they noted that the increase in respiratory burst activity occurred ‘shortly after the parasite reached 50 mg’ (Scharsack *et al.*, 2007), which is considered the minimum weight for plerocercoids that are infective in the final host (Tierney & Crompton, 1992). 67 dpe plerocercoid weight was average at 150 mg (Scharsack *et al.*, 2007), which is higher than the 122 mg mean we achieved 88 dpe. However, our water temperatures was lower (17^{o}C) compared to Scharsack *et al.* (2007, 20^{o}C). Still, this may further indicate lower compatibilities in our host-parasite pairings. This theory is made stronger by Jäger & Schjørring (2006) results who got 182 mg mean plerocercoid weight 13 weeks (91 dpe) with 18^{o}C water temperature. However, they used host and parasite from the same lake source. The role of temperature on plerocercoid growth was investigated by Macnab & Barber (2011) who found after 8 weeks (56 dpe) that plerocercoids mean weight for those in sticklebacks who had stayed in 20^{o}C water was 104.9 mg while it was 26.5 mg in 15 ^{o}C water.

Table 3: Prevalence percentages from selected previous experimental infection studies using *Gasterosteus aculeatus* infected with copepod(s) infected with *S. solidus*

Study	**Sticklebacks from**	***S. solidus* from**	**No. fish infected**	**No. fish with plerocercoid(s)**	**% prevalence**	**Copepod species used**	**Comments**
This study	*Vigdarvatnet, Vigdarvassdraget western Norway*	*Skogseidvatnet, western Norway*	*99*	*3*	*3.0*	*Acanthocyclops* sp.	*Single copepod infected with a single S. solidus*
This study	*Nesavatnet, Vigdarvassdraget, western Norway*	*Skogseidvatnet, western Norway*	*99*	*4*	*4.0*	*Acanthocyclops* sp.	*Single copepod infected with a single S. solidus*
Scharsack (personal communication)	Skogseidvatnet, western Norway	Skogseidvatnet, western Norway	62	30	48.4	*Macrocyclops albilus*	Fish exposed to 3 procercoids
Henrich *et al.*, 2013	Grosser Plöner See, northern Germany	Skogseidvatnet, western Norway	38	17	44.7	*Macrocyclops albilus*	Single copepod infected with a single *S. solidus*
Benesh & Hafer, 2012	Skogseidvatnet, western Norway	Skogseidvatnet, western Norway	100	82	82.0	*Macrocyclops albilus*	Single copepod infected with a single *S. solidus*
Scharsack *et al.*, 2007	Kremper Au, Neustadt, northern Germany	Neustädter Binnenwasser, northern Germany	421	221	52.5	*Macrocyclops albilus*	Single copepod infected with a single *S. solidus*. Different dpe-s.
Hammerschmidt & Kurtz, 2005	Kremper Au, Neustadt, northern Germany	Neustädter Binnenwasser, northern Germany	160	55	33.9	*Macrocyclops albilus*	Single copepod infected with a single *S. solidus*
Jäger & Schjørring, 2006	Neustädter Binnenwasser, northern Germany	Neustädter Binnenwasser, northern Germany	27	12	44.4	*Macrocyclops albilus*	Single copepod infected with a single *S. solidus*
Macnab & Barber, 2011	Carsington Reservoir, Derbyshire, UK	Carsington Reservoir, Derbyshire, UK	25	13	52.0	*Cyclops strenuus abyssorum*	Single copepod infected with a single *S. solidus.*
Barber & Svensson, 2003	Llyn Frongoch, Wales, UK	Llyn Frongoch, Wales, UK	30	5	16.7	*Cyclops strenuus*	Single copepod infected with a single *S. solidus*

Unexpectedly no plerocercoids were found in the mass infection experiment in spite of exposing 244 sticklebacks to 5000 copepods which were mass infected with *S. solidus*. Conditions in the experimental tanks were not comparable to the single-infection experiment, as they resemble more of a mesoscale environment with temperatures following the season rather than controlled, stable temperature in the small aquariums. A low initial temperature (11 ^{o}C), may also have strongly affected host resistance towards the parasite and/or plerocercoid infectivity (Scharsack, personal communication). Another important factor is that not all fish survived the mass infection experiment. The reason for this could be that the experiment was confounded by selective mortality of infected fish during the winter, as 156 of 400 exposed sticklebacks died. Thus we cannot say anything about the confounding factors such as behavioral resistance or differences in distribution that may affect copepod predation in the stickleback morphs. Checking daily for dead sticklebacks and dissecting them would have given more precise results regarding prevalence. An earlier start to the experiment was not feasible due to the hatching failure of *S. solidus* eggs. However, heating the large tanks, while at a considerable cost, might have resolved the problem of the experiment being lengthened and the high fish mortalities.

Parasites often have a strong effect on the evolution of its hosts to the point that strong local adaptations can occur. Hosts such as sticklebacks have evolved efficient and adaptive immune responses such as major histocompatibility complex (MHC) genes in order to combat the dynamic source of selection, i.e. the parasite (Eizaguirre & Lenz, 2010). A costly parasite such as *S. solidus* may be strongly selected against when encountered and sticklebacks have an optimal number of MHC alleles of about 5.8 (Kurtz *et al.*, 2004). High MHC diversity has been correlated with high resistance against infections (Wegner *et al.*, 2003; Kurtz *et al.*, 2004). However maintaining MHC polymorphism is expensive and mechanisms that help maintain this have been summarized by Milinski (2006) and Eizaguirre & Lenz (2010). One such mechanism is the negative frequency dependent selection (NFDS) where rare resistant alleles in the host are favored and increase in frequency until another rare resistant alleles are favored due to the counter-adaptation of the parasite. Loss and fixation of alleles are then selected against at the same time, favoring polymorphism. Such a scenario has been found for three-spined sticklebacks infected with the ectoparasite *Gyrodactylus* sp.; where a specific MHC haplotype was significantly less infected by the parasite the first year increased in frequency in the second year which led to a lower abundance of the parasite (Eizaguirre *et al.*, 2009, Lenz *et al.,* 2009). For the second year however this haplotype did not give a further advantage against the parasite which suggests a counter adaptation by the parasite (Eizaguirre *et al.*, 2009, Lenz *et al.*, 2009). Local adaptations may therefore occur over very short periods of time.

Another mechanism for maintaining MHC polymorphism is habitat heterogeneity, that is, habitat differences creating allele pools from which divergent local adaptations arise (Eizaguirre & Lenz, 2010). Eizaguirre *et al.* (2012) found divergent selection in F2 generation lake and river hybrids when they were brought to the habitat of their grandparents. Independently of their genetic background, MHC genotypes were selected in order to adapt to the local parasites (Eizaguirre *et al.*, 2012). A study on sympatric stickleback population in two lakes of limnetic and benthic species pair suggested that MHC alleles diverged parallelly due to different parasite communities in the pelagic and littoral habitat zones of the lakes (Matthews *et al.*, 2010). Furthermore, a recent field study on Icelandic sticklebacks showed that parasite infections "differed consistently between *G. aculeatus* from different benthic habitats" (Natsopoulou *et al.*, 2012). Here, similar parallel patterns were seen where MHC variation was higher in mud habitat morphs compared to lava habitat morphs, the latter being more heavily infected by parasites (Natsopoulou *et al.*, 2012). In our experiment we therefore expected to see similar shaping of parasite infectivity caused by habitat heterogeneity. We chose sticklebacks of significantly divergent pelvic characteristics, of which maintenance has been attributed to predation (Reimchen, 1980; Vamosi & Schluter, 2004). Pelvic reduction has been attributed to presence of predatory insects (Reimchen, 1980) and lack of calcium resources (Giles, 1983a; Bell *et al.*, 1993). We wanted to explore how a specific, costly parasite would perform in such strikingly different populations and to what degree *S. solidus* may influence distribution of morphs in its host population. Unfortunately our results give no clear answers to these questions.

It may however further highlight that MHC diversity is not necessarily habitat-specific; it can also be parasite-specific (Eizaguirre *et al.*, 2009, Eizaguirre *et al.*, 2010). We may see a parasite-specific frequency-dependent local adaptation scenario unfolding for Vigdarvatnet and Nesavatnet stickleback populations infected with Skogseidvatnet *S. solidus*. This *S. solidus* population has been used in experiments with German sticklebacks, and has shown to be more compatible in the allopatric host combination compared to their sympatric hosts (Kalbe *et al.*, unpublished data). One possible explanation for this is that both Vigdarvatnet and Nesavatnet sticklebacks are put under high selective pressure from the locally adapted

S. solidus strains. This relies on a relatively low migration rate of other parasite strains. The 77 km distance by air (Google Earth, 2013) between parasite site of origin (Lake Skogseidvatnet) and the two lakes in Vigdarvassdraget (Figure 2) suggests that it is within the distance of the feeding areas of non-breeding birds (van der Veen & Kurtz, 2002). The migration rate of birds may however be season-dependent and lower if local colonies are present. Despite no studies of these migratory patterns of birds in the area have been performed, it is reasonable to suggest that the encounter of parasites from local colonies of birds happen at a larger rate. Locally then, a high compatibility between host and parasite may be maintained.

This, like the results of the experiment, does not however explain the morphological variations between the stickleback populations. Piscivorous fish predation in particular has been of interest due to its potential role for disruption of the *S. solidus* life cycle, as this is a not a host. Infected sticklebacks may be at a larger risk of being eaten by fish predators (Jakobsen *et al.,* 1988), which is detrimental for both the sticklebacks and *S. solidus*. We expected that tri-trophic interactions involving fish predators would be a predictor for parasite resistance. However, sticklebacks with complete pelvic girdle have shown the same high resistance towards *S. solidus* similar to those with a reduced pelvis. These surprising results may suggest that tri-trophic interactions play a small role. Perhaps parasitized sticklebacks do not encounter predatory fish to a significant degree either due to their behavior or habitat choice, which would be beneficial for the parasite. However the manipulative stage of the cestode facilitates predation from bird hosts (Giles, 1983b; Tierney *et al.,* 1993; Ness & Foster, 1999; Barber *et al.,* 2004) and the costs of the parasite to the stickleback hosts makes it an easy target for predatory fish as well (Östlund-Nilsson *et al.,* 2007). If predation risk is high, Vigdarvatnet sticklebacks may go through strong selection to maintain a complete pelvis as well as a strong selection on resistance to avoid a costly parasite.

We have compared several studies to observe the prevalence of plerocercoids in lab-bred sticklebacks infected with lab-bred copepods, but did not find experimental infections using Vigdarvatnet and Nesavatnet sticklebacks. Without another experiment which includes Nesavatnet *S. solidus* and Vigdarvatnet *S. solidus* we are no closer to understanding the morphology of these stickleback populations on parasite resistance. For future research a full cross-infection design is recommended where Nesavatnet, Vigdarvatnet as well as Skogseidvatnet sticklebacks are infected with *S. solidus* from all 3 lakes in a 3x3 matrix including controls. This design would test for strong local adaptation of the parasite. While the results from this experiment may suggest strong local adaptation and low compatibility of these hosts and parasites, we cannot be certain of this without further experiments.

5. References

Maps, lake surface area and distance between lakes found through tools in Google Earth (2013).

Observations of birds in lakes found at www.artsobservasjoner.no

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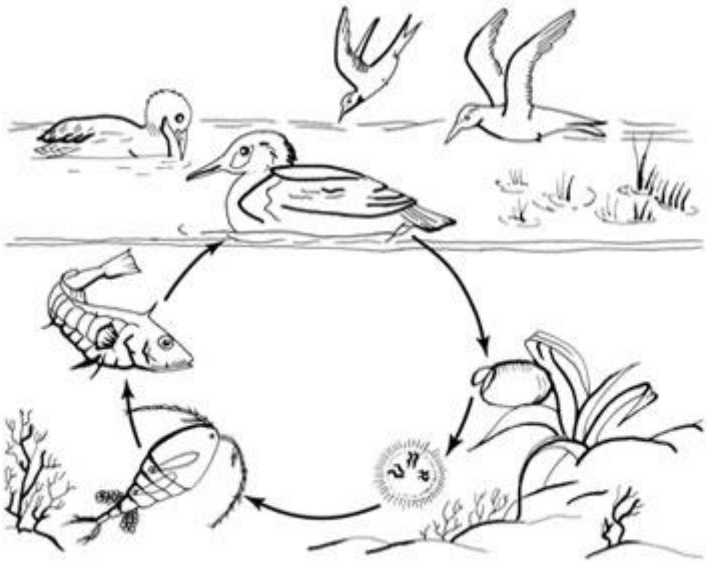
6. Appendix

6.1 Parasite life cycle

S.solidus has a complex lifecycle (Figure 13) with three consecutive hosts, a copepod, the three-spined sticklebacks and any fish eating bird (Smyth, 1946; Bråten, 1966; Dubinina, 1980; Wedekind, 1997). The tapeworm takes all its resources from its two intermediate hosts, which are used as vessels for transmission and die as prey for the next host (Arme & Owen 1967). The parasite does not grow in its definitive host but mature there and produce eggs either sexually or through selfing that are released in water with the bird's faeces. When exposed to light the eggs hatch and become free-swimming coracidia which are eaten by the first intermediate host, a wide range of copepod species (Orr & Hopkins, 1969; Dubinina, 1980; Benesh & Hafer, 2012). In the haemocoel of the copepod *S. solidus* coracidia develops into procercoids, noted as fully developed by the cigar-shaped body and the cercomer with its six hooks on one end. Infected copepods are eaten by sticklebacks which may have no behavioural defence against eating them (Urdal *et al.,* 1995; Wedekind & Milinski, 1996) and rely on their immune system to resist the parasite (Kurtz *et al.*, 2004; Scharsack *et al.*, 2007). Establishment in the body cavity is essential for infection success (Hammerschmidt & Kurtz, 2007). Here it develops into a plerocercoid, the longest stage of the parasite life cycle. The mass of the fast-growing plerocercoid can approach or even exceed that of the host fish (Arme & Owen, 1967) resulting in a characteristic swollen body cavity that bulges to the anterior as well as the posterior of the pelvis.

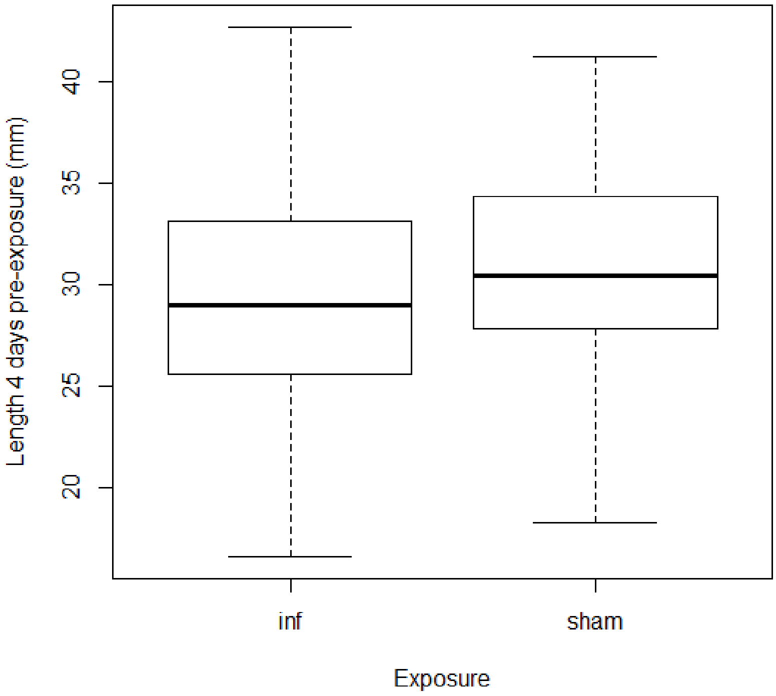
Figure 13: *Schistocephalus solidus* life cycle (The Natural History Museum, 2013).



Growth of plerocercoids poses a massive metabolic demand in stickleback hosts (Meakins & Walkey, 1975). Costs of being infected for the stickleback, other than those already mentioned, include a reduced ability to compete with conspecifics (Milinski, 1984) and reduced reproductive fitness or no reproduction at all (Tierney *et al.*, 1996; Wedekind, 1997; Bagamian *et al.*, 2004). Reproductive disruption has not been found to be caused by the parasite actively castrating its host; rather it seems more likely that it comes as a side effect of the large energy costs of infection (Macnab *et al.*, 2011). A wide range of other potential costs have been summarized by Östlund-Nilsson *et al.* (2007). As successful infections can cause great fitness reduction in hosts, selection against this parasite should therefore be strong.

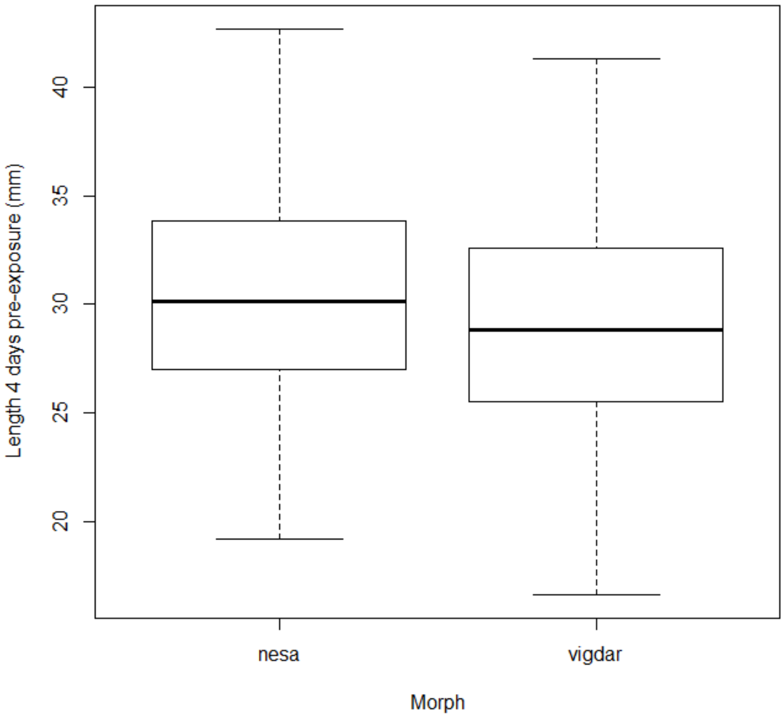
6.2 Fork length 4 days pre-exposure, comparison of *S. solidus*- and sham-infected

Box plots comparing fork length (mm) of sticklebacks infected (inf) with *S. solidus* and sham-infected (sham) 4 days pre-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.



6.3 Fork length 4 days pre-exposure, comparison of morphs

Box plots comparing fork length (mm) of Nesavatnet sticklebacks (nesa) and Vigdarvatnet sticklebacks (vigdar) 4 days pre-exposure. Bold lines show medians with the box representing the middle 50% of the data samples. Upper and lower quartiles are shown with whiskers, outliers are marked by circles.



6.4 Single infection experiment raw data

fam 1-6 refers to *S. solidus* families exposed to. dsn = dorsal spine number, cps = pelvic score, para= parasite found (0 = no, 1 = yes), weightpara = weight of parasite (mg). “time” is categorical, time = 88 is 88 days post-exposure, time = 1 is 4 days pre-exposure.

time	exposure	morph	aquarium	length	weight	dsn	cps	para	weightpara
88	sham	nesa	nesasham	42,8	0,56	2	7	0	
88	sham	nesa	nesasham	39,0	0,41	2	0	0	
88	sham	nesa	nesasham	44,9	0,57	2	6	0	
88	sham	nesa	nesasham	43,0	0,56	2	0	0	
88	sham	nesa	nesasham	43,6	0,58	2	1	0	
88	sham	nesa	nesasham	41,0	0,52	3	0	0	
88	sham	nesa	nesasham	41,9	0,78	3	0	0	
88	sham	nesa	nesasham	37,1	0,38	3	0	0	
88	sham	nesa	nesasham	37,0	0,35	2	2	0	
88	sham	nesa	nesasham	36,6	0,36	2	0	0	
88	sham	nesa	nesasham	39,9	0,51	2	0	0	

88	sham	nesa	nesasham	34,2	0,39	2	0	0
88	sham	nesa	nesasham	35,7	0,33	2	0	0
88	sham	nesa	nesasham	35,7	0,36	2	0	0
88	sham	nesa	nesasham	44,2	0,85	2	0	0
88	sham	nesa	nesasham	44,6	0,56	3	0	0
88	sham	nesa	nesasham	39,0	0,48	2	0	0
88	sham	nesa	nesasham	38,9	0,48	3	8	0
88	sham	nesa	nesasham	39,2	0,45	1	0	0
88	sham	nesa	nesasham	37,0	0,42	2	0	0
88	sham	nesa	nesasham	33,5	0,38	2	0	0
88	sham	nesa	nesasham	41,0	0,47	2	0	0
88	sham	nesa	nesasham	44,9	0,58	3	1	0
88	sham	nesa	nesasham	39,7	0,43	2	0	0
88	sham	nesa	nesasham	37,1	0,4	2	0	0
88	sham	nesa	nesasham	44,0	0,62	2	0	0
88	sham	nesa	nesasham	41,4	0,49	2	0	0
88	sham	nesa	nesasham	38,2	0,4	2	2	0
88	sham	nesa	nesasham	37,7	0,37	2	0	0
88	sham	nesa	nesasham	39,8	0,43	2	0	0
88	sham	nesa	nesasham	41,0	0,52	3	2	0
88	sham	nesa	nesasham	40,0	0,5	2	0	0
88	sham	nesa	nesasham	36,2	0,34	2	0	0
88	inf	nesa	nesafam1	38,1	0,49	3	0	0
88	inf	nesa	nesafam1	42,7	0,59	2	0	0
88	inf	nesa	nesafam1	42,2	0,58	3	2	0
88	inf	nesa	nesafam1	39,0	0,41	3	1	0
88	inf	nesa	nesafam1	44,7	0,62	2	0	0
88	inf	nesa	nesafam1	38,9	0,43	2	0	0
88	inf	nesa	nesafam1	42,8	0,58	3	2	0
88	inf	nesa	nesafam1	41,0	0,51	3	0	0
88	inf	nesa	nesafam1	43,9	0,57	2	1	0
88	inf	nesa	nesafam1	43,3	0,61	2	2	0
88	inf	nesa	nesafam1	44,0	0,64	2	1	0
88	inf	nesa	nesafam1	37,7	0,55	3	0	0
88	inf	nesa	nesafam1	38,6	0,48	2	1	0
88	inf	nesa	nesafam1	36,4	0,37	2	0	0
88	inf	nesa	nesafam1	43,8	0,68	2	4	0
88	inf	nesa	nesafam1	46,1	0,68	3	0	0
88	inf	nesa	nesafam1	44,3	0,66	2	0	0
88	inf	nesa	nesafam1	37,0	0,41	2	0	0
88	inf	nesa	nesafam1	42,8	0,5	2	0	0
88	inf	nesa	nesafam1	40,8	0,5	2	0	0
88	inf	nesa	nesafam1	43,2	0,59	2	1	0
88	inf	nesa	nesafam1	39,7	0,46	2	6	0
88	inf	nesa	nesafam1	43,0	0,55	2	0	0

88	inf	nesa	nesafam1	43,3	0,61	2	0	0	
88	inf	nesa	nesafam1	44,4	0,6	2	5	0	
88	inf	nesa	nesafam1	41,6	0,53	2	0	0	
88	inf	nesa	nesafam1	39,6	0,44	3	0	0	
88	inf	nesa	nesafam1	42,9	0,59	2	7	0	
88	inf	nesa	nesafam1	41,5	0,47	2	0	0	
88	inf	nesa	nesafam1	40,8	0,51	3	1	0	
88	inf	nesa	nesafam1	33,9	0,34	2	0	0	
88	inf	nesa	nesafam1	39,9	0,45	2	0	0	
88	inf	nesa	nesafam1	42,2	0,56	3	0	0	
88	inf	nesa	nesafam2	42,9	0,53	3	2	0	
88	inf	nesa	nesafam2	44,5	0,56	3	2	0	
88	inf	nesa	nesafam2	41,0	0,48	3	0	0	
88	inf	nesa	nesafam2	44,7	0,6	3	2	0	
88	inf	nesa	nesafam2	40,0	0,47	2	0	0	
88	inf	nesa	nesafam2	45,2	0,59	2	0	0	
88	inf	nesa	nesafam2	48,4	0,78	3	0	0	
88	inf	nesa	nesafam2	44,7	0,68	2	4	0	
88	inf	nesa	nesafam2	49,5	0,81	3	4	0	
88	inf	nesa	nesafam2	46,0	0,62	2	0	0	
88	inf	nesa	nesafam2	39,1	0,41	2	2	0	
88	inf	nesa	nesafam2	46,1	0,6	2	0	0	
88	inf	nesa	nesafam2	46,9	0,65	3	2	0	
88	inf	nesa	nesafam2	44,9	0,56	3	1	0	
88	inf	nesa	nesafam2	42,6	0,53	2	0	0	
88	inf	nesa	nesafam2	39,3	0,43	3	0	0	
88	inf	nesa	nesafam2	44,0	0,61	2	0	0	
88	inf	nesa	nesafam2	43,9	0,59	2	1	0	
88	inf	nesa	nesafam2	39,0	0,48	3	0	0	
88	inf	nesa	nesafam2	43,2	0,57	3	0	0	
88	inf	nesa	nesafam2	43,8	0,63	3	0	0	
88	inf	nesa	nesafam2	45,8	0,71	2	0	1	110,02
88	inf	nesa	nesafam2	36,3	0,3	3	0	0	
88	inf	nesa	nesafam2	46,1	0,63	2	0	0	
88	inf	nesa	nesafam2	40,0	0,41	3	0	0	
88	inf	nesa	nesafam2	42,4	0,48	2	0	0	
88	inf	nesa	nesafam2	42,0	0,47	2	0	0	
88	inf	nesa	nesafam2	42,4	0,48	2	0	0	
88	inf	nesa	nesafam2	31,7	0,21	3	0	0	
88	inf	nesa	nesafam2	37,4	0,41	2	0	0	
88	inf	nesa	nesafam2	43,0	0,5	2	0	0	
88	inf	nesa	nesafam2	42,9	0,63	2	0	1	107,41
88	inf	nesa	nesafam2	46,0	0,75	2	0	1	150,65
88	inf	nesa	nesafam3	42,5	0,48	2	0	0	
88	inf	nesa	nesafam3	41,8	0,45	2	0	0	

88	inf	nesa	nesafam3	35,0	0,31	3	0	0
88	inf	nesa	nesafam3	44,9	0,62	2	0	0
88	inf	nesa	nesafam3	40,5	0,45	2	0	0
88	inf	nesa	nesafam3	40,0	0,47	2	0	0
88	inf	nesa	nesafam3	48,0	0,76	3	0	0
88	inf	nesa	nesafam3	39,4	0,73	2	0	0
88	inf	nesa	nesafam3	45,0	0,64	2	3	0
88	inf	nesa	nesafam3	39,0	0,41	3	2	0
88	inf	nesa	nesafam3	45,3	0,62	2	0	0
88	inf	nesa	nesafam3	44,8	0,61	2	0	0
88	inf	nesa	nesafam3	44,0	0,58	2	0	0
88	inf	nesa	nesafam3	40,9	0,47	2	0	0
88	inf	nesa	nesafam3	42,8	0,5	3	0	0
88	inf	nesa	nesafam3	48,1	0,92	3	0	0
88	inf	nesa	nesafam3	33,8	0,25	2	0	0
88	inf	nesa	nesafam3	41,0	0,47	3	0	0
88	inf	nesa	nesafam3	44,0	0,54	2	0	0
88	inf	nesa	nesafam3	39,7	0,46	2	0	0
88	inf	nesa	nesafam3	45,0	0,62	2	0	0
88	inf	nesa	nesafam3	42,1	0,58	2	0	0
88	inf	nesa	nesafam3	43,9	0,56	1	7	0
88	inf	nesa	nesafam3	39,6	0,38	1	0	0
88	inf	nesa	nesafam3	37,0	0,29	2	0	0
88	inf	nesa	nesafam3	43,0	0,54	1	0	0
88	inf	nesa	nesafam3	46,2	0,64	1	0	0
88	inf	nesa	nesafam3	43,2	0,5	1	0	0
88	inf	nesa	nesafam3	39,0	0,4	2	0	0
88	inf	nesa	nesafam3	39,9	0,46	3	8	0
88	inf	nesa	nesafam3	40,3	0,48	3	0	0
88	inf	nesa	nesafam3	39,7	0,43	2	0	0
88	inf	nesa	nesafam3	36,0	0,79	2	0	0
88	sham	vigdar	vigdarsham	31,8	0,52	3	8	0
88	sham	vigdar	vigdarsham	46,9	0,7	3	8	0
88	sham	vigdar	vigdarsham	45,7	0,75	3	8	0
88	sham	vigdar	vigdarsham	40,7	0,57	3	8	0
88	sham	vigdar	vigdarsham	44,8	0,67	3	8	0
88	sham	vigdar	vigdarsham	42,0	0,65	3	8	0
88	sham	vigdar	vigdarsham	46,6	0,71	3	8	0
88	sham	vigdar	vigdarsham	45,8	0,75	3	8	0
88	sham	vigdar	vigdarsham	44,0	0,74	3	8	0
88	sham	vigdar	vigdarsham	36,6	0,43	3	8	0
88	sham	vigdar	vigdarsham	43,1	0,57	3	8	0
88	sham	vigdar	vigdarsham	35,1	0,35	3	8	0
88	sham	vigdar	vigdarsham	43,2	0,53	3	8	0
88	sham	vigdar	vigdarsham	43,0	0,57	3	8	0

88	sham	vigdar	vigdarsham	41,8	0,58	3	8	0	
88	sham	vigdar	vigdarsham	44,7	0,64	3	8	0	
88	sham	vigdar	vigdarsham	37,2	0,5	3	8	0	
88	sham	vigdar	vigdarsham	38,6	0,47	3	8	0	
88	sham	vigdar	vigdarsham	50,5	1,03	3	8	0	
88	sham	vigdar	vigdarsham	38,6	0,51	3	8	0	
88	sham	vigdar	vigdarsham	41,1	0,65	3	8	0	
88	sham	vigdar	vigdarsham	36,1	0,41	3	7	0	
88	sham	vigdar	vigdarsham	39,3	0,48	3	8	0	
88	sham	vigdar	vigdarsham	41,8	0,56	3	8	0	
88	sham	vigdar	vigdarsham	38,6	0,43	3	8	0	
88	sham	vigdar	vigdarsham	41,4	0,49	3	8	0	
88	sham	vigdar	vigdarsham	44,9	0,71	3	8	0	
88	sham	vigdar	vigdarsham	43,9	0,64	3	8	0	
88	sham	vigdar	vigdarsham	34,0	0,39	3	8	0	
88	sham	vigdar	vigdarsham	46,7	0,8	3	8	0	
88	sham	vigdar	vigdarsham	42,0	0,69	3	8	0	
88	sham	vigdar	vigdarsham	37,2	0,55	3	7	0	
88	sham	vigdar	vigdarsham	46,0	0,95	3	8	0	
88	inf	vigdar	vigdarfam1	39,0	0,52	3	8	0	
88	inf	vigdar	vigdarfam1	40,6	0,54	3	8	0	
88	inf	vigdar	vigdarfam1	40,6	0,54	3	8	0	
88	inf	vigdar	vigdarfam1	43,0	0,62	3	8	0	
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88	inf	vigdar	vigdarfam1	44,0	0,75	3	8	0	
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88	inf	vigdar	vigdarfam1	44,8	0,65	3	8	0	
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1	sham	vigdar	vigdarsham	31,7
1	sham	vigdar	vigdarsham	33,4
1	sham	vigdar	vigdarsham	19,7
1	sham	vigdar	vigdarsham	37,5
1	sham	vigdar	vigdarsham	29,0
1	sham	vigdar	vigdarsham	33,1
1	sham	vigdar	vigdarsham	34,8
1	sham	vigdar	vigdarsham	35,0
1	sham	vigdar	vigdarsham	37,1
1	inf	vigdar	vigdarfam1	30,8
1	inf	vigdar	vigdarfam1	29,2
1	inf	vigdar	vigdarfam1	26,9
1	inf	vigdar	vigdarfam1	32,6
1	inf	vigdar	vigdarfam1	18,2
1	inf	vigdar	vigdarfam1	32,0
1	inf	vigdar	vigdarfam1	38,9
1	inf	vigdar	vigdarfam1	21,9
1	inf	vigdar	vigdarfam1	30,3
1	inf	vigdar	vigdarfam1	22,8
1	inf	vigdar	vigdarfam1	26,3
1	inf	vigdar	vigdarfam1	27,2
1	inf	vigdar	vigdarfam1	35,3
1	inf	vigdar	vigdarfam1	18,3
1	inf	vigdar	vigdarfam1	38,5
1	inf	vigdar	vigdarfam1	30,4
1	inf	vigdar	vigdarfam1	31,4
1	inf	vigdar	vigdarfam1	39,7
1	inf	vigdar	vigdarfam1	27,0
1	inf	vigdar	vigdarfam1	31,2
1	inf	vigdar	vigdarfam1	36,1
1	inf	vigdar	vigdarfam1	34,3
1	inf	vigdar	vigdarfam1	35,5
1	inf	vigdar	vigdarfam1	34,1
1	inf	vigdar	vigdarfam1	17,9
1	inf	vigdar	vigdarfam1	34,3
1	inf	vigdar	vigdarfam1	33,4
1	inf	vigdar	vigdarfam1	31,1
1	inf	vigdar	vigdarfam1	24,0
1	inf	vigdar	vigdarfam1	39,4
1	inf	vigdar	vigdarfam1	24,8
1	inf	vigdar	vigdarfam1	18,0

1	inf	vigdar	vigdarfam1	18,0
1	inf	vigdar	vigdarfam1	24,6
1	inf	vigdar	vigdarfam1	23,3
1	inf	vigdar	vigdarfam1	25,6
1	inf	vigdar	vigdarfam1	17,7
1	inf	vigdar	vigdarfam1	28,2
1	inf	vigdar	vigdarfam1	33,6
1	inf	vigdar	vigdarfam1	29,8
1	inf	vigdar	vigdarfam1	29,6
1	inf	vigdar	vigdarfam1	28,1
1	inf	vigdar	vigdarfam1	29,0
1	inf	vigdar	vigdarfam1	18,6
1	inf	vigdar	vigdarfam1	33,1
1	inf	vigdar	vigdarfam1	27,4
1	inf	vigdar	vigdarfam1	30,8
1	inf	vigdar	vigdarfam1	26,3
1	inf	vigdar	vigdarfam1	33,5
1	inf	vigdar	vigdarfam1	22,2
1	inf	vigdar	vigdarfam1	31,6
1	inf	vigdar	vigdarfam1	28,6
1	inf	vigdar	vigdarfam1	27,2
1	inf	vigdar	vigdarfam1	20,3
1	inf	vigdar	vigdarfam1	20,8
1	inf	vigdar	vigdarfam1	28,6
1	inf	vigdar	vigdarfam1	26,7
1	inf	vigdar	vigdarfam1	27,2
1	inf	vigdar	vigdarfam1	27,6
1	inf	vigdar	vigdarfam1	28,6
1	inf	vigdar	vigdarfam2	24,7
1	inf	vigdar	vigdarfam2	27,3
1	inf	vigdar	vigdarfam2	24,1
1	inf	vigdar	vigdarfam2	37,0
1	inf	vigdar	vigdarfam2	25,6
1	inf	vigdar	vigdarfam2	34,4
1	inf	vigdar	vigdarfam2	38,6
1	inf	vigdar	vigdarfam2	36,7
1	inf	vigdar	vigdarfam2	23,6
1	inf	vigdar	vigdarfam2	30,4
1	inf	vigdar	vigdarfam2	25,4
1	inf	vigdar	vigdarfam2	23,3
1	inf	vigdar	vigdarfam2	29,1
1	inf	vigdar	vigdarfam2	25,0
1	inf	vigdar	vigdarfam2	22,3
1	inf	vigdar	vigdarfam2	37,5
1	inf	vigdar	vigdarfam2	41,3

1	inf	vigdar	vigdarfam2	27,4
1	inf	vigdar	vigdarfam2	24,0
1	inf	vigdar	vigdarfam2	29,8
1	inf	vigdar	vigdarfam2	16,6
1	inf	vigdar	vigdarfam2	38,7
1	inf	vigdar	vigdarfam2	24,6
1	inf	vigdar	vigdarfam2	35,7
1	inf	vigdar	vigdarfam2	20,0
1	inf	vigdar	vigdarfam2	38,4
1	inf	vigdar	vigdarfam2	30,1
1	inf	vigdar	vigdarfam2	24,1
1	inf	vigdar	vigdarfam2	31,9
1	inf	vigdar	vigdarfam2	20,5
1	inf	vigdar	vigdarfam2	27,4
1	inf	vigdar	vigdarfam2	26,8
1	inf	vigdar	vigdarfam2	30,1
1	inf	vigdar	vigdarfam2	30,0
1	inf	vigdar	vigdarfam2	24,5
1	inf	vigdar	vigdarfam2	32,8
1	inf	vigdar	vigdarfam2	23,6
1	inf	vigdar	vigdarfam2	33,9
1	inf	vigdar	vigdarfam2	26,0
1	inf	vigdar	vigdarfam2	30,6
1	inf	vigdar	vigdarfam2	31,2
1	inf	vigdar	vigdarfam2	27,0
1	inf	vigdar	vigdarfam2	26,7
1	inf	vigdar	vigdarfam2	36,0
1	inf	vigdar	vigdarfam2	32,1
1	inf	vigdar	vigdarfam2	25,9
1	inf	vigdar	vigdarfam2	31,1
1	inf	vigdar	vigdarfam2	32,0
1	inf	vigdar	vigdarfam2	35,4
1	inf	vigdar	vigdarfam2	24,2
1	inf	vigdar	vigdarfam2	38,2
1	inf	vigdar	vigdarfam2	26,6
1	inf	vigdar	vigdarfam2	32,4
1	inf	vigdar	vigdarfam2	32,4
1	inf	vigdar	vigdarfam2	29,4
1	inf	vigdar	vigdarfam2	22,2
1	inf	vigdar	vigdarfam2	24,6
1	inf	vigdar	vigdarfam2	32,1
1	inf	vigdar	vigdarfam2	28,0
1	inf	vigdar	vigdarfam2	38,3
1	inf	vigdar	vigdarfam3	31,1
1	inf	vigdar	vigdarfam3	23,8

1	inf	vigdar	vigdarfam3	26,2
1	inf	vigdar	vigdarfam3	34,8
1	inf	vigdar	vigdarfam3	36,5
1	inf	vigdar	vigdarfam3	23,6
1	inf	vigdar	vigdarfam3	31,9
1	inf	vigdar	vigdarfam3	31,4
1	inf	vigdar	vigdarfam3	32,2
1	inf	vigdar	vigdarfam3	26,3
1	inf	vigdar	vigdarfam3	21,4
1	inf	vigdar	vigdarfam3	28,6
1	inf	vigdar	vigdarfam3	23,7
1	inf	vigdar	vigdarfam3	30,9
1	inf	vigdar	vigdarfam3	28,5
1	inf	vigdar	vigdarfam3	25,4
1	inf	vigdar	vigdarfam3	29,5
1	inf	vigdar	vigdarfam3	23,9
1	inf	vigdar	vigdarfam3	24,8
1	inf	vigdar	vigdarfam3	29,5
1	inf	vigdar	vigdarfam3	17,5
1	inf	vigdar	vigdarfam3	26,5
1	inf	vigdar	vigdarfam3	28,0
1	inf	vigdar	vigdarfam3	37,5
1	inf	vigdar	vigdarfam3	34,3
1	inf	vigdar	vigdarfam3	40,0
1	inf	vigdar	vigdarfam3	24,8
1	inf	vigdar	vigdarfam3	28,4
1	inf	vigdar	vigdarfam3	28,1
1	inf	vigdar	vigdarfam3	30,2
1	inf	vigdar	vigdarfam3	33,1
1	inf	vigdar	vigdarfam3	23,0
1	inf	vigdar	vigdarfam3	29,3
1	inf	vigdar	vigdarfam3	27,4
1	inf	vigdar	vigdarfam3	24,0
1	inf	vigdar	vigdarfam3	27,3
1	inf	vigdar	vigdarfam3	26,4
1	inf	vigdar	vigdarfam3	20,1
1	inf	vigdar	vigdarfam3	32,6
1	inf	vigdar	vigdarfam3	20,6
1	inf	vigdar	vigdarfam3	31,3
1	inf	vigdar	vigdarfam3	27,7
1	inf	vigdar	vigdarfam3	33,8
1	inf	vigdar	vigdarfam3	21,8
1	inf	vigdar	vigdarfam3	30,4
1	inf	vigdar	vigdarfam3	28,1
1	inf	vigdar	vigdarfam3	29,7

1	inf	vigdar	vigdarfam3	21,4
1	inf	vigdar	vigdarfam3	30,7
1	inf	vigdar	vigdarfam3	25,7
1	inf	vigdar	vigdarfam3	29,2
1	inf	vigdar	vigdarfam3	33,1
1	inf	vigdar	vigdarfam3	27,9
1	inf	vigdar	vigdarfam3	23,8
1	inf	vigdar	vigdarfam3	25,5
1	inf	vigdar	vigdarfam3	24,7
1	inf	vigdar	vigdarfam3	34,3
1	inf	vigdar	vigdarfam3	22,1

6. 5 Mass infection experiment raw data

“kontroll1-2” are sham-infected tanks while “inf1-2” are *S. solidus* exposed. ”morph” was assessed based on pelvic score (cps) and dorsal spine numbers (dsn). time = 153 is 153 days post-exposure. No separate columns for parasites as none were found.

time	exposure	tank	cps	dsn	morph	length	weight
153	sham	kontroll1	8	3	v	26,8	0,2
153	sham	kontroll1	8	3	v	28,4	0,2
153	sham	kontroll1	8	2	v	29,2	0,23
153	sham	kontroll1	8	3	v	26,1	0,16
153	sham	kontroll1	0	2	n	27,3	0,21
153	sham	kontroll1	0	2	n	27,7	0,19
153	sham	kontroll1	0	3	n	27,9	0,23
153	sham	kontroll1	0	2	n	31,1	0,26
153	sham	kontroll1	8	3	v	29,4	0,21
153	sham	kontroll1	8	3	v	33,5	0,34
153	sham	kontroll1	8	3	v	25,7	0,15
153	sham	kontroll1	8	3	v	30,7	0,22
153	sham	kontroll1	8	3	v	34,4	0,38
153	sham	kontroll1	8	3	v	33,8	0,37
153	sham	kontroll1	8	3	v	34,0	0,38
153	sham	kontroll1	8	3	v	32,6	0,28
153	sham	kontroll1	8	3	v	28,0	0,2
153	sham	kontroll1	0	3	n	27,7	0,21
153	sham	kontroll1	8	3	v	26,4	0,17
153	sham	kontroll1	8	3	v	25,9	0,15
153	sham	kontroll1	8	3	v	34,6	0,38
153	sham	kontroll1	8	3	v	37,2	0,41
153	sham	kontroll1	8	2	v	24,1	0,13
153	sham	kontroll1	0	2	n	26,2	0,15
153	sham	kontroll1	0	2	n	25,3	0,15
153	sham	kontroll1	8	3	v	25,2	0,13

153	sham	kontroll1	0	2	n	26,0	0,16
153	sham	kontroll1	8	3	v	25,5	0,12
153	sham	kontroll1	0	2	n	32,1	0,3
153	sham	kontroll1	0	2	n	27,3	0,19
153	sham	kontroll1	8	3	v	21,7	0,08
153	sham	kontroll1	8	3	v	26,9	0,18
153	sham	kontroll1	8	3	v	32,2	0,28
153	sham	kontroll1	8	3	v	29,5	0,26
153	sham	kontroll1	0	2	n	30,0	0,23
153	sham	kontroll1	8	3	v	26,8	0,13
153	sham	kontroll1	0	2	n	27,5	0,17
153	sham	kontroll1	0	2	n	32,3	0,27
153	sham	kontroll1	8	3	v	27,1	0,13
153	sham	kontroll1	0	3	n	30,4	0,28
153	sham	kontroll1	8	3	v	29,6	0,21
153	sham	kontroll1	0	3	n	32,2	0,27
153	sham	kontroll1	8	3	v	31,0	0,22
153	sham	kontroll1	0	3	n	29,1	0,2
153	sham	kontroll1	8	3	v	33,5	0,31
153	sham	kontroll1	8	3	v	32,1	0,26
153	sham	kontroll1	0	2	n	29,2	0,21
153	sham	kontroll1	0	2	n	30,1	0,23
153	sham	kontroll1	8	3	v	25,7	0,14
153	sham	kontroll1	8	3	v	26,0	0,15
153	sham	kontroll1	8	3	v	21,7	0,08
153	sham	kontroll1	8	3	v	23,1	0,11
153	sham	kontroll1	0	2	n	31,0	0,22
153	sham	kontroll1	8	3	v	32,1	0,25
153	sham	kontroll1	8	3	v	29,1	0,19
153	sham	kontroll1	0	2	n	29,2	0,2
153	sham	kontroll1	8	3	v	26,6	0,15
153	sham	kontroll1	8	3	v	28,8	0,19
153	sham	kontroll1	0	2	n	25,8	0,12
153	sham	kontroll1	0	2	n	30,2	0,19
153	sham	kontroll1	8	3	v	34,5	0,38
153	sham	kontroll1	0	2	n	33,6	0,29
153	sham	kontroll1	0	2	n	26,7	0,14
153	sham	kontroll1	8	3	v	32,0	0,27
153	sham	kontroll1	0	2	n	30,7	0,21
153	sham	kontroll1	0	3	n	33,3	0,31
153	sham	kontroll1	0	2	n	40,8	0,49
153	sham	kontroll1	0	3	n	37,0	0,36
153	sham	kontroll1	8	3	v	32,2	0,33
153	sham	kontroll1	0	2	n	35,4	0,31
153	sham	kontroll1	8	3	v	33,0	0,31

153	sham	kontroll1	8	3	v	32,7	0,27
153	sham	kontroll1	0	2	n	32,1	0,25
153	sham	kontroll1	8	2	v	23,6	0,11
153	sham	kontroll1	0	2	n	25,7	0,11
153	sham	kontroll1	0	3	n	34,0	0,35
153	sham	kontroll1	0	2	n	29,1	0,19
153	sham	kontroll1	0	2	n	29,2	0,18
153	sham	kontroll1	8	3	v	31,6	0,25
153	sham	kontroll1	4	3	n	32,4	0,32
153	sham	kontroll1	0	2	n	28,6	0,15
153	sham	kontroll1	8	3	v	33,8	0,35
153	sham	kontroll1	8	3	v	24,0	0,13
153	sham	kontroll1	0	2	n	26,0	0,13
153	sham	kontroll1	8	3	v	26,6	0,15
153	sham	kontroll1	8	3	v	26,8	0,16
153	sham	kontroll1	0	2	n	27,0	0,14
153	sham	kontroll1	0	2	n	27,3	0,16
153	sham	kontroll1	8	3	v	32,6	0,32
153	sham	kontroll1	8	3	v	24,8	0,12
153	sham	kontroll1	8	3	v	28,2	0,19
153	sham	kontroll1	0	2	n	28,4	0,2
153	sham	kontroll1	8	3	v	24,6	0,12
153	sham	kontroll1	0	3	n	32,0	0,24
153	sham	kontroll1	0	2	n	24,0	0,11
153	sham	kontroll1	8	3	v	25,3	0,14
153	sham	kontroll1	0	3	n	28,8	0,22
153	sham	kontroll1	8	3	v	30,3	0,23
153	sham	kontroll1	8	3	v	31,8	0,27
153	sham	kontroll1	0	3	n	28,4	0,18
153	sham	kontroll1	0	3	n	21,5	0,14
153	sham	kontroll1	8	3	v	20,6	0,07
153	sham	kontroll1	0	2	n	29,2	0,2
153	sham	kontroll1	0	2	n	30,5	0,23
153	sham	kontroll1	0	3	n	29,9	0,2
153	sham	kontroll1	0	3	n	31,6	0,24
153	sham	kontroll1	8	3	v	30,7	0,22
153	sham	kontroll1	0	2	n	34,6	0,34
153	sham	kontroll1	8	3	v	29,1	0,18
153	sham	kontroll1	7	3	v	31,4	0,25
153	sham	kontroll1	8	3	v	30,5	0,22
153	sham	kontroll1	8	3	v	34,3	0,34
153	sham	kontroll1	0	2	n	28,7	0,21
153	sham	kontroll1	8	3	v	32,0	0,27
153	sham	kontroll1	8	3	v	31,5	0,26
153	sham	kontroll1	0	2	n	30,9	0,25

153	sham	kontroll1	8	3	v	30,4	0,23
153	sham	kontroll1	0	2	n	29,7	0,21
153	sham	kontroll1	0	2	n	24,4	0,1
153	sham	kontroll1	8	3	v	29,0	0,19
153	sham	kontroll1	0	2	n	27,1	0,14
153	sham	kontroll1	2	2	n	29,3	0,17
153	sham	kontroll1	0	2	n	31,8	0,25
153	sham	kontroll1	0	2	n	30,9	0,24
153	sham	kontroll2	8	3	v	33,3	0,29
153	sham	kontroll2	0	2	n	25,7	0,13
153	sham	kontroll2	0	2	n	27,3	0,15
153	sham	kontroll2	0	3	n	31,1	0,27
153	sham	kontroll2	8	3	v	35,6	0,43
153	sham	kontroll2	8	3	v	29,0	0,23
153	sham	kontroll2	0	2	n	29,1	0,19
153	sham	kontroll2	1	3	n	36,1	0,38
153	sham	kontroll2	8	3	v	21,0	0,09
153	sham	kontroll2	0	2	n	25,2	0,12
153	sham	kontroll2	8	3	v	23,4	0,09
153	sham	kontroll2	8	2	v	22,2	0,09
153	sham	kontroll2	0	2	n	28,2	0,18
153	sham	kontroll2	0	2	n	31,6	0,24
153	sham	kontroll2	0	3	n	31,9	0,25
153	sham	kontroll2	0	2	n	30,4	0,21
153	sham	kontroll2	8	3	v	26,5	0,16
153	sham	kontroll2	8	3	v	29,8	0,25
153	sham	kontroll2	8	3	v	27,1	0,15
153	sham	kontroll2	8	3	v	32,2	0,29
153	sham	kontroll2	8	3	v	29,9	0,23
153	sham	kontroll2	0	3	n	21,8	0,13
153	sham	kontroll2	8	3	v	27,5	0,19
153	sham	kontroll2	8	3	v	25,7	0,14
153	sham	kontroll2	8	3	v	31,9	0,27
153	sham	kontroll2	8	3	v	27,2	0,17
153	sham	kontroll2	8	3	v	24,8	0,14
153	sham	kontroll2	0	3	n	30,5	0,24
153	sham	kontroll2	0	2	n	27,9	0,2
153	sham	kontroll2	0	2	n	27,9	0,2
153	sham	kontroll2	0	2	n	27,2	0,15
153	sham	kontroll2	0	3	n	26,9	0,16
153	sham	kontroll2	8	3	v	25,6	0,15
153	sham	kontroll2	0	2	n	27,7	0,2
153	sham	kontroll2	8	3	v	33,6	0,33
153	sham	kontroll2	8	3	v	23,6	0,12
153	sham	kontroll2	8	3	v	33,8	0,31

153	sham	kontroll2	0	3	n	31,4	0,24
153	sham	kontroll2	0	3	n	31,1	0,23
153	sham	kontroll2	0	2	n	29,4	0,21
153	sham	kontroll2	0	3	n	27,5	0,2
153	sham	kontroll2	0	2	n	29,5	0,21
153	sham	kontroll2	8	3	v	30,4	0,23
153	sham	kontroll2	0	3	n	23,6	0,12
153	sham	kontroll2	8	3	v	21,9	0,09
153	sham	kontroll2	8	3	v	29,2	0,22
153	sham	kontroll2	0	2	n	33,3	0,29
153	sham	kontroll2	0	3	n	28,2	0,22
153	sham	kontroll2	0	3	n	30,5	0,24
153	sham	kontroll2	0	2	n	38,0	0,58
153	sham	kontroll2	8	3	v	22,2	0,11
153	sham	kontroll2	0	2	n	28,7	0,23
153	sham	kontroll2	8	3	v	30,4	0,33
153	sham	kontroll2	8	3	v	24,9	0,13
153	sham	kontroll2	0	2	n	26,8	0,19
153	sham	kontroll2	0	3	n	31,9	0,37
153	sham	kontroll2	8	3	v	22,3	0,09
153	sham	kontroll2	0	2	n	28,5	0,2
153	sham	kontroll2	0	2	n	33,6	0,26
153	sham	kontroll2	0	2	n	36,9	0,41
153	sham	kontroll2	8	3	v	36,2	0,41
153	sham	kontroll2	0	3	n	31,8	0,25
153	sham	kontroll2	0	2	n	27,1	0,18
153	sham	kontroll2	6	3	v	30,3	0,22
153	sham	kontroll2	0	2	n	30,5	0,21
153	sham	kontroll2	8	3	v	27,6	0,2
153	sham	kontroll2	0	2	n	30,0	0,2
153	sham	kontroll2	0	3	n	30,2	0,21
153	sham	kontroll2	0	2	n	30,8	0,23
153	sham	kontroll2	0	2	n	31,0	0,24
153	sham	kontroll2	0	2	n	33,5	0,33
153	sham	kontroll2	0	2	n	29,6	0,2
153	sham	kontroll2	0	2	n	25,4	0,15
153	sham	kontroll2	0	3	n	27,0	0,18
153	sham	kontroll2	8	3	v	26,9	0,17
153	sham	kontroll2	0	3	n	29,0	0,23
153	sham	kontroll2	0	2	n	27,4	0,2
153	sham	kontroll2	8	3	v	24,2	0,13
153	sham	kontroll2	0	2	n	27,1	0,15
153	sham	kontroll2	0	3	n	34,3	0,36
153	sham	kontroll2	0	2	n	29,8	0,21
153	sham	kontroll2	8	3	v	32,3	0,33

153	sham	kontroll2	0	2	n	28,0	0,17
153	sham	kontroll2	8	3	v	33,6	0,31
153	sham	kontroll2	8	3	v	25,4	0,13
153	sham	kontroll2	0	3	n	31,4	0,23
153	sham	kontroll2	0	2	n	29,0	0,24
153	sham	kontroll2	8	3	v	29,2	0,2
153	sham	kontroll2	0	2	n	33,4	0,29
153	sham	kontroll2	0	2	n	31,1	0,31
153	sham	kontroll2	0	2	n	30,4	0,23
153	sham	kontroll2	8	2	v	26,1	0,15
153	sham	kontroll2	8	3	v	26,0	0,17
153	sham	kontroll2	0	2	n	31,5	0,26
153	sham	kontroll2	0	2	n	31,9	0,27
153	sham	kontroll2	0	2	n	31,5	0,24
153	sham	kontroll2	0	2	n	31,7	0,24
153	sham	kontroll2	0	2	n	27,5	0,18
153	sham	kontroll2	8	3	v	25,9	0,14
153	sham	kontroll2	8	3	v	29,0	0,21
153	sham	kontroll2	8	3	v	33,3	0,39
153	sham	kontroll2	0	2	n	27,3	0,17
153	sham	kontroll2	8	3	v	31,6	0,31
153	sham	kontroll2	0	3	n	34,2	0,33
153	sham	kontroll2	0	3	n	35,1	0,39
153	sham	kontroll2	0	2	n	32,6	0,27
153	sham	kontroll2	0	2	n	32,4	0,29
153	sham	kontroll2	0	2	n	27,0	0,17
153	sham	kontroll2	8	3	v	26,0	0,18
153	sham	kontroll2	8	3	v	27,2	0,21
153	sham	kontroll2	0	2	n	27,0	0,17
153	sham	kontroll2	0	2	n	26,2	0,15
153	sham	kontroll2	8	3	v	33,5	0,35
153	sham	kontroll2	8	3	v	27,4	0,18
153	sham	kontroll2	8	3	v	31,9	0,3
153	sham	kontroll2	0	3	n	34,7	0,38
153	sham	kontroll2	8	3	v	22,2	0,12
153	inf	inf1	8	3	v	36,1	0,39
153	inf	inf1	0	2	n	29,2	0,24
153	inf	inf1	0	2	n	29,1	0,18
153	inf	inf1	8	3	v	27,3	0,22
153	inf	inf1	0	2	n	26,5	0,22
153	inf	inf1	8	3	v	32,0	0,3
153	inf	inf1	8	3	v	29,2	0,23
153	inf	inf1	8	3	v	24,6	0,13
153	inf	inf1	8	3	v	21,5	0,08
153	inf	inf1	0	2	n	34,7	0,34

153	inf	inf1	8	3	v	27,4	0,19
153	inf	inf1	8	3	v	34,6	0,42
153	inf	inf1	0	2	n	28,2	0,21
153	inf	inf1	8	3	v	20,3	0,07
153	inf	inf1	0	2	n	29,3	0,23
153	inf	inf1	8	3	v	35,5	0,4
153	inf	inf1	8	3	v	29,4	0,23
153	inf	inf1	0	2	n	30,5	0,22
153	inf	inf1	8	3	v	29,7	0,25
153	inf	inf1	8	3	v	24,8	0,14
153	inf	inf1	0	3	n	24,9	0,14
153	inf	inf1	8	3	v	24,5	0,1
153	inf	inf1	0	2	n	26,8	0,17
153	inf	inf1	0	3	n	31,9	0,28
153	inf	inf1	8	2	v	36,6	0,36
153	inf	inf1	0	3	n	31,8	0,26
153	inf	inf1	8	3	v	28,5	0,19
153	inf	inf1	8	3	v	22,4	0,1
153	inf	inf1	8	3	v	28,2	0,2
153	inf	inf1	8	3	v	27,0	0,16
153	inf	inf1	0	2	n	30,6	0,26
153	inf	inf1	8	3	v	26,2	0,19
153	inf	inf1	0	3	n	28,4	0,2
153	inf	inf1	8	3	v	22,7	0,11
153	inf	inf1	0	3	n	26,5	0,18
153	inf	inf1	8	3	v	22,4	0,12
153	inf	inf1	8	3	v	34,7	0,34
153	inf	inf1	0	2	n	28,1	0,2
153	inf	inf1	0	2	n	30,8	0,24
153	inf	inf1	0	3	n	31,4	0,26
153	inf	inf1	0	2	n	31,2	0,25
153	inf	inf1	8	3	v	25,8	0,19
153	inf	inf1	7	3	v	31,6	0,26
153	inf	inf1	0	3	n	27,4	0,23
153	inf	inf1	8	3	v	33,5	0,3
153	inf	inf1	0	3	n	27,9	0,19
153	inf	inf1	0	2	n	30,5	0,23
153	inf	inf1	8	3	v	25,0	0,14
153	inf	inf1	8	3	v	25,4	0,15
153	inf	inf1	8	3	v	29,7	0,23
153	inf	inf1	7	3	v	28,3	0,21
153	inf	inf1	8	3	v	32,1	0,26
153	inf	inf1	0	2	n	29,4	0,2
153	inf	inf1	0	2	n	27,0	0,16
153	inf	inf1	8	3	v	29,5	0,2

153	inf	inf1	8	3	v	28,5	0,19
153	inf	inf1	8	3	v	28,9	0,21
153	inf	inf1	0	2	n	26,4	0,13
153	inf	inf1	6	2	v	24,6	0,12
153	inf	inf1	0	2	n	29,0	0,18
153	inf	inf1	8	3	v	24,7	0,12
153	inf	inf1	1	3	n	43,9	0,97
153	inf	inf1	8	3	v	34,6	0,41
153	inf	inf1	8	3	v	32,7	0,27
153	inf	inf1	8	3	v	24,6	0,15
153	inf	inf1	8	3	v	29,2	0,26
153	inf	inf1	8	3	v	35,6	0,49
153	inf	inf1	0	1	n	29,3	0,17
153	inf	inf1	8	3	v	26,4	0,15
153	inf	inf1	8	3	v	27,4	0,17
153	inf	inf1	8	3	v	26,0	0,13
153	inf	inf1	8	3	v	22,1	0,15
153	inf	inf1	0	2	n	21,8	0,15
153	inf	inf1	0	2	n	29,2	0,2
153	inf	inf1	0	2	n	28,8	0,19
153	inf	inf1	8	3	v	29,8	0,2
153	inf	inf1	8	3	v	30,0	0,22
153	inf	inf1	0	2	n	31,8	0,25
153	inf	inf1	8	3	v	32,9	0,39
153	inf	inf1	0	2	n	30,4	0,25
153	inf	inf1	0	3	n	25,1	0,18
153	inf	inf1	0	2	n	26,1	0,19
153	inf	inf1	0	3	n	24,8	0,17
153	inf	inf1	0	2	n	29,5	0,28
153	inf	inf1	0	2	n	33,1	0,32
153	inf	inf1	8	3	v	27,9	0,19
153	inf	inf1	0	2	n	33,7	0,34
153	inf	inf1	4	3	v	34,0	0,31
153	inf	inf1	0	2	n	29,4	0,25
153	inf	inf1	8	3	v	30,4	0,28
153	inf	inf1	8	3	v	33,3	0,34
153	inf	inf1	8	3	v	32,4	0,26
153	inf	inf1	8	3	v	33,0	0,34
153	inf	inf1	0	2	n	30,2	0,29
153	inf	inf1	8	3	v	23,6	0,1
153	inf	inf1	0	3	n	27,8	0,19
153	inf	inf1	0	2	n	29,4	0,26
153	inf	inf1	8	3	v	23,5	0,14
153	inf	inf1	0	2	n	39,1	0,52
153	inf	inf1	0	2	n	29,2	0,19

153	inf	inf1	0	2	n	28,3	0,24
153	inf	inf1	0	2	n	29,6	0,23
153	inf	inf1	7	2	v	28,2	0,2
153	inf	inf1	8	3	v	31,8	0,28
153	inf	inf1	8	2	v	31,9	0,28
153	inf	inf1	8	3	v	34,4	0,4
153	inf	inf1	8	3	v	28,0	0,17
153	inf	inf1	8	3	v	29,7	0,25
153	inf	inf1	0	2	n	25,8	0,15
153	inf	inf1	0	2	n	31,5	0,3
153	inf	inf1	0	2	n	30,6	0,33
153	inf	inf1	8	3	v	33,5	0,29
153	inf	inf1	0	2	n	28,1	0,26
153	inf	inf1	8	3	v	30,3	0,23
153	inf	inf1	8	3	v	27,9	0,21
153	inf	inf1	0	2	n	31,0	0,28
153	inf	inf1	8	3	v	24,9	0,14
153	inf	inf1	8	3	v	35,3	0,44
153	inf	inf1	0	2	n	27,3	0,24
153	inf	inf1	0	2	n	25,5	0,18
153	inf	inf1	0	3	n	25,5	0,17
153	inf	inf1	8	3	v	30,0	0,32
153	inf	inf1	0	3	n	26,7	0,17
153	inf	inf1	0	3	n	27,6	0,24
153	inf	inf2	8	3	v	27,6	0,15
153	inf	inf2	8	3	v	27,5	0,2
153	inf	inf2	8	3	v	34,0	0,34
153	inf	inf2	7	3	v	32,1	0,22
153	inf	inf2	0	2	n	27,6	0,17
153	inf	inf2	0	2	n	23,9	0,11
153	inf	inf2	8	3	v	32,8	0,3
153	inf	inf2	0	3	n	28,1	0,18
153	inf	inf2	0	2	n	27,1	0,17
153	inf	inf2	0	2	n	32,6	0,28
153	inf	inf2	8	3	v	33,6	0,32
153	inf	inf2	0	2	n	27,0	0,15
153	inf	inf2	0	3	n	31,3	0,22
153	inf	inf2	0	2	n	28,1	0,2
153	inf	inf2	0	2	n	26,6	0,17
153	inf	inf2	0	3	n	28,2	0,19
153	inf	inf2	8	3	v	25,5	0,16
153	inf	inf2	8	3	v	29,2	0,24
153	inf	inf2	8	3	v	29,4	0,22
153	inf	inf2	0	3	n	32,1	0,29
153	inf	inf2	0	2	n	32,2	0,34

153	inf	inf2	8	3	v	23,6	0,11
153	inf	inf2	0	1	n	27,2	0,17
153	inf	inf2	8	3	v	33,2	0,34
153	inf	inf2	0	3	n	35,1	0,35
153	inf	inf2	0	2	n	32,1	0,28
153	inf	inf2	0	2	n	32,1	0,28
153	inf	inf2	0	2	n	21,1	0,08
153	inf	inf2	8	3	v	34,1	0,36
153	inf	inf2	0	2	n	35,2	0,4
153	inf	inf2	8	3	v	33,5	0,35
153	inf	inf2	8	3	v	30,1	0,35
153	inf	inf2	8	3	v	32,0	0,28
153	inf	inf2	8	3	v	26,7	0,19
153	inf	inf2	0	2	n	29,0	0,21
153	inf	inf2	0	2	n	24,8	0,1
153	inf	inf2	0	3	n	31,3	0,24
153	inf	inf2	8	3	v	26,1	0,18
153	inf	inf2	8	3	v	25,5	0,16
153	inf	inf2	0	2	n	27,2	0,17
153	inf	inf2	8	3	v	29,3	0,21
153	inf	inf2	0	2	n	29,0	0,2
153	inf	inf2	0	3	n	23,4	0,13
153	inf	inf2	0	2	n	28,1	0,16
153	inf	inf2	8	3	v	27,2	0,2
153	inf	inf2	8	3	v	34,5	0,38
153	inf	inf2	8	3	v	36,7	0,44
153	inf	inf2	0	2	n	25,5	0,14
153	inf	inf2	8	3	v	33,7	0,29
153	inf	inf2	8	3	v	34,7	0,38
153	inf	inf2	8	3	v	27,0	0,25
153	inf	inf2	0	2	n	28,2	0,18
153	inf	inf2	8	3	v	23,3	0,12
153	inf	inf2	8	3	v	22,6	0,09
153	inf	inf2	0	2	n	27,1	0,14
153	inf	inf2	0	2	n	26,0	0,16
153	inf	inf2	8	3	v	26,7	0,14
153	inf	inf2	8	3	v	36,6	0,42
153	inf	inf2	0	2	n	35,8	0,43
153	inf	inf2	8	3	v	33,5	0,37
153	inf	inf2	8	3	v	28,2	0,21
153	inf	inf2	0	3	n	30,5	0,26
153	inf	inf2	0	3	n	29,0	0,2
153	inf	inf2	8	3	v	32,5	0,3
153	inf	inf2	8	3	v	32,8	0,28
153	inf	inf2	8	3	v	30,5	0,25

153	inf	inf2	8	3	v	33,6	0,32
153	inf	inf2	0	2	n	27,1	0,21
153	inf	inf2	8	3	v	25,1	0,15
153	inf	inf2	0	2	n	35,2	0,42
153	inf	inf2	0	2	n	28,4	0,19
153	inf	inf2	0	2	n	36,1	0,4
153	inf	inf2	0	3	n	32,2	0,3
153	inf	inf2	8	3	v	32,5	0,26
153	inf	inf2	0	3	n	28,2	0,2
153	inf	inf2	0	2	n	31,7	0,3
153	inf	inf2	8	3	v	28,2	0,22
153	inf	inf2	8	3	v	30,3	0,22
153	inf	inf2	0	2	n	27,8	0,2
153	inf	inf2	8	3	v	26,8	0,17
153	inf	inf2	0	2	n	22,0	0,09
153	inf	inf2	8	3	v	31,7	0,26
153	inf	inf2	0	2	n	25,0	0,13
153	inf	inf2	0	3	n	27,2	0,17
153	inf	inf2	8	3	v	32,2	0,29
153	inf	inf2	8	3	v	38,3	0,51
153	inf	inf2	8	3	v	35,7	0,38
153	inf	inf2	8	3	v	33,5	0,33
153	inf	inf2	0	2	n	34,0	0,3
153	inf	inf2	8	3	v	33,5	0,34
153	inf	inf2	8	3	v	32,7	0,32
153	inf	inf2	8	3	v	26,0	0,14
153	inf	inf2	8	3	v	40,5	0,55
153	inf	inf2	8	3	v	33,3	0,33
153	inf	inf2	8	3	v	27,2	0,18
153	inf	inf2	8	3	v	34,9	0,45
153	inf	inf2	8	3	v	32,5	0,34
153	inf	inf2	8	3	v	21,0	0,1
153	inf	inf2	0	2	n	30,4	0,24
153	inf	inf2	8	3	v	33,3	0,32
153	inf	inf2	8	3	v	28,0	0,2
153	inf	inf2	8	3	v	31,0	0,26
153	inf	inf2	8	3	v	30,1	0,31
153	inf	inf2	8	3	v	32,6	0,29
153	inf	inf2	0	2	n	32,8	0,27
153	inf	inf2	0	2	n	32,0	0,28
153	inf	inf2	0	2	n	29,8	0,21
153	inf	inf2	0	2	n	28,6	0,22
153	inf	inf2	8	3	v	29,9	0,2
153	inf	inf2	8	3	v	27,3	0,18
153	inf	inf2	0	2	n	27,9	0,21

153	inf	inf2	0	2	n	28,9	0,2
153	inf	inf2	8	3	v	37,2	0,44
153	inf	inf2	0	2	n	29,6	0,22
153	inf	inf2	8	3	v	38,2	0,48
153	inf	inf2	8	3	v	32,3	0,29
153	inf	inf2	0	2	n	28,0	0,2
153	inf	inf2	8	3	v	25,4	0,14
153	inf	inf2	0	2	n	24,8	0,12
153	inf	inf2	8	3	v	30,0	0,25

6.6 R-syntax

6.6.1 t-test example

```
#CPS t-test

 cpsmorph.df<-read.table('clipboard',header=T,dec=',')

attach (cpsmorph.df)

names(cpsmorph.df)

 t.test(cps~morph)
```

6.6.2 linear mixed effect models example

```
#several fish in each tank = no independent observations

#tanks are random effect factor = source behind cluster

#"treatment" is a fixed effect predictor

#= linear mixed effect model

stortankendlength.df<-read.table('clipboard',header=T,dec=',')

attach(stortankendlength.df)

#call on nlme-function (must be downloaded if not already there)

library(nlme)

fit.lme<-lme(length~exposure,random=~+1|tank)

anova(fit.lme)

summary (fit.lme)
```

6.6.3 Tukey contrast

```
to.df<-read.table('clipboard',header=T,dec=",")

attach(to.df)

names(to.df)

oneway.lm<-lm(weight~group)

anova(oneway.lm)

#if treatment is significant, call on the required packages to perform Tukey contrast tests to test which treatment group differs from each other

library(multcomp)

 mc<-glht(oneway.lm,linfct=mcp(group="Tukey"))

summary(mc)
```