Comparison of virulence in different European isolates of the pseudophyllidean cestode *Schistocephalus solidus* (Müller 1776) in the three-spined stickleback *Gasterosteus aculeatus* (Linnaeus 1758).

Max Planck Institute

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Bachelor thesis

In the Research Group Parasitology, department Evolutionary Ecology of the Max Planck Institute for Evolutionary Biology in Plön to achieve the degree of

Bachelor of Science

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1 Zusammenfassung

Die am Max-Planck-Institut für Evolutionsbiologie in Plön durchgeführte Bachelorarbeit untersuchte im Rahmen des ersten Teiles eines reziproken Kreuzungsversuch die Fragestellung: Nimmt die Virulenz von Parasiten in allopatrischen Kombinationen gemessen über die geographische Distanz der Populationen hin ab? Und korreliert die Abnahme oder Zunahme der Virulenz entlang des Breitengrades in nördlicher Richtung? Als Versuchmodel diente das Wirt- Parasit- System des höchst wirtsspezifischen Cestoden *Schistocephalus solidus* mit den Zwischenwirten *Macrocyclops albidus* (Hüpferling) und *Gasterosteus aculeatus* (dreistachliger Stichling).

Innerhalb von zwei Wochen wurden in vier Infektionsrunden 2611 Hüpferlinge mit *S. solidus* Familien aus Spanien, Schottland, Norwegen, Schweden und dreien aus Deutschland infiziert. Je Runde wurde eine Familie pro Population verwendet. Die erfolgreich Infizierten wurden 413 Stichlingen aus einer Plöner Population (4 Familien) exponiert. Die Fische wurden mit einer Besatzdichte von 20 Individuen pro 16 L Aquarium für eine Wachstumsphase von sieben Wochen gehältert. Bei der Sezierung sind Körpermaße, Organgewichte ermittelt, Würmer entnommen und gewogen, sowie die Kopfnieren zur weiteren Analyse entfernt worden. Aus den erfassten Daten wurden der hepatosomatische und splenosomatische Index, der Conditionfactor, sowie das Lymphozyten/ Granulozyten Verhältnis gebildet und verglichen. Die Virulenz der Parasiten wurde mittels des Wurmgewichtes korrelierend mit der Fekundität, des Parasitenindexes und der Infektionsrate als direktem Erfolgsmaß ermittelt.

Die Ergebnisse dieser Kreuzungsreihe zeigen, dass es keinen signifikanten Zusammenhang zwischen der Virulenz und der Herkunft entlang des geographischen Breitengrades gibt. Ferner lässt sich teilweise ein leichter Einfluss, aber kein signifikanter Zusammenhang zwischen den Konditionsindices und dem Breitengrad erkennen. Zudem lässt sich aufgrund der geringen sympatrischen Versuchsgruppengröße keine genaue Aussage zu einer vermuteten optimalen Virulenz machen. Außerdem müssen vielfältige Einflüsse, wie die Prävalenz der verschiedenen Parasitenarten in einer Population oder der Räuberdruck, berücksichtigt werden.

Erste phylogeographische Untersuchungen (Samonte-Padilla et al., in Vorbereitung) zeigen, dass sich ein signifikanter Zusammenhang zwischen Virulenz und Breitengrad herstellen lässt, wenn man die im Experiment genutzten *S. solidus* Populationen in drei genetische Gruppen- atlantisch, baltisch und kontinental- unterteilt. Dabei wird deutlich, dass sich die atlantische Gruppe (Spanien, Schottland und Norwegen) durch sehr hohe Virulenz (hohe Infektionsraten und Wurmgewicht) auszeichnet, gefolgt von der Kontinentalen (Ibbenbühren, NRW, Deutschland). Wohingegen die baltische Gruppe (Schweden und Norddeutsche Populationen) eine geringe Virulenz aufweist.

2 Introductions

Parasites, “organism that obtains nourishment and shelter on another organism” (www.encyclo.co.uk/define/Parasite), exercise one of the most successful ways of living in nature. “Parasites are everywhere, affecting almost every aspect imaginable in the life of their hosts including physiology, behavior, life histories and, by implication, the structure of entire ecosystems” (Schmid-Hempel, 2011). This is stressed by the fact that for nearly every creature, whether bacteria, plant or animal a huge number of parasites exist. More than that over half of all living species of plants and animals are parasitic (Price, 1980; Windsor, 1998). And nearly all higher organisms are affected by several, sometimes highly specialized parasites. Thereby often organs with less or even none immune defense like eyes, brain, gonads or gut seem to be preferred sites for parasites.

To stay unaffected in the host parasites involved strategies of immune evasion like coating with an for the host immune system undetectable layer of host similar proteins. Or just by changing their surface proteins and antigens so that the immune system has no chance to be *up to date*, for example by *Plasmodium falciparum* showing “an extreme degree of antigenic diversity” (Marsh & Howard, 1986) and additionally by presenting the host own surface proteins.

In evolutionary biology parasite-host-systems became very important study objects in the last decades, because they allow to investigate evolutionary questions on coevolution and reciprocal adaptation in a manageable time due to fast alternation even within a single parasite and host generation (Eizaguirre et al., 2012).

The interaction between the three- spined stickleback *Gasterosteus aculeatus* (Linnaeus 1758) and its tapeworm, the pseudophyllidean cestode *Schistocephalus solidus* (Müller 1776) is an experimental model for host-parasite research (Hammerschmidt & Kurtz, 2009; Barber & Scharsack, 2010).

Schistocephalus solidus is a tapeworm with three hosts in its life cycle (figure 1). After three weeks at 18 °C the coracidium, the first larval stage, hatches from the eggs. According to the water temperature the coracidium has a few hours (Wedekind, 1997) to be ingested by the copepod, the first intermediate host. Here it loses its outer ciliated cells while getting as so-called oncosphere through the gut in the body cavity where it undergoes the formation into the procercoid, the second larval stage, with its characteristic cercomer.

Achieving the procercoid stage the parasite starts to influence the behavior of its host promoting ingestion by the second intermediate host (Hammerschmidt et al., 2009), the three-spined stickleback.

When the infected copepod is eaten by the fish, the parasite "switches coats" to evade the host immune system (Hammerschmidt & Kurtz, 2007) and escapes from the fish's gut into its body cavity. After about two to three months of development the plerocercoid becomes infective for the final host, a fish eating bird (Smith, 1946). Like in Copepods the cestode influences the stickleback's behavior and movement abilities (Giles, 1983; Milinski, 1990). In its final homoeothermic host (Smith, 1946) the hermaphroditic *Schistocephalus* matures and within two days it starts to produce eggs for a few days up to two weeks. The eggs are released into the water and the adults die.

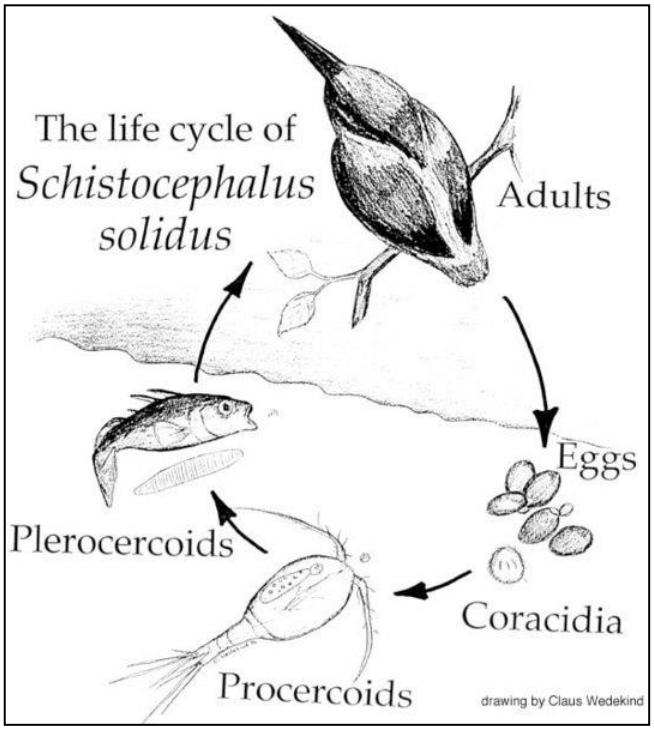

Figure 1: The lifecycle of *S. solidus*
Out of the released eggs hatched coracidia which are ingested by copepods. After growing to the Procercoid stage the copepods are ingested by sticklebacks. Here it becomes the Plerocercoid stage and through influencing the host they are ingested by a fish eating bird, where they mate.

Important in the relationship between fish and tapeworm is the high host specificity of the parasite having no other 2nd intermediate host to switch to. In addition to that there is only one parasite generation per host generation. So if only one parasite generation fails the whole population is threatened with extinction. For example it might be necessary for a stable population of the parasite to find the best degree of virulence to secure the surviving of the involved species including themselves.

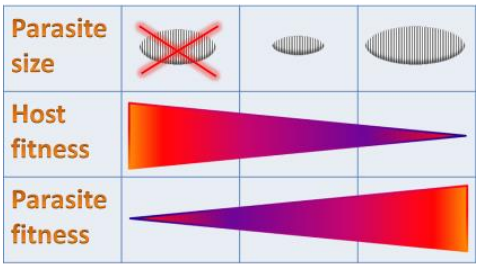

Figure 2: Trade-off
Trade-off between body size and fitness of parasite and host

An analysis of mass mortality of a three-spined Stickleback population in Walby Lake, Alaska, over winter 1996-97, has stressed the importance of an optimal virulence, because it has ended an epizootic caused by the predominant parasite *S. solidus.* Reasonable for this were the decrease of host reproduction, the increase of host predation and possible environmental influences of the fish condition (Heins et al., 2010).

Optimal virulence means an optimal balance between the costs and benefits of harming the host. It is assumed that there is a trade-off between different fitness components for example infection or transmission rate and longevity of the host (Anderson & May, 1982).

In the sticklebacks for example the growth of the tapeworm is limited by the size of the body cavity and the available nutrients. If there is more than one tapeworm in a fish they have to share the available space. If *S. solidus* has grown very fast in a short time post infection, but not reached the plerocercoid stage - after several weeks, depending on the temperature -, the host might not be able anymore to take up enough food, because of the reduced ability to swim and by losing the competition for food with its conspecific (Barber & Ruxton, 1998). This would lead to a decreased chance of transmission due to the decreased body condition of the fish. In addition to that, a fast growing tapeworm in a small fish has not enough available space to reach its full size. The body size correlates to the fecundity. The result is a lower reproductive success, subject to the condition that the host survives long enough and is ingested by the last host, the fish- eating bird. On the other hand when a juvenile fish ingests a procercoid showing a normal growing both grow normally (Arnott et al., 2000).

According to the Red-Queen-Hypothesis, saying that host and parasite adapt genetically to each other in a constant mutual natural selection (Lively & Dybdahl, 2000), the aim of this study was to test whether the virulence of the parasite decreases in allopatric combinations correlated to increasing geographical distance / latitude from the host population.

To verify the adaptation of the “parasite to the locally common host genotype” (Lively & Dybdahl, 2000) the infection rate and the fecundity, correlating to the body size respectively body weight of the tapeworm, were measured.

3 Material and Methods

3.1. Materials

Microscopes

MZ6	Leica
MZ 7,5	Leica
KL1500 LCD	Leica
DMLB 100S	Leica

Centrifuges

Centrifuge 5804R	Eppendorf
Sigma Lab Centriguge 4-15C	Qiagen
Spectrafuge Mini Centrifuge C1301B	Labnet international, Inc.

Sequencing

Thermomixer Comfort	eppendorf
ThermoCell Cooling & Heating Block HB 202	Bioer Technologies

PCR

Thermocycler: Labcycler gradient	SensoQuest
Spectrophotometer NanoDrop ND1000	PEQLAB

Else

FACS Calibur	Becton, Dickinson
Magnetic stirrer R100	Roth
Microplate shaker MTS 4	IKA
Scales BP 610	Sartorius
Vertex Genie2 G560 E	Scientific Industries

Software

Genmarker 3.0	Softgenetics
Statistica 10.0	StatSoft

3.2 Laboratory Animals

All used animals were lab-bred and never were in contact with any parasite before. The abbreviations of the families (*G. aculeatus* and *S. solidus*) is based on the origin of the population, like GPS for Grosser PLöner See, and the numbers refer to the original identification numbers of the parents (like e.g. 220x236)

3.2.1 *Schistocephalus solidus*

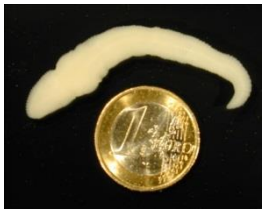

Figure 3: ***Schistocephalus solidus*****,** plerocercoid stage

For the infection series were four *Schistocephalus solidus* (figure 3) families out of seven populations from Xinzo de Lima in Spain (SP), North Uist, outer Hebrides, in Scotland (NU), Skogseidvatnet near Bergen in Norway (NO), Obbola in Sweden (OBB), Neustädter Binnenwasser - a brackish lagoon of the Baltic Sea- in Germany (NST), Ibbenbührener Aa, near Münster in Germany (IBB) and one family from the Grosser Plöner See in the North of Germany (GPS) used.

3.2.2 *Macrocyclops albidus*

Figure 4: ***Macrocyclops albidus***

The origin of the lab- bread population of *Macrocyclops albidus* (Jurine 1830; figure 4) is the Neustädter Binnenwasser. They were cultured in the copepod-room of the Max-Planck-Institute in Plön. The water and copepod room temperature is 18 °C and the light was on for 16 hours, from 6 am to 10 pm. They were fed with living paramecia. For the infection we filtered out the larval stages CI and CII.

3.2.3 *Gasterosteus aculeatus*

Figure 5: ***Gasterosteus aculeatus***

Gasterosteus aculeatus (figure 5) is a teleost fish widely destributet in the northern hemisphere. The freshwater fish lives in rivers and lakes, but also in brackish water like the Baltic Sea. The sticklebacks used in this experiment were from four lab-bred families, hatched between the 12th and the 29th December 2012, GPS 220x236, GPS 1x2, GPS 73x74 and GPS 219x223 from the Grosser Plöner See.

3.3 Map

The map of Europe (figure 6) shows the origin of the lab- breaded *S. solidus* used in this experiment. The origin areas range from the 42° to the 63° north. For the transition of the coordinates the website http://transition.fcc.gov/mb/audio/bickel/DDDMMSS-decimal.html was used.

Coordinates:	Longitudes decimal:
GPS: 54°09' 21"N, 10°25'49E	54.155833
NST: 54°06'40"N, 10°48'50"E	54.111111
IBB: 52°17'31"N, 7°36'46"E	52.291944
NO: 60°14'44"N, 5°55'03"E	60.245556
OBB: 63°39'21"N, 20°17'25"E	63.655833
SP: 42°08'01"N, 7°39'47"W	42.133611
NU: 57°34'27"N, 7°17'44"W	57.574167

Figure 6: Map of Europe
Map of Europe with the origin of the experimental tapeworm *S. solidus***.** The lab fish (*G. aculeatus*) have the same origin from GPS like *S. solidus.*

3.4 Infection series

3.4.1 *Macrocyclops albidus* infection

The copepods were taken out of the laboratory culture (van der Veen & Kurtz, 2002) and selected by size with help of micro sieves, mesh size 180 µm, and set in 24-tissue culture test plates (Biochrom AG, Berlin), one per well. The copepods in the culture plates were stored in the culture room and fed each with three *Artemia* spec. two times and *Paramecium* spec. once a week.

The *S. solidus* eggs were incubated for three weeks at 20 °C. In the evening of the day before exposure the eggs were taken out and transferred into Petri dishes (90x15 mm, SARSTEDT).

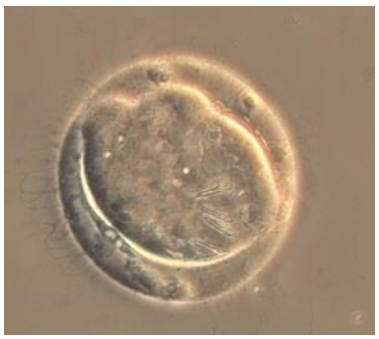

Figure 7: *S. solidus*
The free-living coracidium freshly hatched, 44 µm in diameter

In the morning when the tapeworm larvae were freshly hatched (figure 7) they were picked up with an Eppendorf micropipette and individually added to one copepod (Hammerschmidt & Kurtz, 2005). Nine days after exposure all exposed copepods were checked for successful infection under the microscope (figure 8) with 100 x amplification for visual inspection.

Figure 8: *M. albidus*
M. albidus infected *with S. solidus* (red cycled) in the procercoid stage, 9 days after exposure, visual inspection using 100x amplification.

3.4.2 *Gasterosteus aculeatus* infection

The fish infection was carried out in 4 rounds -one per fish family and two rounds per week- in which each fish family was exposed to one of the four worm families from each *S. solidus* origin. Three weeks after the copepods infection the three-spined sticklebacks were taken out of their 200 liter "family-tank" and separated in half filled 2 liter tanks (figure 9). On the next day one infected *Macrocyclops*, transferred in small Petri dishes (35x10 mm, SARSTEDT), was exposed to each of the singled sticklebacks starved for a day. After 24 hours the fishes were set in 16 liter plastic tanks (figure 10, 11). In order to reach the same density of 20 fishes, the tanks were filled up with additional fish marked by cutting off one pelvic spine. Therefore 64 fishes were marked and added. Marked fish were not used for analyses. In cases not enough infected copepods or fish were available, fish from the same family but infected with different worm families were mixed.

Figure 9: *G. aculeatus*
separated in 2L tanks, one infected *Macrocyclops*, transferred in small Petri dishes, was exposed to each of the singled sticklebacks starved for a day.

The water from the 2 liter tanks was filtered with micro strains – one per 10 tanks of one family- to find out the exact number of copepods that had been eaten. The four control groups (one per round) were treated the same except exposure and water filtering. The copepods that were not eaten were counted and subtracted from the total number of infected fishes. In total we had 560 Sticklebacks in 28 tanks, among these were 413 exposed-, 80 control- and 67 restock fishes.

The room and the water temperature were 18 °C the light was on for 17 hours, from 6 am to 11 pm. The Sticklebacks were fed three times per week with frozen *Chironomidae* larvae ad libidum for seven weeks.

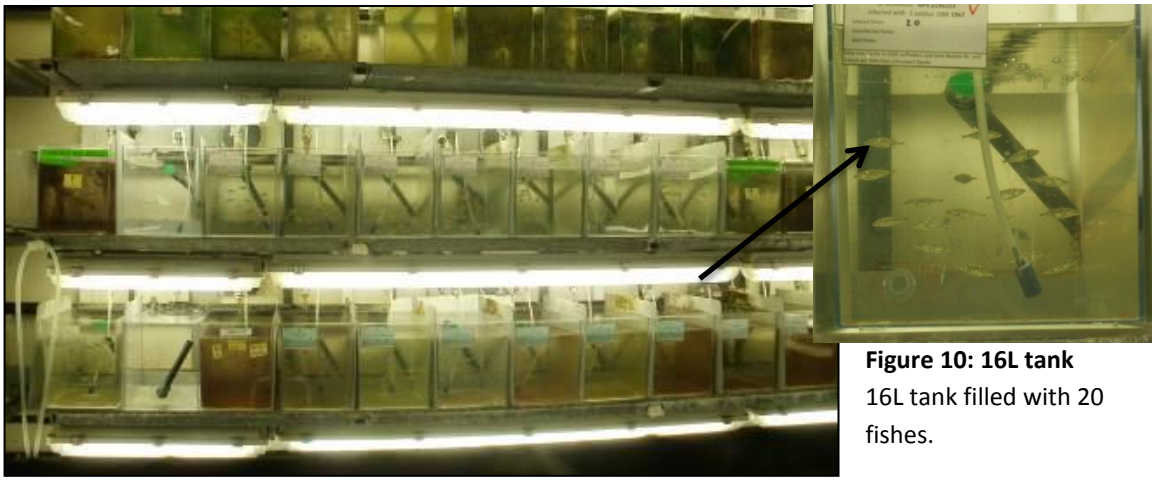

Figure 10: 16L tank
16L tank filled with 20 fishes.

Figure 11: Storage of *G. aculeatus*
Storage of *G. aculeatus* at 18 °C room and water temperature and with 17 hours artificial daylight (summertime). All tanks were connected to the same water and oxygen supply system.

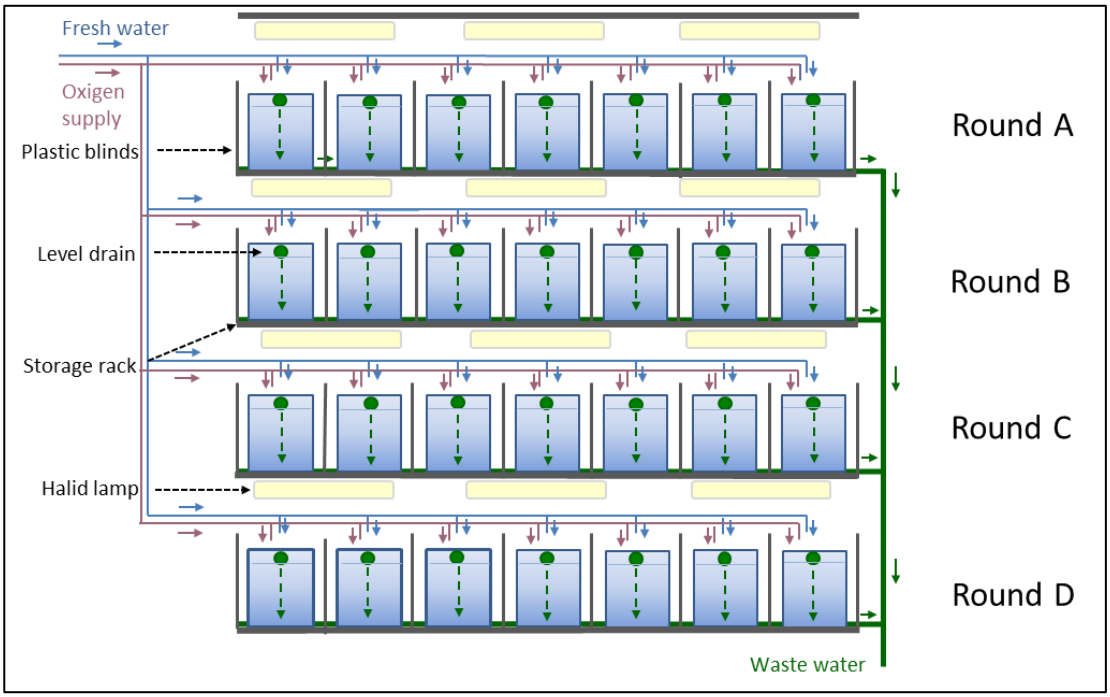

Figure 12: Schematic drawing of the storage
Schematic drawing of the storage of *G. aculeatus* in the fish room of the Max-Plank Institute in Plön (Germany). Every 16 l tank was equipped with the same level drain. The air supply tube ended in air diffuser stones placed at the bottom of each tank.

3.5 Fish dissection

The dissection of one round (one infected *G. aculeatus* family) was progressed on two days, ten fishes from each tank of one round per day. In order to have the same time line like the infection series, two rounds per week were dissected. For the dissection the fish were caught with a small fishing net and separated in groups of five in 2 liter plastic tanks. The procedure was repeated in the noon with further 5 fishes from each tank of the round. To reduce the stress while transport to the lab the tanks were covered with a dark towel.

The dissection in the lab was organized in three workplaces. At the first station the fish were killed individually with an overdose of MS 222 (1g per L) filled in a one Liter beaker glass. As soon as no sign of life was detectable the fish were softly dried with paper towels, weighed and the total length (with tail fin) and the standard length (without tail fin, used for Condition factor analyses) were measured with help of scale paper. Blood samples were taken with blood capillary right after cutting off the tail fin with a scalpel by gentle pressuring the body. The fish parts were laid in numbered small Petri dishes and stored in thermo boxes filled with ice (figure 13).

Figure 13: Dissected *G. aculeatus* Body parts of the dissected Sticklebacks in serially numbered Petri dishes cooled on ice between the workplaces.

In the second step, the head was cut off with scissors and the body cavity was opened by two lateral incisions in the body wall to extract the head kidneys with help of forceps under a binocular. The head kidneys were weight and separated for further immune cell analyses.

Then liver and spleen were removed and the weighed and the sex was determined under a binocular. When present, the tapeworms were carefully taken out of the body cavity with a special tissue forceps (with a ring at the tip). For weighting, the worms were transferred into a pre-weighed petri dish with a culture medium on the scales. For the cases it was not clear which family the tapeworm originates from, a part of the ‘tail’ was cut off for genotyping them using microsatellite markers. The last step of the dissection was to conserve the fish parts and the worms in 85% ethanol filled plastic tubes.

3.6 Blood analyses

3.6.1 Hematocrit

The numbered serially blood capillaries were set in 2 ml centrifuge tubes and cooled by a thermo box filled with ice during dissection and afterwards. The tubes with the capillaries were centrifuged by 10.000 rpm for 5 min whereby the blood plasma lymphocyte and red blood cell (RBC) content dissolute. The lengths of each part were measured with an electric measuring caliper. The hematocrit, the volume percentage of erythrocytes, was calculated by the formula:

Total length of blood sample / length of packed red blood cell.

Because of the small amount of plasma due to the small size of the fish, it was not possible to measure the titer of antibodies by Enzyme-Linked ImmunoSorbent Assay (ELISA).

3.7 Microsatellite genotyping

“Microsatellites are di-, tri-, or tetra nucleotide tandem repeats in DNA sequences. The number of repeats is variable in populations of DNA and within the alleles of an individual.” (www.lifesciences.sourcebioscience.com)

3.7.1 DNA isolation

The DNA of the tapeworms from the tanks with mixed populations was isolated with the QIAmp Micro Kit (50) subject to the producer’s protocol (appendix 9.3)

DNA lysate was pipetted into the three prepared 96 wells PCR plates. In the first plate the primers Schistoplex 1 and 2, in the second MP4, MP6a and MP10 and the third one MP8 were added. The following markers were used Scso22, Scso33, Scso29, Scso24, Scso34 (Andris et al., 2012) and Schistoplex 1 and 2 (Binz et al., 2000). The plates were set in three Thermocycler (LabCycler, SensoQuest) and programmed according to the manufacturer’s instruction (appendix 9.4).

3.7.2 Preparation for Microsatellite Fragment Analyzer

For one MicroAmpOptical 96 well Reaction plate (applied biosystems) 1000 µl Hi-Di formamide were mixed with 50 µl Rox Standard 350 and 10.2 µl per well distributed. Thereby it was taken care not to fully press the pipette in order to avoid air bubbles in the wells. Then 1 µl PCR sample was added and denatured for 2 minutes at 90 ^{o}C. Immediately afterwards the plate was put inside the refrigerator for 5 minutes at 4 ^{o}C. For sequencing the plate was handed over to the sequencing team of the Max-Planck Institute in Plön.

3.8 Immune parameters

In the humeral immune system lymphocytes and granulocytes are the two main groups of leukocytes which are responsible for the detection and destruction or neutralization of invading pathogens and parasites. An increase of these cells is an indication of an infection. Thereby lymphocytes are the most important cells of the acquired immune system, whereas granulocytes are involved in the innate immune system e.g. by releasing reactive oxygen species (ROS). The ratio between granulocytes and lymphocytes (G/L ratio) is used to estimate the relative activity of the immune systems (Kurtz et al., 2004). To count and classify the cells flow cytometry was used, whereby the characteristics of the immune cells were detected by their optical characteristics. In brief, when a cell passes a laser beam in a flow cell, the reduction of light measured with a detector opposit to the light soure (forwardscatter, FSC) indicates the size of a particel, whereas another detector positioned in a 90° angle to the laser beam records the scattered light (sidescatter, SSC), which gives information about the granularity of a cell. To quantify the cells, a definded number of flourescent labeled latex beads where added to the cell suspension, acting as an internal standard for calibration. The flow cytometry of the sticklebacks head kidney leucocytes was performed by BTA Withe Derner with the Becton and Dickinson FACSCalibur as described by Scharsack et al. (2004).

Vital cells= events (vital) x number (standard beads)/events (standard beats)

The respiratory burst (RB) activity of head kidney leucocytes was analysed in a lucigenin enhanced chemilumineszenz assay (Kurtz et al.,2004, modified after Scott Klesius, 1981). The production of reactive oxygen species (ROS) was initiated by adding zymosan, immune-stimulating particles from yeast, that get phagocytized by activated granulocytes, monocytes or macrophages. The ROS produced during phagocytosis react with lucigenin under emmission of photons, which are quantified in a luminescence plate reader (Berthold).

Head kidney cells were attuned on 1.25 x 10^6/ml R-90 (=Standard RPMI cell culture medium, diluted with 10% sterile water) . A 96 microtitre plate were set up with 80 µl R-90 plus 20 µl lucigenin (Sigma M 8010) per well. 80 µl head kidney cell suspension were added per well. Then the plate were incubated for 30 minutes at 18°C and 2% CO_2. After preparing the luminescence reader 20 µl zymosan (Sigma Z 4250) were added and the luminescence plate (Greiner bio-one) was put into the luminescence reader. Total measuring time was 3:30 hours at 20°C.

3.9 Data analyze and indices

To analyze the measured parameters of the fish dissection (size, weights, immune activity), an analyses of variance (ANOVA) with the used indices as effect variables was made. Multivariate analyses of variance (MANOVA) considering the indices was calculated in order to compare the infected, not infected and control group. The honest significant difference (HSD) test for unequal N was used to compare the means of sibships and control group among each other and with each other. As the level of significance $p<0.05$ was considered. The analyses were performed with the software statistika for windows (statsoft).

3.9.1 Infection rate

Infected copepod or stickleback were those which had a procercoid respectively plerocercoid in their body cavity and survived. In the *M. albidus* infection series multi infected copepods occurred and were counted as infected. But only single infected copepods were used for the *G. aculeatus* infection series. All infected fish had only one tapeworm inside. As Exposed only those fish were counted which ingested one infected copepod. The infection rate is calculated for the total populations, not the families, by the formula:

Number of exposed/number of infected x100

3.9.2 Parasite index

The parasite index (PI) is a value to describe the growth of the parasite in ratio to the fish weight. The net fish weight is the fish weight after drying with a paper towel measured to the nearest of 0,1gr and without the parasite weight (Kurtz et al., 2004). It was calculated:

Parasite weight [mg]/fish weight [mg] x100

3.9.3 Hepatosomatic index

The liver is an important store of energy reserves in fish and its weight in relation to the body weight helps to estimate the energy status of the fish (Chellappa et al., 1995). Especially the hepatosomatic index (HSI) makes it more suitable to compare different sized non fatty fishes. The HSI is calculated with the fish weight without worm according to Bolger & Connolly, 1988:

Liver weight [mg]/fish weight [mg] x100

3.9.4 Splenosomatic index

The spleen is an immunological highly active organ. The spleen can be divided in two areas the red pulp responsible for blood filtering and the white pulp responsible for the production of immune cells like lymphocytes, storage of monocytes and filtering antibody marked pathogens. The Splenosomatic index (SSI) is calculated according to Bolger & Connolly, 1988:

Spleen weight [mg]/fish weight [mg] x100

3.9.5 Conditon Factor

The Condition factor (CF) is an index "to estimate the body condition in a fish and is calculated as a ratio between the weight and length". The exponent x is the value considering the isometric growth "in which weight increases as the cube of length". (Chellappa et al., 1995) The exponent is determined in a regression analysis (cf. appendix 7.6). For the fish used in this experiment x was determined as 3,015 (Wootton, 1976). The length was measured from the snout to the base of the tail to the nearest of 0.1 mm. The fish weight is without the worm weight. Formula:

Weight [mg]/length [mm]^{x}

4 Results

4.1 Results *Macrocyclops albidus* infection series

From the 2611 copepods exposed to coracidia 586 were infected, of which only 24 were multiply infected. 413 infected *Macrocyclops* were exposed to the three-spined Stickleback, 387 were ingested and 26 were found by filtering the water from the 2L tanks. As presented in figure 14, the highest infection rate is shown by the Norwegian population (39.06 %) and the lowest by the Plön population (4.69 %).The Neustadt population, which was the sympatric combination in case of the copepods, has an average infection rate of 21.38 %, nearly half of the rate presented by the allopatric combination NO *S. solidus*- NST *G. aculeatus.* In addition to that the two other German populations, from Ibbenbühren (14.30 %) and Plön (4.69%), with the smallest geographical distance to the sympatric combination present a significant lower infection rate than the ones with the widest distance, like the ones with Spanish (21.09 %) and Swedish (25.52 %) origin.

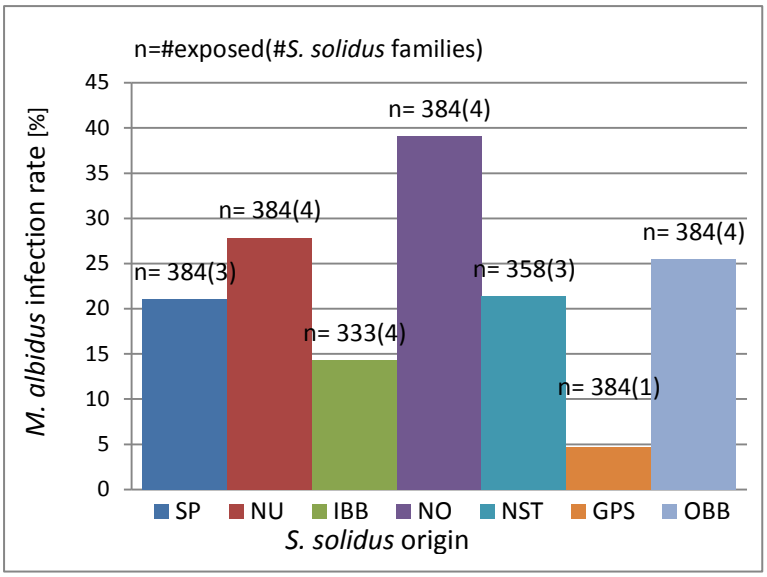

Figure 14: *Macrocyclops albidus* infection rate
Infection status measured 3 weeks post infection.

The mortality rate (figure 15) highlights the fact that the most infective ones, the Norwegians, cause the lowest host mortality with 16.93 %. The highest mortality is shown by the North Uist population with 34.38 %. The other populations are close to the total average mortality rate of 24.40 %.

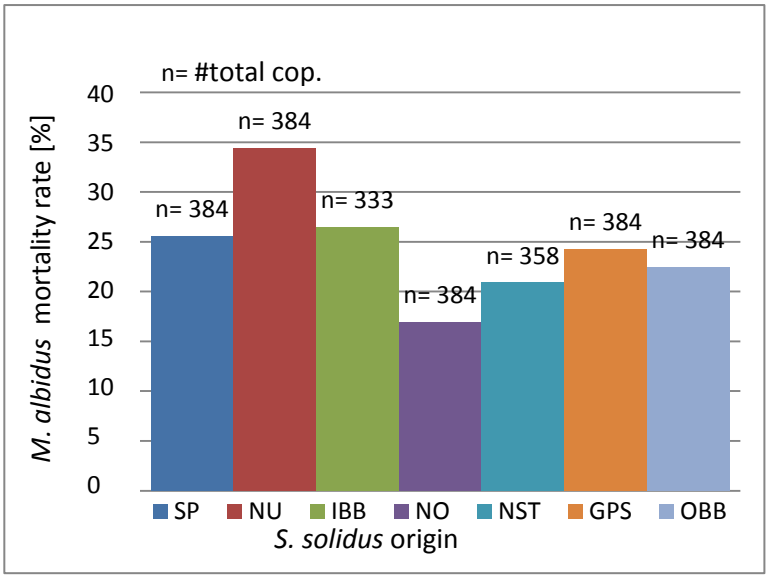

Figure 15: *Macrocyclops albidus* mortality

4.2 Results *Gasterosteus aculeatus* dissection

One stickleback infected with SP 1x37 had a fungi infection at the tail fin and was dissected one week earlier than planned. One tank infected with NO 94x103 collapsed because of a blockage in the freshwater supply overnight. All 20 fishes died and were dissected right after they were found. For analyze only the infection rate was used. Otherwise there were only 6 isolated cases of death (one NU 3x6, one NO 40x21 and in total four from control group C).

4.2.1 Infection rate

The highest infection (figure 16) rate is shown by the allopatric combination of Norwegian tapeworm (NO) in the stickleback population from the Großer Plöner See with 72.37%, followed by the Scottish ones (NU) with 61.90%. The lowest infection rate is shown by the Spanish (SP) population with 30.65% followed by Neustdt (NST) with 33.90%. The sympatric combinations GPS fish with GPS worm reaches 44.44%. Close to this are the Swedish (OBB) with 43.55% and the ones from Ibbenbühren (IBB) with 46.81%. The average infection rate is 49.10%.

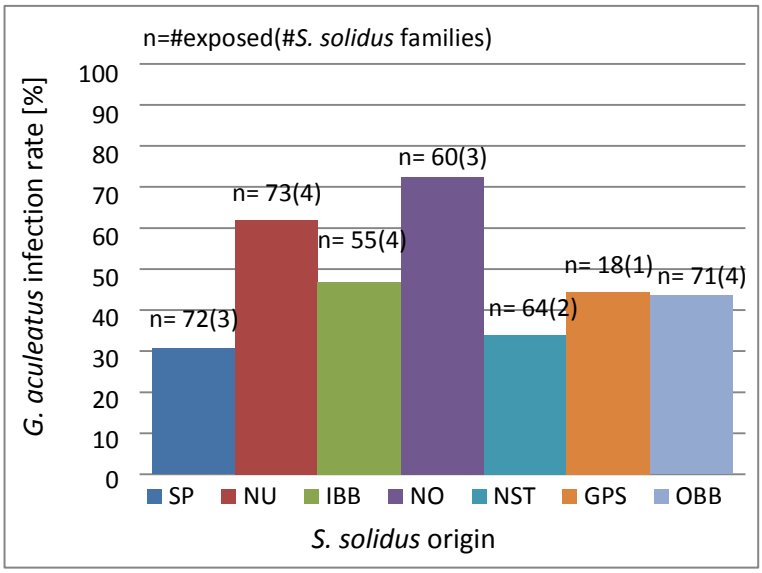

Figure 16: *Gasterosteus aculeatus* infection rate
Infection status measured 7 weeks post infection.

4.2.2 Weight of *Schistocephalus solidus*

As presented in appendix 9.9 (p. 41) the worm weights show a high variance within the populations as well between the families. The biggest different weight of about 42 mg is found in the Spanish population (SP 28x29 with 75.02 mg in mean against SP 1x37 C with 117.10 mg in mean). The NST population has nearly the same difference inside with 40.60 mg between the families NST 7x8 (51.00 mg in mean) and NST 1x3 C (10.40 mg in mean). The GPS population reveals the lowest variance between the rounds A and C. But in detail GPS 1x2 C with 28.53 mg in mean has a worm weight margin from 7.90 mg to 84.20 mg and with it the widest margin of all families and populations. The maximum worm weight of the GPS population is smaller than the minimum worm weight of the NO population (85.70 mg).

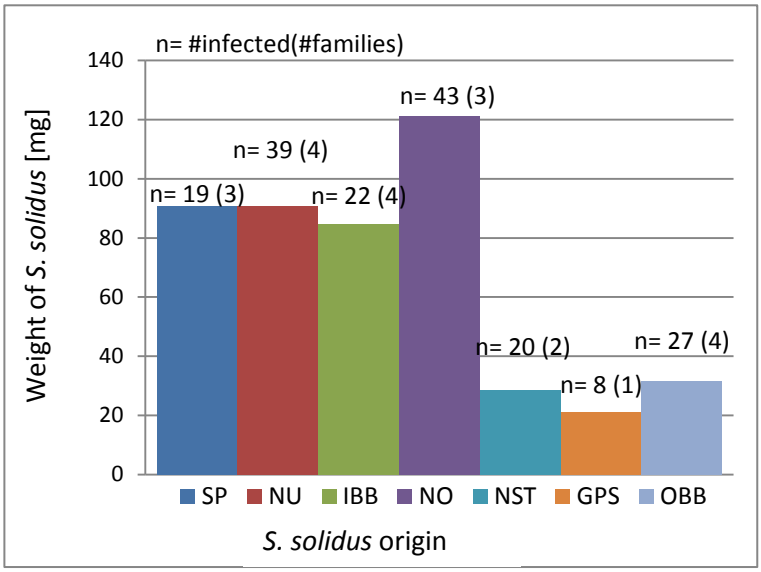

Figure 17: Weight of *S. solidus*
The average weight of the seven tapeworms populations, 7 weeks post infection

The highest variance within the family is shown in NU 10x14 with 883.88 by 11 measured tapeworms. The cause of that is the wide difference between a single 5.90 mg and a 120.60 mg heavy worm. The smallest worm of the experiment was a Swedish one (OBB 2x23) with 3.10 mg, and with 156.70 mg a Norwegian (NO 40x21) the biggest one.

The 12 tapeworms from the collapsed fish tank were weighted 26 days after infection and were between 1.80 mg and 10.10 mg.

The bar chart (figure 17) illustrates the differences between the mean worm weights of the experimental populations. The allopatric combination Norwegian *S. solidus* in *G. aculeatus* from GPS results in the biggest tapeworms with an average weight of 121.32 mg. Whereas the worms from the sympatric combination (GPS) are only as sixth as heavy than the Norwegians with 21.21 mg, followed by the geographically neighbored NST population with 28.69 mg in average. From the one GPS family (GPS 1x2) used for the four infection rounds only two out of four infected some fish, NST three out of four. For NO also just three of four families are presented, but because of a technical defect.

4.2.3 Parasite index

The bar chart (figure 18) shows the parasite index (PI) from the origins of the seven analyzed populations. The populations out of the Baltic area, NST, OBB and GPS have the lowest PI. The PI's of SP, IBB and NU are also close together. With a PI of 22.18 the Norwegians (NO) are the biggest/fastest growing worms. The comparison of the PI among each tapeworm population indicates no significant differences between SP, IBB and NU as well as between NST, GPS and OBB. Except these two clusters significant differences are shown between all other populations. The Norwegians (NO) significantly differ from all other population. The range of the parasite index varies a lot within the families of the populations except of the Norwegian population (cf. appendix 7.11.1). NST reveals the widest range of 1.80 – 8.78. But there is no obvious influence on the PI caused by the stickleback families.

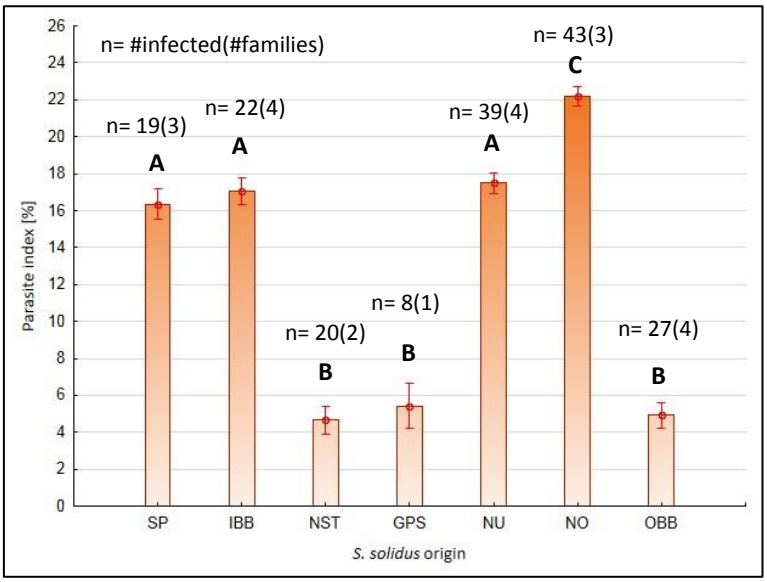

Figure 18: Parasite index

Worm weight in relation to fish weight, from the origins of the seven analyzed populations. Data are mean ± SEM, different letters indicate significant differences.

4.2.4 Hepatosomatic index

The fish infected with the most virulent parasites, from the populations NO, NU and SP, show significant differences compared to the control group, but no significant differences to the other population. These populations, IBB, GPS, NST and OBB also don't differ from the control group (cf. appendix 7.10.2). In mean the Hepatosomatic indices (figure 19) of control fish are higher than from the infected fish. Whether the exposed but not infected (p = 0.5987; r = -0.0359; r^2 = 0.0013) nor the infected (p = 0.1917; r = 0.09830; r^2 = 0.0097) reveal a significant change among latitude (cf. appendix 7.11.2).

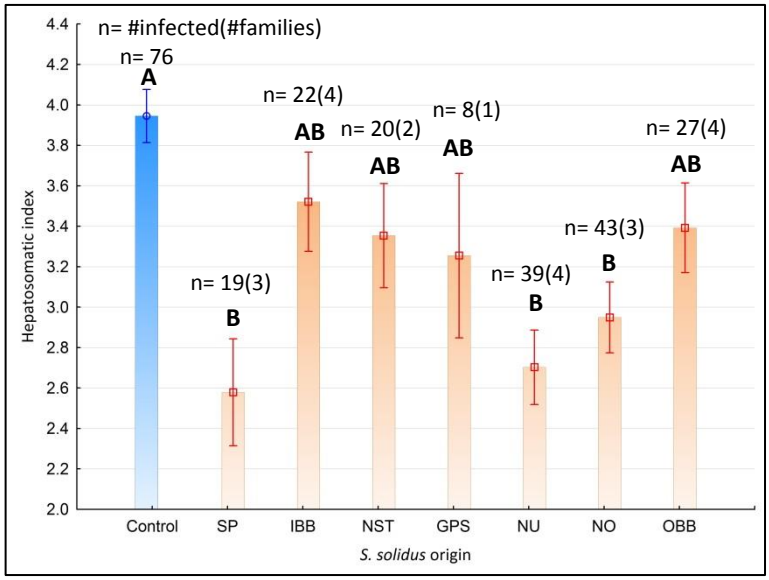

Figure 19: Hepatosomatic index
Data are mean ± SEM, different letters indicate significant differences.

4.2.5 Splenosomatic index

The spleens of the infected sticklebacks are bigger than from the not infected ones. As presented in figure 20 the control group has the smallest SSI. Only IBB and OBB differ significantly from control group. Although the SSI of the SP population is nearly as big as the IBB and OBB population there is no significant difference to them or to control group. OBB, highest SSI, and NU, lowest SSI, also show sicnificant differences (cf. appendix 7.10.3). The SSI of the infected fish has its mean at 0.0946 ±0.0027 (n= 178), exposed but not infected at 0.0775 ±0.0775 (n= 217) and Control at

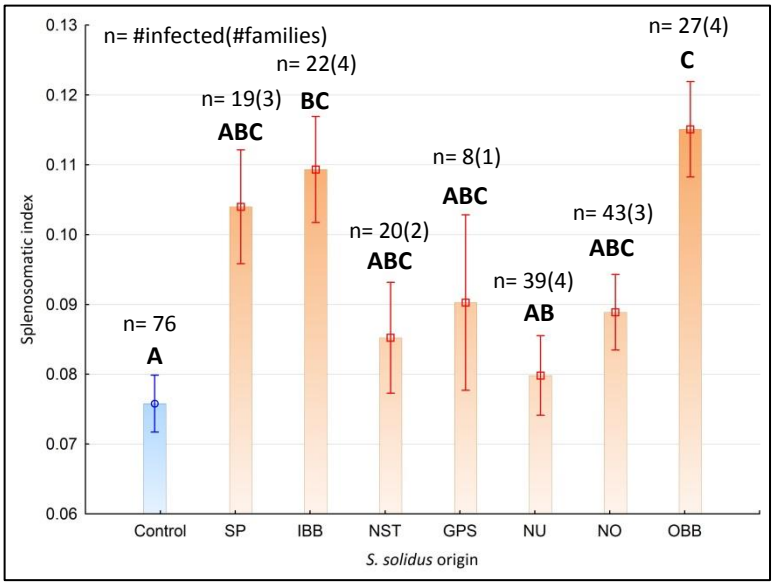

Figure 20: Splenosomatic index
Data are mean ± SEM, different letters indicate significant differences.

0,0757 ±0.0041 (n= 76). No significant change is given among latitude (p = 0.7549; r = -0.0236; r^2 = 0.0006). The same counts for the exposed but not infected fish (p = 0.8460; r = 0.0133; r^2 = 0.0002, cf. appendix 7.11.3).

4.2.6 Conditon Factor

The Condition factors, presented in figure 21, indicates that the fish infected with the worms from IBB, GPS, NO, NU and SP reveal lower CF's than control group. But the fish infected with worms from NST and OBB had a slightly but not signicantly higher CF. A significant difference is given only between IBB and OBB (cf. appendix 7.10.4). The infected fish have its mean at 1.0118 ±0.0072, exposed but not infected at 1.0544 ±0.0065 and Control at 1.0216 ±0.0110. No significant change among latitude is on the hand (not infected: p = 0.4322; r = 0.0535; r^2 = 0.0029; infected: p = 0.3163; r = 0.0755; r^2 = 0.0057; cf. appendix 7.11.4).

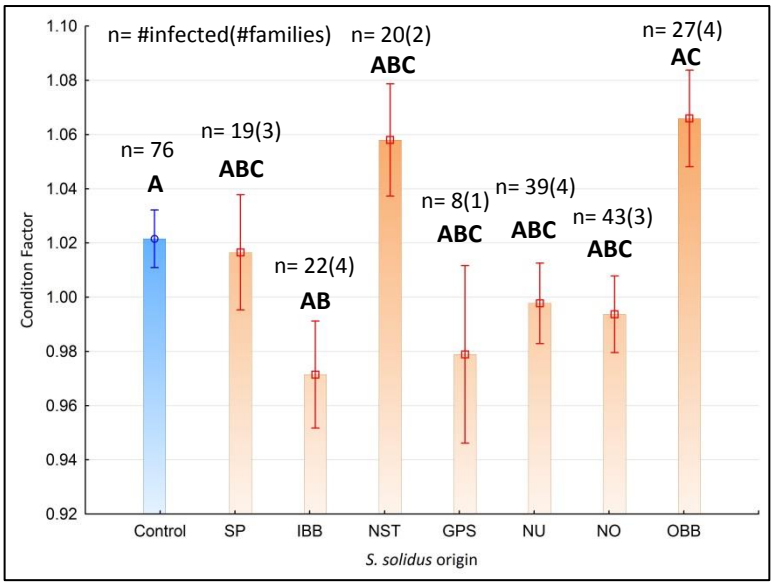

Figure 21: Condition factor
Data are mean ± SEM, different letters indicate significant differences.

4.2.7 Respiratory burst and lymphocytes / granulocytes ratio

Figure 22 presents the respiratory burst (RB) activity of the stickleback head kidney leucocytes (HKL). It is obvious that the infected ones show a higher RB activity than the control group. Significant differences are shown by SP, NU and NO compared to control group and between NO and OBB (cf. appendix 7.10.5).

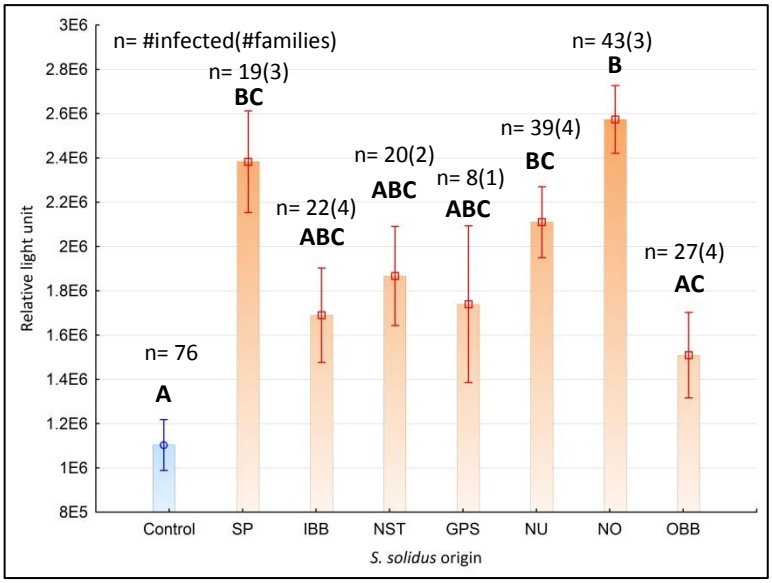

Figure 22: Relative light unit
The respiratory burst (RB) activity of head kidney leucocytes after *in vitro* cultivation was analysed in a lucigenin enhanced chemilumineszenz assay. Data are mean ± SEM, different letters indicate significant differences.

Particularly interesting is the RB activity of fish infected with NO being most active compared to exposed but not infected fish (1.34E+06 ±8.12E+05 RLU) and control group. Furthermore the HKL of nearly all not infected fish- except GPS- are more active than the ones from control group.

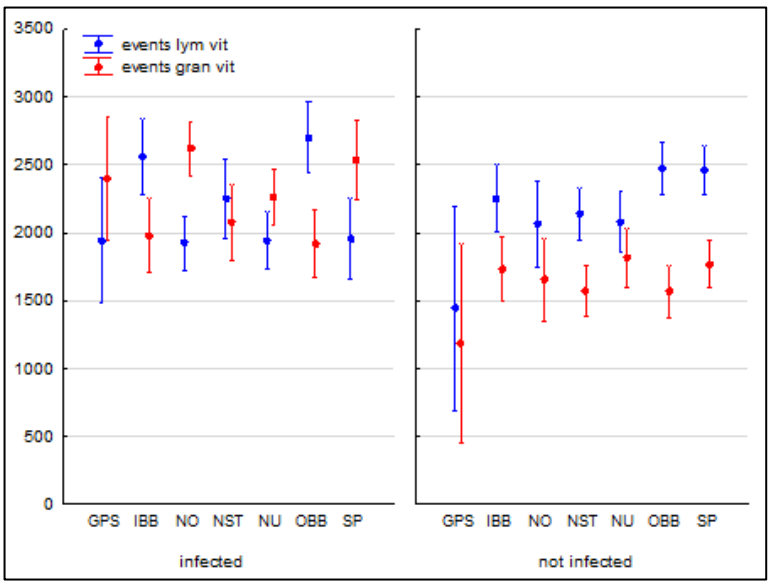

Figure 23: Number of granulocytes and lymphocytes
The figure shows the number of measured vital granulocytes (red bars) and vital lymphocytes (blue bars) of the dissected headkidneys splitted into infected and not infected. Data are mean ± SEM

The distribution of the total numbers of vital lymphocytes (L) and vital granulocytes (G) differ (figure 23) among fish groups infected with the different worm populations. IBB, OBB and NST infected sticklebacks have more lymphocytes than granulocytes. That indicates a higher activity of the acquired immune system (G<L), contrary to GPS, NO, NU and SP (G>L). But this statement is relative considering that the immune cells migrate in different quantity into the harmed body area during the infection progress. In not infected fish lymphocytes equally outnumber the granulocytes.

5 Discussion

5.1 Infection rates and parasite index

During the *M. albidus* infection it became obvious that the hatched coracidia differed in their swimming activity. The slowest ones were the GPS 1x2. In addition to that they had the lowest hatch rate. The most active coracidia seemed to be the NO and NU populations. The hatching success and swim activity seems to be correlated to the infection success in the copepod. Furthermore the size of the procercoid may influence the infection success, but this was not object to this experiment. The assumption that a disadvantage in infecting the first host may lead to a better advantage in the second intermediate host, as described in Hammerschmidt & Kurtz (2005), is not confirmed by this study. Lively & Dybdahl (2000) demonstrated that the host is attuned to its parasite in sympatric combinations leading to higher infection success respectively balanced host mortality, but this cannot be proofed in this experiment. Most successful was the allopatric combination, GPS fish and NO worm, in infecting (39% in copepod and 72.4% in fish) and causing mortality (17%). The sympatric combination, GPS fish and GPS worm, reveals much lower rates (4.7% and 44.4%) respectively higher mortality (24.2%). Hammerschmidt & Kurtz (2007) found that between 50 and 75% of the parasites fail to infect the fish the present infection rates mostly reach the target. In this study, only the Norwegians and the North Uists failed less.

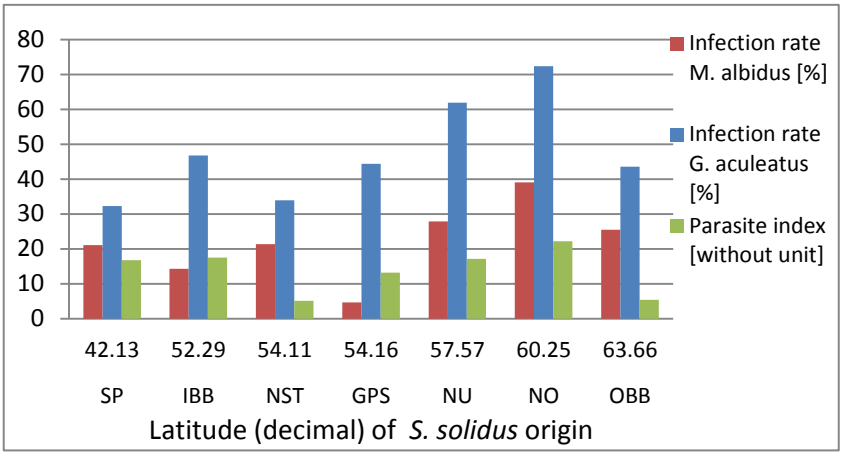

Figure 24: Infection rates and Parasite index
Infection rates of *M. albidus* and *G. aculeatus* in addition to parasite index plotted against latitude of the *S. solidus* origins.

Ebert (1994) found in experiments with *Daphnia sp*. that an "increasing geographic distance between host and parasite origin [...] [correlates] with a decrease in spore production and virulence". Quite contrary to this are the present results. The furthest linear distance between the origins of GPS fish and SP worms is 1892 km and features the lowest infection rate with 32.3%. GPS and NST are 26 km away from each other, but having nearly the same infection rate (33.9%). Also comparable air-line distances show discrepancies: GPS - OBB 1200km with an infection rate of 43.6%, in contrast to GPS - NU 1163km, 61.9%. The top position is in between with 750 km as the crow flies, the worms originated from Norway with 72.4%.

The hypothesis that the virulence increases among the latitude or distance can be negated for this study (first part of a reciprocal crossing experiment), because parasite index and infection rate do not increase or decrease among latitude. The most northern population, the Swedish, grew smaller than the most southern, the Spanish population. The sympatric combination GPS fish plus GPS worm showed a low PI and infectivity, although it was expected as balanced due to mutual adaptation (Lively & Dybdahl, 2000; Lambrechts et al., 2006). Also the combination NST in GPS fish, assumed as sympatric because of the vicinity of their habitats was not successfull. Contrary to the scientific results of the last decades the sympatric combinations failed, the allopatric combination succeeded. An influence on the worm weight might have the water respectively the room temperature in the aquarium room of the Institute. Basically the metabolism of poikilothermic animals depends on the environmental temperature. The influence of temperature was shown by MacNab and Barber in 2011. A possible preference or adaptation to warmer or colder water was not tested. Because of the same water temperature a direct influence on the metabolism can be neglected. But an adaptation to seasonal effect like for periods an ice layer covers the habitat, meaning that no bird could ingest the 2nd host, is assumable. This could partly explain firstly the faster growth of populations near the Atlantic Ocean where the Gulfstream delivers warm water for the whole year. Secondly due to the cold winters around the Baltic Sea it could explain the slow growth of the nearby populations attuned to a reduced growth rate due to a longer cold spell or maybe to hibernation inside the host.

Barber proofed in 2004 that “parasites grow larger in faster growing fish hosts”. With his experiment he found that the growth of *S. solidus* is independent from the fish’s competition for food, whereby this refers to the intraspecific competition within one parasite species, but dependent of the growth rate of the stickleback. In the present experiment the chosen fish families had nearly the same size at the beginning. Comparing the weights of fish and tapeworms from this experiment it is obvious that there is no relation. Just for example, one GPS fish population with an average weight of 662.1 mg contained 13.9 mg tapeworm whereas another one with 531.97 mg had 114.03 mg in average worm inside. Because of this different between the parasite populations I assume that parasite growth is mostly influenced by genetical interactions between involved species. This is also indicated by the huge variance of parasite indices of the families with the same origin in different stickleback families.

5.2 HSI, SSI, CF, G/L ratio and RB activity

The liver, an energy reservoir, of the infected fish is on average smaller than the ones from the not infected. The spleen, an immunologically active organ, is bigger in the infected sticklebacks. The condition factor shows that most fish have the same cubic growth, except that the not infected fish have a slightly higher CF. All of the indices used in this experiment reveal no significant correlation to the geographical distance or latitude.

The granulocytes/ lymphocytes ratio, as an indication for the activity of the innate- in relation to the acquired immune system, also shows no change across latitude. On the other hand the proportion of the two cell types raises up some questions. As shown in figure 23, the fish reacts depending on the *S. solidus* population, with the innate- (G>L) or the acquired immune system (G<L) against the invaders. Normally "[the] elimination of the invading parasite *S. solidus* has to be carried out in the early stages of the infection, because it becomes impossible due to the fast growth of the parasite" (Scharsack et al., 2004). So the innate immune system as the first line of defense is responsible and should answer with the proliferation of a large number of granulocytes including eosinophils defending against parasites e.g. by releasing reactive oxygen species. Four out of seven cases follow this pattern, but in three cases the number of lymphocytes predominates. For two of these three cases (IBB and OBB, figure 23) the respiratory burst activity stresses the assumption that the acquired immune system is strongly acticvated. However, in *in vitro* HKL-cultures stimulated with antigens from *S. solidus* Franke et al. (2013) found evidence that the respiratory burst activity induced by different isolates of *S. solidus* is quite low. It is thinkable that some populations might trigger an earlier switch from innate to acquired immune system in order to evade the more dangerous immune response. Immune evasion of parasites is quite common in all hosts, especially when the worm has to spend months undetected in the host (Franke at al.,2013). Probably the results can be explained with by different strategies the parasite populations evolved in order to adapt to the specific requirements of host and habitat. The frequency of infections in a stickleback population and the costs of mutual adaptations to a high number of different parasite genotypes play also an important role. Additionally the responsivness of the stickleback leucocytes to the parasite increase with the prevalence of *S. solidus* populations (Franke at al.,2013).

The fact that the immune cells migrate in different quantities into the body cavity where the parasite grows has to be considered when testing cells derived from the head kidneys. In conclusion there is no clear information to the exact number or whereabouts of active immune cells. All in all there is no clear explanation.

5.3 Prevalence, predators and other biotic factors

In the Großer Plöner See (GPS), the origin of the experimental host sticklebacks of this study, prevalence of *S. solidus* is extremely low (Kalbe, personal communication). A parasite needing more time to develope than others, is threatened with extinction, because it might not have enough time to lay the foundation for the following generation. There are about 30 parasites (Kalbe, personal communication) affecting the three-spined stickleback in GPS. In addition to this the tapeworm needs a relative long time to reach the necessary size to initiate the last host switch. In this period other parasites like eye fluke (*Diplostomum pseudospathaceum*) might cause too much damage to the fish's fitness, enhance too early transmission to the final host or even cause death. But normally sticklebacks can accommodate several parasites as long as the fish fitness decreases not dramatically and most parasites are specialized on different body areas. So one imaginable trigger could be the available energy or nutrient the parasites need. A rapid growing worm in a small fish consumes a big part of the energy resources the fish has. When the fish e.g. becomes blind due to eye flukes (Chappell et al., 1994) its ability to take up food decreases dramatically. Other parasites might compete with *S. solidus* for host resources. When an infection of the skin occurs predators locate him easier or when the tails are infested he loses his ability to escape. There are many more examples underlining the importance of the interspecific competition between parasites.

An important aspect is the pressure of predators. Predators fish, sharing their habitat with the sticklebacks, increase the likelyhood particularly of *S. solidus*-infected sticklebacks to being preyed upon by predator that is not a suitable host for the parasite e.g. by lowering the margin for failures. Every single disadvantage makes the stickleback to easy prey. Jakobsen et al. (1988) have shown that introduction of predatory fish decreases prevalence of *S. solidus* dramatically in a Norwegian lake population. Unfortunately the fish species in the origin habitats are not fully characterized.

Another aspects is also considerable. McPhail et al. (1983) found in Fuller Lake, Vancouver Island, that the highest infection rate is shown in Autumn and the lowest in spring during breeding season. This suggests that *S. solidus* plerocercoids are adapted to delay the inappropiate effects into the postreproductive period. So the selective impact of *Schistocephalus* on *Gasterosteus* might be quite low in some areas.

Or a mix of the mentioned factors could have led to the problem that host and parasite did not properly adapt to each other in order to secure a stable *S. solidus* population in the Großer Plöner See. Furthermore it could partly explain the divergence between the success of the same *S. solidus* population in different *G. aculeatus* families.

5.4 Genetical connection

A very interesting aspect came out by reordering the *S. solidus* populations in genetic groups (Samonte-Padilla et al., in preparation). Plotting the three genetic groups Atlantic (NO, NU, SP), Baltic (GPS,NST, OBB) and Continental (IBB) against worm weight results in a highly significant relation (F(2;175) = 204,7057; *p*=0.0000). That indicates the worm size depends on the genetic relationship whereby the Atlantic populations grew biggest and the Baltic ones grew smallest. This might be influenced by the connectivity of the habitats also with regard to the Continental group isolated from the Baltic population by land barrier. A possible influence on the genetic exchange between the *S. solidus* populations across the routes taken by migratory birds is also thinkable. The studied populations are partly connected through the flight corridors of the migrant birds except the Continental population.

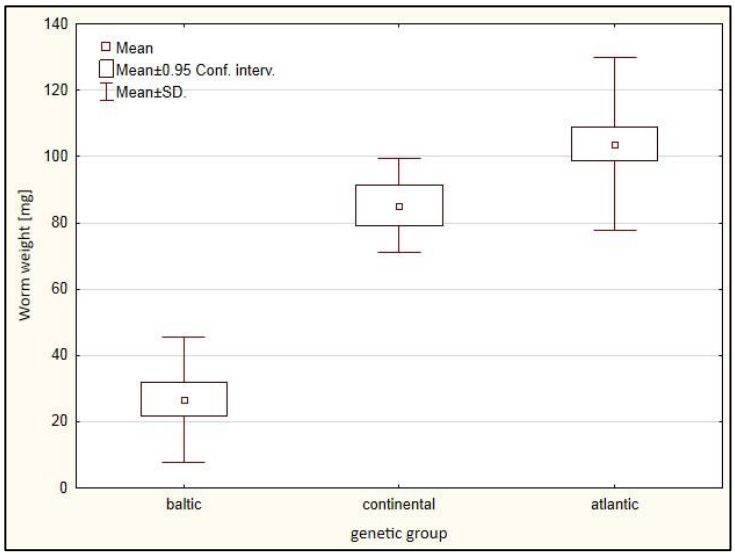

figure 25: Genetic groups plotted against worm weight
Plotting the three genetic groups Atlantic (NO, NU, SP), Baltic (GPS, NST, OBB) and Continental (IBB) against worm weight results in a highly significant relation (F(2;175) = 204,7057; p = 0.0000).

Further research, i.e. the influence of climate distinctions on host and parasite genotypes, the selection pressure of predatory fish and other parasites on *S. solidus* growth and virulence as well as the connectivity of habitats leading to a possible gene transmission among the involved species due to natural or human impact, is needed in order to obtain an adequate and comprehensive understanding of the host- parasite relationship. And of course the fulfillment of the reciprocal cross infection experiment I started is required.

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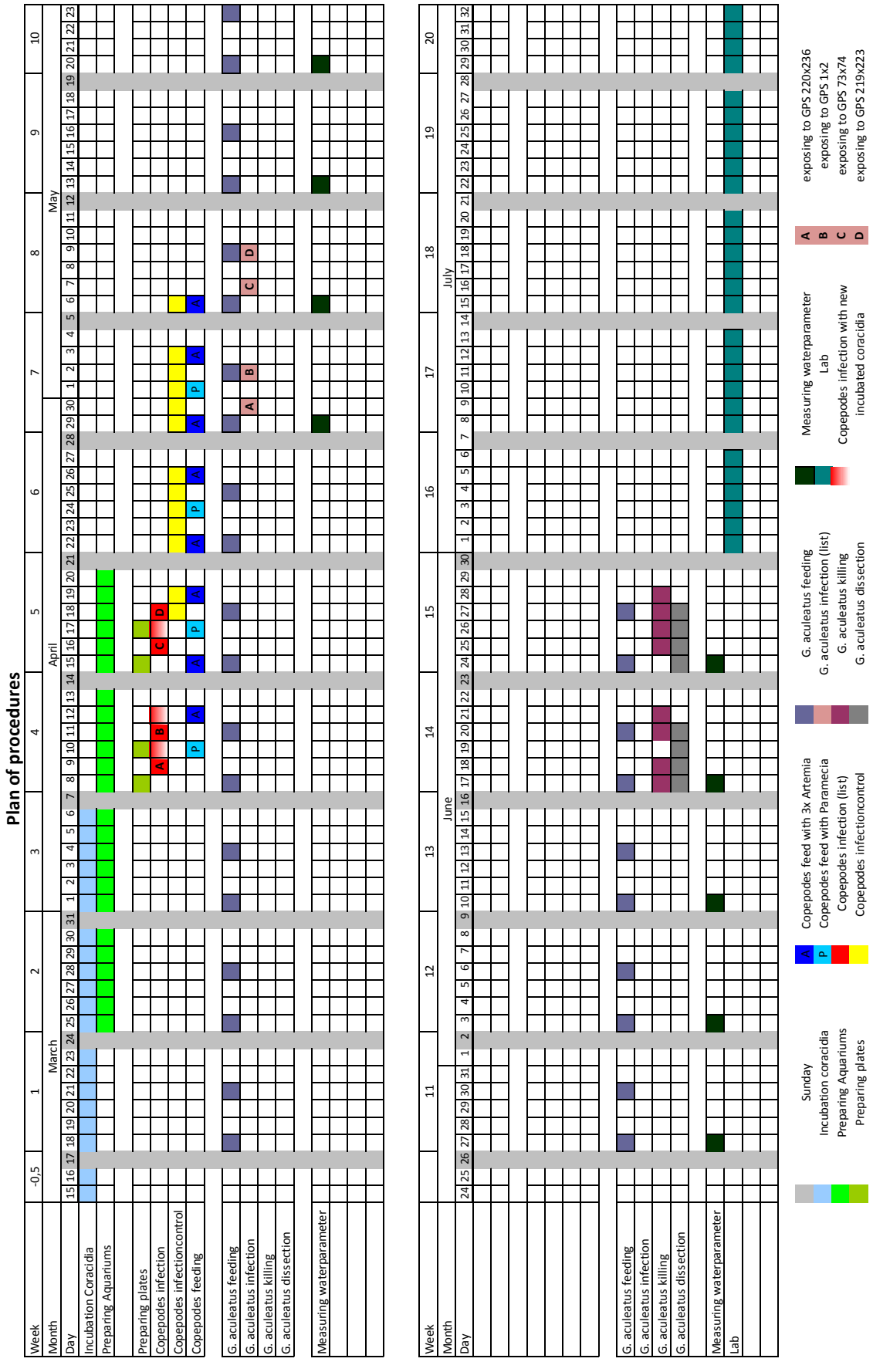
7 Appendix

7.1 Plan of procedure



7.2 Protocol: Isolation of Genomic DNA from Tissues

QIAamp DNA Micro Handbook 5/2010 25-**27**

This protocol is for isolation of genomic DNA from less than 10 mg tissue.
Important points before starting
■ Perform all centrifugation steps at room temperature (15–25°C)
■ If isolating DNA from very small amounts of tissue, carrier RNA is required (see page 15).
■ Prepare tissue samples on a cold surface (e.g., a glass, steel, or aluminum plate placed on top of a block of dry ice).
■ If using frozen tissue, ensure that the sample does not thaw out before addition of Buffer ATL in step 2.
Things to do before starting
■ Equilibrate Buffer AE or distilled water for elution to room temperature (15–25°C).
■ Set a thermomixer or heated orbital incubator to 56°C for use in step 4. If a thermomixer or heated orbital incubator is not available, a heating block or water bath can be used instead.
■ If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
■ Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 14.

Procedure
1. Transfer a tissue sample of less than 10 mg in weight to a 1.5 ml microcentrifuge tube (not provided).

2. Immediately add 180 µl Buffer ATL, and equilibrate to room temperature (15–25°C).

3. Add 20 µl proteinase K and mix by pulse-vortexing for 15 s.

4. Place the 1.5 ml tube in a thermomixer or heated orbital incubator, and incubate at 56°C overnight until the sample is completely lysed.
For small amounts of tissue, lysis is complete in 4–6 h, but best results are achieved after overnight lysis.

5. Add 200 µl Buffer AL, close the lid, and mix by pulse-vortexing for 15 s.
To ensure efficient lysis, it is essential that the sample and Buffer AL are thoroughly mixed to yield a homogenous solution.
Note: If carrier RNA is required (see page 13), add 1 µg dissolved carrier RNA to 200 µl Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

6. Add 200 µl ethanol (96–100%), close the lid, and mix thoroughly by pulse-vortexing for 15 s. Incubate for 5 min at room temperature (15–25°C).
Note: If room temperature exceeds 25°C, cool the ethanol on ice before adding to the tube.
7. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.

8. Carefully transfer the entire lysate from step 7 to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim. Close the lid, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean

2 ml collection tube, and discard the collection tube containing the flow-through.
If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute column is empty.

9. Carefully open the QIAamp MinElute column and add 500 µl Buffer AW1 without wetting the rim. Close the lid, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

10. Carefully open the QIAamp MinElute column and add 500 µl Buffer AW2 without wetting the rim. Close the lid, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
Contact between the QIAamp MinElute column and the flow-through should be avoided. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, coming into contact with the QIAamp MinElute column. Take care when removing the QIAamp MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute column.

11. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min to dry the membrane completely.
This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

12. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 20–100 µl Buffer AE or distilled water to the center of the membrane.
If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 12).
Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.
QIAamp MinElute columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of the solution applied to the column.

13. Close the lid and incubate at room temperature (15–25°C) for 1 min. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min.
Incubating the QIAamp MinElute column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

7.3 PCR programs

MP4	µl	no.	µl
	1 sample	96 samples	total
10 x buffer	1,00	10	10
dNTP's	1,00	10	10
MgCl2	0,30	10	3
Scso 22f	0,40	10	4
Scso 22r	0,40	10	4
Taq (Invitek)	0,05	10	0,5
HPLC	5,85	10	58,5
	9,00	10	90
	+ 1.00 µl template		

Program:		
Cycle	**Temp**	**min**
1 pre-denat.	95°C	15:00
2 denat	94°C	00:30
3 anneal	50°C	00:90
4 ext	72°C	01:00
5 repeat 2-3		34x
6 final ext.	60°C	30:00
7	4°C	forever

MP6a	µl	no.	µl
	1 sample	96 samples	total
10 x buffer	1,00	10	10
dNTP's	1,00	10	10
MgCl2	0,30	10	3
Scso 33f	0,40	10	4
Scso 33r	0,40	10	4
Taq (Invitek)	0,05	10	0,5
HPLC	5,85	10	58,5
	9,00	10	90
	+ 1.00 µl template		

Program:		
Cycle	**Temp**	**min**
1 pre-denat.	95°C	15:00
2 denat	94°C	00:30
3 anneal	50°C	00:90
4 ext	72°C	01:00
5 repeat 2-3		34x
6 final ext.	60°C	30:00
7	4°C	forever

MP10	µl	no.	µl
	1 sample	96 samples	total
10 x buffer	1,00	10	10
dNTP's	1,00	10	10
MgCl2	0,30	10	3
Scso 34f	0,40	10	4
Scso 34r	0,40	10	4
Taq (Invitek)	0,05	10	0,5
HPLC	5,85	10	58,5
	9,00	10	90

+ 1.00 µl template

Program:		
Cycle	**Temp**	**min**
1 pre-denat.	95°C	15:00
2 denat	94°C	00:30
3 anneal	50°C	00:90
4 ext	72°C	01:00
5 repeat 2-3		34x
6 final ext.	60°C	30:00
7	4°C	forever

MP8	µl	no.	µl
	1 sample	96 samples	total
10 x buffer	1,00	10	10
dNTP's	1,00	10	10
MgCl2	0,30	10	3
Scso 24f	0,40	10	4
Scso 24r	0,40	10	4
Scso 29f	0,40	10	4
Scso 29r	0,40	10	4
Taq (Invitek)	0,05	10	0,5
HPLC	5,05	10	50,5
	9,00	10	90

+ 1.00 µl template

Program:		
Cycle	Temp	min
1 pre-denat.	95°C	15:00
2 denat	94°C	00:30
3 anneal	53°C	00:90
4 ext	72°C	01:00
5 repeat 2-3		34x
6 final ext.	60°C	30:00
7	4°C	∞

SchistoplexI	µl	no.	µl
	1 sample	75 samples	total
10x buffer	1,00	10	10
dNTPs	1,00	10	10
1% BSA	1,00	10	10
MgCl2	0,30	10	3
SsCAA22-F	0,30	10	3
SsCAA22-R	0,30	10	3
SsCAB6- F	0,20	10	2
SsCAB6-R	0,20	10	2
SsCAA77-F1	0,30	10	3
SsCAA77-R1	0,30	10	3
Taq (invitek)	0,05	10	0,5
	4,95	10	
HPLC water	4,05	10	40,5
DNA	1,00		90

Program (Schistoplex1):		
Cycle	Temp	min
1 Denat.	94oC	03:00
2 Denat.	94oC	00:30
3 Anneal.	57oC	00:15
4 Ext.	72oC	01:00
5 repeat 2-3		34x
6 Final Ext.	72oC	7 min
7	4°C	forever

SchistoplexII	µl	no.	µl
	1 sample	75 samples	total
10x buffer	1,00	10	10
dNTPs	1,00	10	10
1% BSA	1,00	10	10
MgCl2	0,30	10	3
SsCTB24 F	0,30	10	3
SsCTB24 R	0,30	10	3
SsCA58 F	0,30	10	3
SsCA58 R	0,30	10	3
Taq (invitek)	0,05	10	0,5
	4,55	10	
HPLC water	4,45	10	44,5
DNA	1,00	10	90

Program (Schistoplex1):		
Cycle	Temp	min
1 Denat.	94oC	03:00
2 Denat.	94oC	00:30
3 Anneal.	57oC	00:15
4 Ext.	72oC	01:00
5 repeat 2-3		34x
6 Final Ext.	72oC	7 min
7	4°C	forever

7.4 Results Genotype sepuencing

		Scso22		Scso33_IS		Scso29		Scso24		Scso34		SsCaa22		SsCaa77		SSCTB24		SSCA58		Result
IBB 8x13 + NU 4x12	MSI_mSat_V51_140_MP4_C05_2013-07-19.fsa	114	128	122	125	93	93	156	156	125	134	149	149	195	209	164	168	135	135	IBB
IBB 8x13 + NU 4x12	MSI_mSat_V51_141_MP4_D05_2013-07-19.fsa	132	132	122	125	96	96	159	159	119	131	147	149	199	201	160	160	160	160	NU
IBB 8x13 + NU 4x12	MSI_mSat_V51_161_MP4_E05_2013-07-19.fsa	132	132	122	125	96	99	159	159	122	122	**		**	**	162	178	137	137	NU
IBB 8x13 + NU 4x12	MSI_mSat_V51_162_MP4_F05_2013-07-19.fsa	128	132	131	131	96	96	153	153	122	122	159	161	199	201	162	178	133	133	NU
IBB 8x13 + NU 4x12	MSI_mSat_V51_195_MP4_G05_2013-07-19.fsa	128	132	122	125	96	99	159	159	119	131	159	161	195	199	160	160	133	182	NU
IBB 8x13 + NU 4x12	MSI_mSat_V51_196_MP4_H05_2013-07-19.fsa	132	132	122	125	96	96	153	159	122	122	149	161	199	201	162	178	133	133	NU
IBB 8x13 + NU 4x12	MSI_mSat_V51_197_MP4_A06_2013-07-19.fsa	126	132	122	131	96	96	153	159	119	131	159	161	195	199	160	162	133	137	NU
IBB 8x13 + NU 4x12	MSI_mSat_V51_199_MP4_B06_2013-07-19.fsa	128	132	122	131	96	96	153	159	125	131	147	159	**	**	160	160	133	182	NU
IBB 8x13 + NU 4x12	MSI_mSat_V51_201_MP4_C06_2013-07-19.fsa	114	128	122	125	93	93	156	156	125	125	144	144	207	209	162	164	**	**	IBB
IBB 8x13 + NU 4x12	MSI_mSat_V51_202_MP4_D06_2013-07-19.fsa	128	128	113	122	93	93	156	156	125	134	144	149	195	195	160	168	**	**	IBB
IBB 8x13 + NU 4x12	MSI_mSat_V51_203_MP4_E06_2013-07-19.fsa	128	132	122	125	96	96	159	159	119	122	147	159	199	201	160	162	**	**	NU
	MSI_mSat_V51_4x12A	125	131	125	131	96	96	153	159	**		162	162	**		160	160	**	**	
	MSI_mSat_V51_4x12B	127	131	122	131	96	99	153	159	**		163	163	**		160	160	**	**	
GPS 1x2 + IBB 11x12	MSI_mSat_V51_398_MP4_F06_2013-07-19.fsa	114	128	137	137	93	93	159	159	125	125	149	149	207	209	162	164	135	144	IBB
GPS 1x2 + IBB 11x12	MSI_mSat_V51_433_MP4_G06_2013-07-19.fsa	114	128	113	137	93	93	156	159	125	125	149	149	201	209	162	164	**	**	IBB
	MSI_ESTLineagecheck_plate01_MP5a_GPS_2011_001_B03_2011-06-28.fsa	130	132	135	135	99	99	150	156	125	132	142	144	194	194	160	166			
	MSI_ESTLineagecheck_plate01_MP5a_GPS_2011_002_C03_2011-06-28.fsa	114	130	132	135	96	99	159	162	129	129	140	149	196	216	160	160			
	MSI_ESTLineagecheck_plate01_MP5a_GPS_2011_003_A03_2011-06-28.fsa	120	130	135	135	96	96	162	168	123	123	144	146	198	200	160	166			
GPS 1x2 + OBB 9x6	MSI_mSat_V51_42_MP4_A05_2013-07-19.fsa	128	128	128	128	**	**	156	156	125	125	144	151	193	193	164	168	135	135	OBB
GPS 1x2 + OBB 9x6	MSI_mSat_V51_43_MP4_B05_2013-07-19.fsa	**	**	116	116	**	**	156	159	**	**	**	**	**	**	162	164	**	**	GPS
	MIS_BoxTina2_MP4_repeat_12Ob9x6A_MP4_E07_2013-06-03.fsa	118	128	128	128	96	99	150	156	123	125	144	150	190	190	160	160	SSCA58		
	MIS_BoxTina2_MP4_repeat_12Ob9x6b_MP4_F07_2013-06-03.fsa	128	132	128	128	99	107	153	156	125	125	150	154	190	192	160	160			

7.5 Water parameter

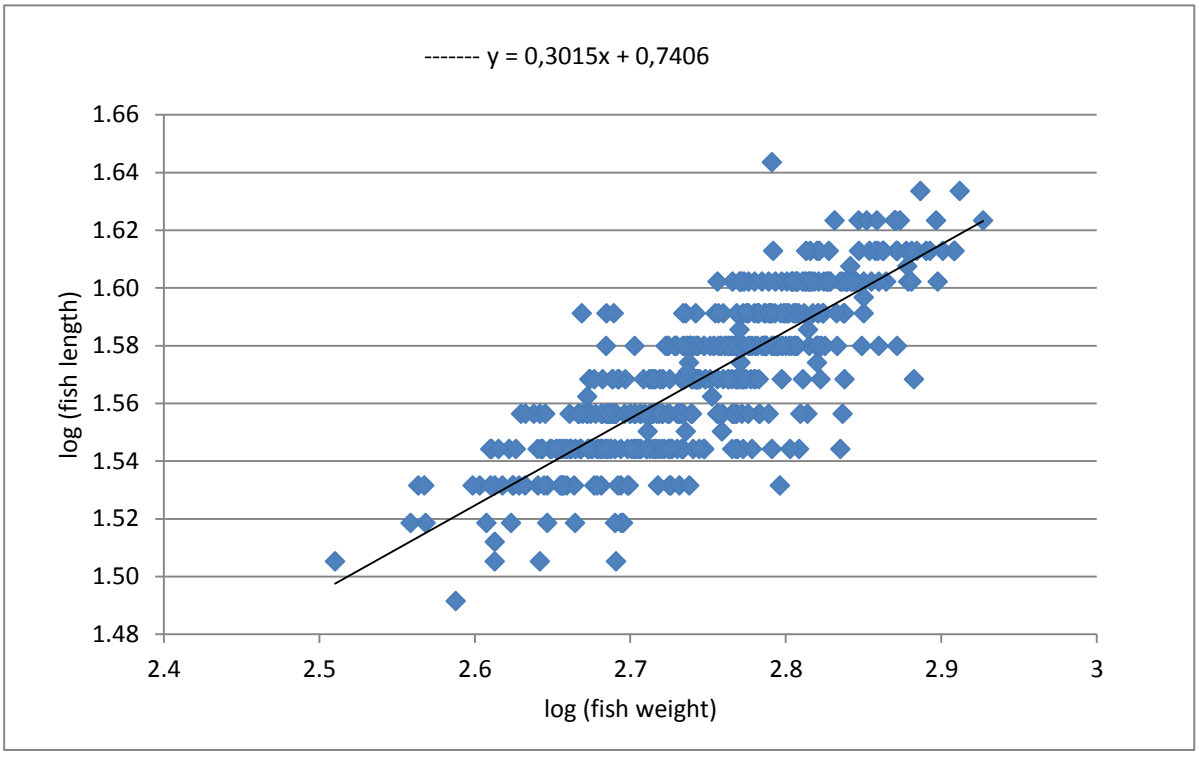
The water used for the aquariums in this experiment were taken out of the Schöhsee, right behind the Max-Planck-Institute in Plön from 10 meters depth. The water treatment contains micro filtering and heating/cooling to a temperature of 18 °C. According to German water standards the lake has pure fresh water.

Tabel 1 : Water parameter of Schoehsee

COND: Conductivity; PP: Particulate phosphorus; TDP: Total dissolved phosphorus; TP: Total phosphorus; PN: Particulate nitrogen; TDN: Total dissolved nitrogen; TN: Total nitrogen; PC: Particulate carbon; DOC: Dissolved organic carbon

test center	Date	Year	pH	COND	PP	TDP	PO4-P	TP	NH4-N	NO2-N	NO3-N	PN	TDN	TN	PC	DOC
Schöhsee				uS/cm	ug P/l	ug P/l	ug P/l	ug P/l	ug N/l	ug N/l	ug N/l	ug N/l	ug N/l	ug N/l	mg C/l	mg C/l
Uni Kiel	3.12.	2007	7,7	241	31	20	9	51	159	6	24	0	554	554		
	8.12.	2008	8	213	68	16	24	84	91	2	83	93	77	170	0,35	5,6
	06.-07.12.	2009	7,23	274	40	19	6	59	77	4	101	870			0	
	05.-07.12.	2010	7,81	278	84	21	17	105	116	2	55	3	553	556	0,03	
LUFA	04.-05.12.	2011	7,6	284			<0,06	<50	114	<0,02	<110			<1000		5,6

7.6 Regression analyse CF



7.7 Results *Macrocyclops albidus* infection series

Table 1: Results of the *Macrocyclops albidus infection* series

Per line is shown the total number of infections (inf.) per family. Multi infected means more than one procercoid was detected in *M. albidus.* Those were not used for the next infection series. Multi and single infected were sum up for the total number of infections (Total inf.). The last two columns show the number of to *Gasterosteus aculeatus* exposed and from him assimilated *M.*

S. solidus family	Exposed to *S. solidus*	Dead	Not infected	Multi infected	Single infected	Total infected	Infection rate	Mortality	Exposed to *G. aculeatus*	Assimilated by *G. aculeatus*
SP 4x20	96	28	49	0	19	19	19.79%	29.17%	17	16
SP 28x29	96	46	29	0	21	21	21.88%	47.92%	19	16
SP 1x37 C	96	15	57	0	24	24	25.00%	15.63%	20	19
SP 1x37 D	96	9	70	0	17	17	17.71%	9.38%	15	11
Total	384	98	205	0	81	81	21.09%	25.52%	71	62
NU 3x6	96	46	30	1	19	20	20.83%	47.92%	14	12
NU 4x12	96	38	31	12	15	27	28.13%	39.58%	12	12
NU 10x14	96	15	49	0	32	32	33.33%	15.63%	20	20
NU 5x13	96	33	35	0	28	28	29.17%	34.38%	20	19
Total	384	132	145	13	94	107	27.86%	34.38%	66	63
IBB 6x7	96	37	39	0	20	20	20.83%	38.54%	20	18
IBB 8x13	69	22	43	0	4	4	5.80%	31.88%	5	4
IBB 9x10	96	18	58	1	19	20	20.83%	18.75%	19	19
IBB 11x12	72	12	53	0	7	7	9.72%	16.67%	6	6
Total	333	89	193	1	50	51	14.30%	26.46%	50	47
NO 40x21	96	18	39	1	38	39	40.63%	18.75%	20	20
NO 94x103	96	22	44	2	28	30	31.25%	22.92%	20	18
NO 4x19	96	11	48	0	37	37	38.54%	11.46%	20	20
NO 134x130	96	14	38	0	44	44	45.83%	14.58%	20	18
Total	384	65	169	3	147	150	39.06%	16.93%	80	76
NST 7x8	96	17	49	2	28	30	31.25%	17.71%	20	18
NST 2x4	70	22	37	0	11	11	15.71%	31.43%	11	11
NST 1x3 C	96	10	68	0	18	18	18.75%	10.42%	14	14
NST 1x3 D	96	23	54	0	19	19	19.79%	23.96%	17	16
Total	358	72	208	2	76	78	21.38%	20.88%	62	59
GPS 1x2 A	96	31	62	0	3	3	3.13%	32.29%	3	3
GPS 1x2 B	96	31	62	0	3	3	3.13%	32.29%	3	3
GPS 1x2 C	96	17	70	0	9	9	9.38%	17.71%	9	9
GPS 1x2 D	96	14	79	0	3	3	3.13%	14.58%	3	3
Total	384	93	273	0	18	18	4.69%	24.22%	18	18
OBB 9x6	96	27	53	2	14	16	16.67%	28.13%	8	8
OBB 25x24	96	30	41	2	23	25	26.04%	31.25%	18	15
OBB 2x23	96	10	51	1	34	35	36.46%	10.42%	20	19
OBB 19x7	96	19	52	0	25	25	26.04%	19.79%	20	20
Total	384	86	197	5	96	101	26.30%	22.40%	66	62
Total	**2611**	**635**	**1390**	**24**	**562**	**586**	**22.10%**	**24.40%**	**413**	**387**

7.8 Fish dissection data

S. solidus family	n	Fish weight [mg]			Short Length [mm]			Total Length [mm]		
		Mean	Stdev.	Variance	Mean	Stdev.	Variance	Mean	Stdev.	Variance
SP 4x20	19	665.44	116.36	13539.69	39.32	2.16	4.64	44.58	2.76	7.59
SP 28x29	19	537.15	102.05	10414.12	36.50	1.95	3.81	41.18	2.08	4.34
SP 1x37 C	19	571.88	84.45	7130.97	37.45	1.88	3.52	42.53	1.97	3.87
SP 1x37 D	15	571.43	94.70	8967.91	36.60	1.92	3.69	41.73	2.02	4.07
NU 3x6	19	608.26	94.61	8951.60	39.11	1.76	3.10	44.26	1.98	3.93
NU 4x12	14	536.46	62.32	3884.34	37.11	1.39	1.93	42.00	1.83	3.35
NU 10x14	20	536.36	75.57	5711.37	36.63	1.65	2.71	41.78	1.72	2.96
NU 5x13	20	548.91	111.41	12411.33	36.00	1.99	3.97	41.30	2.15	4.62
IBB 6x7	19	661.98	68.45	4685.47	39.68	1.38	1.89	45.11	1.67	2.79
IBB 8x13	3	429.83	82.65	6831.54	36.33	1.15	1.33	41.00	1.73	3.00
IBB 9x10	19	529.92	74.41	5536.64	36.71	1.91	3.65	41.89	2.13	4.54
IBB 11x12	9	563.67	89.45	8000.53	36.06	1.33	1.78	41.33	1.66	2.75
NO 40x21	20	604.25	77.57	6016.34	39.15	1.35	1.82	44.30	1.76	3.09
NO 4x19	20	531.97	74.34	5527.07	37.20	1.61	2.59	42.33	1.59	2.53
NO134x130	20	537.25	71.73	5145.87	35.70	2.05	4.22	40.88	2.13	4.55
NST 7x8	20	616.25	81.42	6628.44	38.90	1.59	2.52	44.23	1.76	3.09
NST 2x4	14	554.00	84.09	7071.54	37.43	2.03	4.11	42.29	2.01	4.03
NST 1x3 C	14	546.89	72.83	5304.12	36.71	1.68	2.84	41.89	1.94	3.78
NST 1x3 D	16	522.92	48.24	2327.55	35.00	1.03	1.07	40.41	1.20	1.44
GPS 1x2 A	1	662.10	0.00	0.00	40.00	0.00	0.00	45.00	0.00	0.00
GPS 1x2 C	10	537.43	80.17	6427.33	36.90	1.52	2.32	42.35	2.06	4.23
OBB 9x6	15	598.69	80.71	6513.89	38.40	2.72	7.40	43.70	2.74	7.49
OBB 25x24	17	555.92	90.66	8219.74	36.85	2.01	4.06	41.71	2.14	4.60
OBB 2x23	19	572.69	86.52	7486.04	37.47	1.95	3.82	42.63	2.03	4.11
OBB 19x7	20	559.29	94.02	8840.05	35.73	2.11	4.46	41.00	2.44	5.97
Control A	20	622.35	74.04	5482.56	39.20	1.70	2.91	44.43	1.83	3.35
Control B	20	525.25	94.56	8941.57	37.05	2.09	4.37	41.93	2.33	5.43
Control C	16	545.63	89.90	8081.18	37.22	2.04	4.17	42.34	2.39	5.69
Control D	20	528.95	63.70	4057.52	35.28	1.52	2.30	40.48	1.72	2.96

7.9 Worm weights

S. solidus Family	Mean	N	Standard deviation	Variance	Standard error
Control A		0			
Control B		0			
Control C		0			
Control D		0			
GPS 1x2 A	13.9000	1	0.00000	0.000	
GPS 1x2 C	28.5286	7	25.62497	656.639	9.685328
IBB 11x12	92.5000	2	9.05097	81.920	6.400000
IBB 6x7	86.1333	3	10.00017	100.003	5.773599
IBB 8x13	74.1667	3	3.94250	15.543	2.276205
IBB 9x10	86.3929	14	15.99286	255.771	4.274271
NO 134x130	115.6100	10	14.99737	224.921	4.742584
NO 40x21	134.3188	16	15.79321	249.426	3.948304
NO 4x19	114.0353	17	14.98912	224.674	3.635395
NST 1x3 C	10.4000	9	4.76078	22.665	1.586926
NST 1x3 D	24.6600	5	11.58266	134.158	5.179923
NST 2x4		0			
NST 7x8	51.0000	6	13.78811	190.112	5.628973
NU 10x14	81.4364	11	29.73006	883.877	8.963951
NU 3x6	75.5000	6	10.36976	107.532	4.233438
NU 4x12	107.9375	8	18.19207	330.951	6.431866
NU 5x13	98.7000	14	23.56800	555.451	6.298814
OBB 19x7	16.6143	7	12.97272	168.291	4.903227
OBB 25x24	36.0500	6	20.50744	420.555	8.372126
OBB 2x23	27.2231	13	15.68912	246.149	4.351380
OBB 9x6	46.7000	1	0.00000	0.000	
SP 1x37 C	117.1000	2	2.68701	7.220	1.900000
SP 1x37 D	89.9286	7	8.00556	64.089	3.025819
SP 28x29	75.0200	5	13.99704	195.917	6.259665
SP 4x20	81.1400	5	12.98260	168.548	5.805997
ALL	77.6826	178	41.43765	1717.079	3.105883

7.10 Descriptive statistic and HSD with unequal N

7.10.1 Parasite index

S. solidus Family	Mean	N	Standard deviation	Variance	Standard error
GPS 1x2 A	2.09938	1	0.000000	0.00000	
GPS 1x2 C	5.89392	7	6.025204	36.30308	2.277313
IBB 11x12	20.55634	2	0.681275	0.46414	0.481734
IBB 6x7	14.59241	3	1.227968	1.50791	0.708968
IBB 8x13	17.73090	3	3.793250	14.38875	2.190034
IBB 9x10	16.95233	14	2.406793	5.79265	0.643243
NO 134x130	22.41975	10	3.515177	12.35647	1.111596
NO 40x21	22.51908	16	4.481953	20.08790	1.120488
NO 4x19	21.72661	17	2.370724	5.62033	0.574985
NST 1x3 C	1.79543	9	0.699918	0.48989	0.233306
NST 1x3 D	4.86171	5	2.200654	4.84288	0.984162
NST 7x8	8.77511	6	1.909029	3.64439	0.779358
NU 10x14	15.98059	11	5.449691	29.69913	1.643144
NU 3x6	13.53619	6	1.494185	2.23259	0.609999
NU 4x12	20.12932	8	1.935018	3.74429	0.684132
NU 5x13	18.88721	14	2.617253	6.85002	0.699490
OBB 19x7	3.05640	7	2.509538	6.29778	0.948516
OBB 25x24	6.48794	6	2.906068	8.44523	1.186397
OBB 2x23	5.04174	13	2.990077	8.94056	0.829298
OBB 9x6	6.79470	1	0.000000	0.00000	
SP 1x37 C	19.48534	2	0.885848	0.78473	0.626389
SP 1x37 D	16.70963	7	1.414614	2.00113	0.534674
SP 28x29	15.52862	5	1.615850	2.61097	0.722630
SP 4x20	15.45451	5	3.466000	12.01315	1.550042
ALL	14.56021	178	7.606166	57.85376	0.570106

HSD test for unequal N; Variable Parasite index;
Approximated propability for Post-hoc-tests
Error: MQ(between) = 12.061, FG = 171.00

S. solidus origin	GPS	IBB	NO	NST	NU	OBB	SP
GPS		0.000026	0.000026	0.999454	0.000026	0.999950	0.000026
IBB	0.000026		0.000045	0.000026	0.999609	0.000026	0.996050
NO	0.000026	0.000045		0.000026	0.000026	0.000026	0.000030
NST	0.999454	0.000026	0.000026		0.000026	0.999986	0.000026
NU	0.000026	0.999609	0.000026	0.000026		0.000026	0.951878
OBB	0.999950	0.000026	0.000026	0.999986	0.000026		0.000026
SP	0.000026	0.996050	0.000030	0.000026	0.951878	0.000026	

7.10.2 Hepatosomatic index

S. solidus Family	Mean	N	Standard deviation	Variance	Standard error
Control A	5.008345	20	1.441663	2.078392	0.322366
Control B	3.771802	20	1.100740	1.211628	0.246133
Control C	4.822152	16	1.288859	1.661157	0.322215
Control D	2.353668	20	0.547506	0.299763	0.122426
GPS 1x2 A	4.878417	1	0.000000	0.000000	
GPS 1x2 C	3.132491	10	1.000891	1.001782	0.316509
IBB 11x12	3.069448	9	1.755062	3.080244	0.585021
IBB 6x7	5.034901	19	1.338378	1.791257	0.307045
IBB 8x13	3.076114	3	1.266705	1.604542	0.731333
IBB 9x10	3.721032	19	0.875820	0.767061	0.200927
NO 134x130	2.149538	20	0.405592	0.164505	0.090693
NO 40x21	3.645431	20	0.918414	0.843485	0.205364
NO 4x19	3.417993	20	0.918416	0.843489	0.205364
NST 1x3 C	3.666212	14	0.811182	0.658016	0.216797
NST 1x3 D	2.371488	16	0.770430	0.593562	0.192607
NST 2x4	3.810449	14	0.896462	0.803645	0.239590
NST 7x8	4.444946	20	1.094853	1.198702	0.244816
NU 10x14	3.525892	20	0.947387	0.897541	0.211842
NU 3x6	4.954907	19	1.194030	1.425709	0.273929
NU 4x12	3.148744	14	0.886009	0.785013	0.236796
NU 5x13	2.145076	20	0.560548	0.314214	0.125342
OBB 19x7	2.487759	20	0.610092	0.372212	0.136421
OBB 25x24	3.838456	17	1.033308	1.067725	0.250614
OBB 2x23	4.436336	19	1.164420	1.355875	0.267136
OBB 9x6	4.816776	10	1.158190	1.341403	0.366252
SP 1x37 C	4.771760	19	1.010757	1.021629	0.231883
SP 1x37 D	2.153466	15	0.500582	0.250582	0.129250
SP 28x29	2.871777	19	0.658685	0.433865	0.151113
SP 4x20	4.547463	18	1.445934	2.090726	0.340810
ALL	3.638049	471	1.374051	1.888016	0.063313

HSD test for unequal N; Variable Hepatosomatic index;
Approximated propability for Post-hoc-tests
Error: MQ(between) = 1.3245, FG = 246.00

S. solidus origin	Control	GPS	IBB	NO	NST	NU	OBB	SP
Control		0.932217	0.925672	0.001557	0.735408	0.000079	0.643948	0.006171
GPS	0.932217		0.999799	0.999501	1.000000	0.979924	0.999998	0.939245
IBB	0.925672	0.999799		0.720677	0.999809	0.261918	0.999955	0.185225
NO	0.001557	0.999501	0.720677		0.954460	0.981477	0.850830	0.975696
NST	0.735408	1.000000	0.999809	0.954460		0.627354	1.000000	0.430662
NU	0.000079	0.979924	0.261918	0.981477	0.627354		0.350453	0.999979
OBB	0.643948	0.999998	0.999955	0.850830	1.000000	0.350453		0.364572
SP	0.006171	0.939245	0.185225	0.975696	0.430662	0.999979	0.364572	

7.10.3 Splenosomatic index

S. solidus Family	Mean	N	Standard deviation	Variance	Standard error
Control A	0.078541	20	0.031961	0.001021	0.007147
Control B	0.076975	20	0.031213	0.000974	0.006979
Control C	0.061201	16	0.020432	0.000417	0.005108
Control D	0.083511	20	0.036811	0.001355	0.008231
GPS 1x2 A	0.030207	1	0.000000	0.000000	
GPS 1x2 C	0.078013	10	0.046011	0.002117	0.014550
IBB 11x12	0.085976	9	0.016521	0.000273	0.005507
IBB 6x7	0.075545	19	0.027728	0.000769	0.006361
IBB 8x13	0.119752	3	0.033732	0.001138	0.019475
IBB 9x10	0.110659	19	0.041360	0.001711	0.009489
NO 134x130	0.082472	20	0.037617	0.001415	0.008412
NO 40x21	0.077937	20	0.042718	0.001825	0.009552
NO 4x19	0.098393	20	0.029547	0.000873	0.006607
NST 1x3 C	0.072562	14	0.030358	0.000922	0.008113
NST 1x3 D	0.078390	16	0.033831	0.001145	0.008458
NST 2x4	0.076042	14	0.033366	0.001113	0.008917
NST 7x8	0.075485	20	0.031054	0.000964	0.006944
NU 10x14	0.089450	20	0.038598	0.001490	0.008631
NU 3x6	0.074244	19	0.052277	0.002733	0.011993
NU 4x12	0.078238	14	0.027235	0.000742	0.007279
NU 5x13	0.072683	20	0.030882	0.000954	0.006905
OBB 19x7	0.086636	20	0.030349	0.000921	0.006786
OBB 25x24	0.105941	17	0.053676	0.002881	0.013018
OBB 2x23	0.100592	19	0.034441	0.001186	0.007901
OBB 9x6	0.087821	10	0.061191	0.003744	0.019350
SP 1x37 C	0.093183	19	0.032469	0.001054	0.007449
SP 1x37 D	0.086028	15	0.044829	0.002010	0.011575
SP 28x29	0.089510	19	0.032739	0.001072	0.007511
SP 4x20	0.074340	18	0.034473	0.001188	0.008125
ALL	0.083722	471	0.037279	0.001390	0.001718

HSD test for unequal N; Variable Splenosomatic index;
Approximated propability for Post-hoc-tests
Error: MQ(between) = .00126, FG = 246.00

S. solidus origin	Control	GPS	IBB	NO	NST	NU	OBB	SP
Control		0.992348	0.037058	0.682209	0.990794	0.999656	0.001262	0.218983
GPS	0.992348		0.962429	1.000000	0.999993	0.999028	0.858937	0.994485
IBB	0.037058	0.962429		0.544412	0.385862	0.106886	0.999458	0.999799
NO	0.682209	1.000000	0.544412		0.999982	0.951614	0.119212	0.894952
NST	0.990794	0.999993	0.385862	0.999982		0.999741	0.135856	0.733695
NU	0.999656	0.999028	0.106886	0.951614	0.999741		0.006474	0.417222
OBB	0.001262	0.858937	0.999458	0.119212	0.135856	0.006474		0.979520
SP	0.218983	0.994485	0.999799	0.894952	0.733695	0.417222	0.979520	

7.10.4 Conditon factor

S. solidus Family	Mean	N	Standard deviation	Variance	Standard error
Control A	0.009751	20	0.000509	0.000000	0.000114
Control B	0.009699	20	0.000581	0.000000	0.000130
Control C	0.009967	16	0.000617	0.000000	0.000154
Control D	0.011396	20	0.000701	0.000000	0.000157
GPS 1x2 A	0.009788	1	0.000000	0.000000	
GPS 1x2 C	0.010070	10	0.000667	0.000000	0.000211
IBB 11x12	0.011334	9	0.001068	0.000001	0.000356
IBB 6x7	0.010014	19	0.000723	0.000001	0.000166
IBB 8x13	0.008419	3	0.000888	0.000001	0.000513
IBB 9x10	0.010122	19	0.000795	0.000001	0.000182
NO 134x130	0.011203	20	0.001108	0.000001	0.000248
NO 40x21	0.009513	20	0.000918	0.000001	0.000205
NO 4x19	0.009757	20	0.000793	0.000001	0.000177
NST 1x3 C	0.010426	14	0.000499	0.000000	0.000133
NST 1x3 D	0.011542	16	0.000554	0.000000	0.000138
NST 2x4	0.009951	14	0.000567	0.000000	0.000152
NST 7x8	0.009884	20	0.000815	0.000001	0.000182
NU 10x14	0.010305	20	0.000863	0.000001	0.000193
NU 3x6	0.009565	19	0.000623	0.000000	0.000143
NU 4x12	0.009926	14	0.000618	0.000000	0.000165
NU 5x13	0.011062	20	0.001407	0.000002	0.000315
OBB 19x7	0.011531	20	0.000596	0.000000	0.000133
OBB 25x24	0.010446	17	0.000504	0.000000	0.000122
OBB 2x23	0.010284	19	0.000892	0.000001	0.000205
OBB 9x6	0.010073	11	0.000817	0.000001	0.000246
SP 1x37 C	0.010257	19	0.000566	0.000000	0.000130
SP 1x37 D	0.010961	15	0.000609	0.000000	0.000157
SP 28x29	0.010344	19	0.000770	0.000001	0.000177
SP 4x20	0.010215	18	0.000755	0.000001	0.000178
ALL	0.010330	472	0.000981	0.000001	0.000045

HSD test for unequal N; Variable Conditon Factor;
Approximated propability for Post-hoc-tests
Error: MQ(between) = .00858, FG = 246.00

S. solidus origin	Control	GPS	IBB	NO	NST	NU	OBB	SP
Control		0.984114	0.623904	0.859899	0.918851	0.949179	0.646167	1.000000
GPS	0.984114		1.000000	0.999984	0.682207	0.999915	0.564561	0.992478
IBB	0.623904	1.000000		0.993342	0.062298	0.981942	0.016357	0.807690
NO	0.859899	0.999984	0.993342		0.354590	0.999999	0.079340	0.995016
NST	0.918851	0.682207	0.062298	0.354590		0.443534	0.999995	0.866855
NU	0.949179	0.999915	0.981942	0.999999	0.443534		0.120542	0.998554
OBB	0.646167	0.564561	0.016357	0.079340	0.999995	0.120542		0.722566
SP	1.000000	0.992478	0.807690	0.995016	0.866855	0.998554	0.722566	

7.10.4 Respiratory burst activity

HSD test for unequal N; Variable RLU
Approximated propability for Post-hoc-tests
Error: MQ(between) = 100E10, FG = 246.00

S. solidus origin	Control	GPS	IBB	NO	NST	NU	OBB	SP
Control		0.910123	0.522461	0.000032	0.235491	0.000264	0.813560	0.002102
GPS	0.910123		1.000000	0.708932	0.999997	0.995745	0.999810	0.904351
IBB	0.522461	1.000000		0.067030	0.999283	0.860802	0.998928	0.392343
NO	0.000032	0.708932	0.067030		0.332821	0.451715	0.002400	0.999038
NST	0.235491	0.999997	0.999283	0.332821		0.994712	0.950417	0.757822
NU	0.000264	0.995745	0.860802	0.451715	0.994712		0.348978	0.990807
OBB	0.813560	0.999810	0.998928	0.002400	0.950417	0.348978		0.125747
SP	0.002102	0.904351	0.392343	0.999038	0.757822	0.990807	0.125747	

7.11 Indices plotted against latitude

7.11.1 Parasite index against latitude

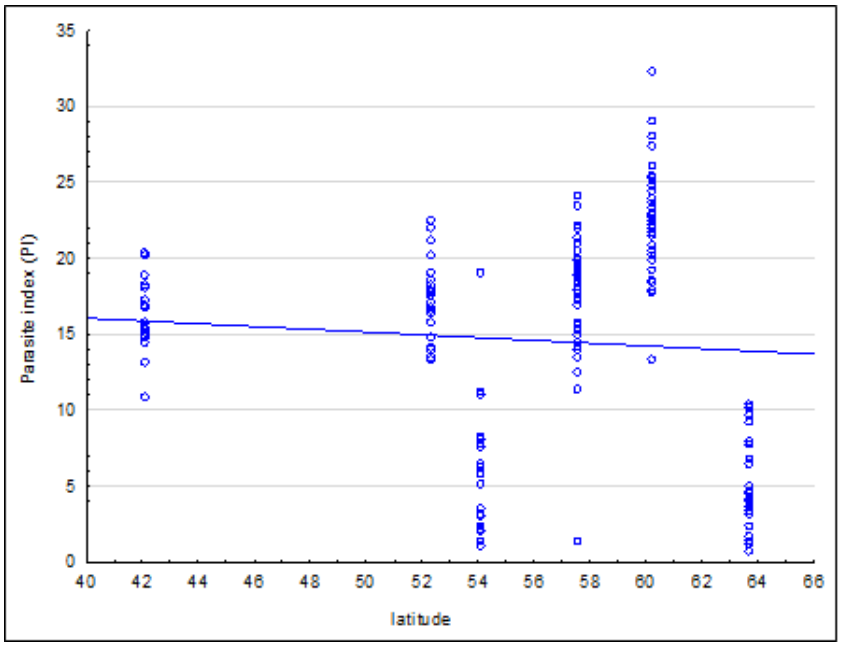


Figure 26: Parasite index

The diagram shows the Parasite index plotted against latitude. There is no significant relation between the parasite index and latitude (p= 0.3357; r = -0.0726), more than that the graph indicates the contrary a decrease among latitude.

7.11.2 Hepatosomatic index against latitude

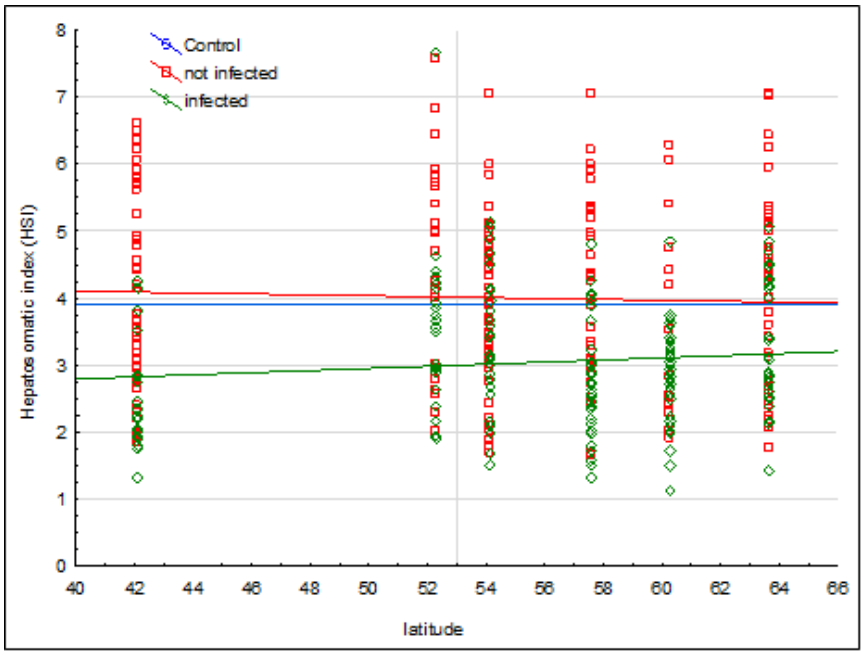


Figure 27: Hepatosomatic index

The diagram shows the Hepatosomatic index plotted against latitude. The index, liver weight relative to the body size, of the not infected (4.0106 ±0.0881) and control fish (3.9451 ± 0.1489) are one point higher than from the infected fish (3.0526 ±0.0973). The regression line of the not infected is quite stable whereas the infected show a slight increase of the hepatosomatic index among latitude.

7.11.3 Splenosomatic index against latitude

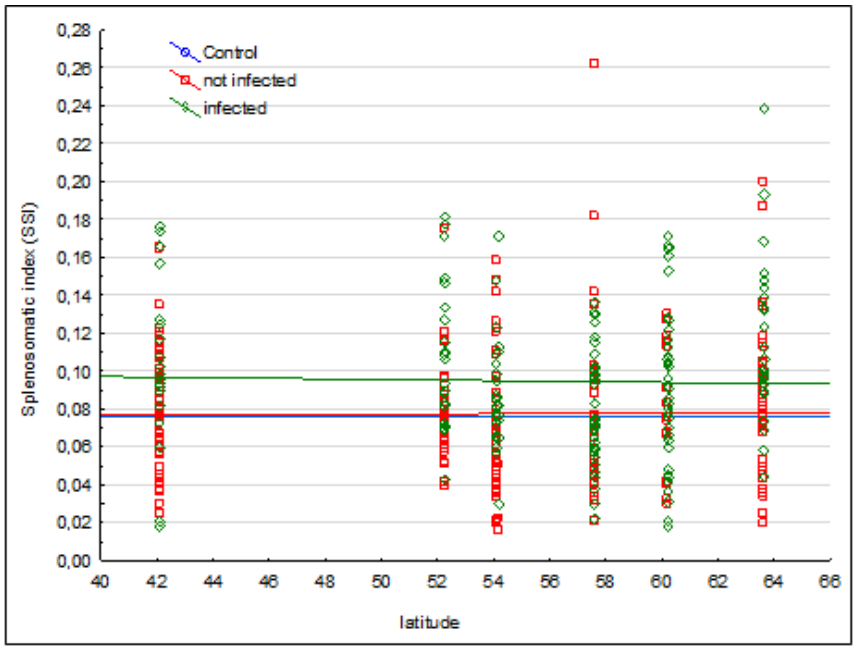


Figure 28: Splenosomatic index

The diagram shows the Splenosomatic index plotted against latitude. The spleen of the infected stickleback is smaller than in the not infected ones. The splenosomatic index of infected fish has its mean at 0.0946 ±0.0027, not infected at 0.0775 ±0.0775 and control at 0,075786 ±0.0041.

7.11.4 Conditon factor against latitude

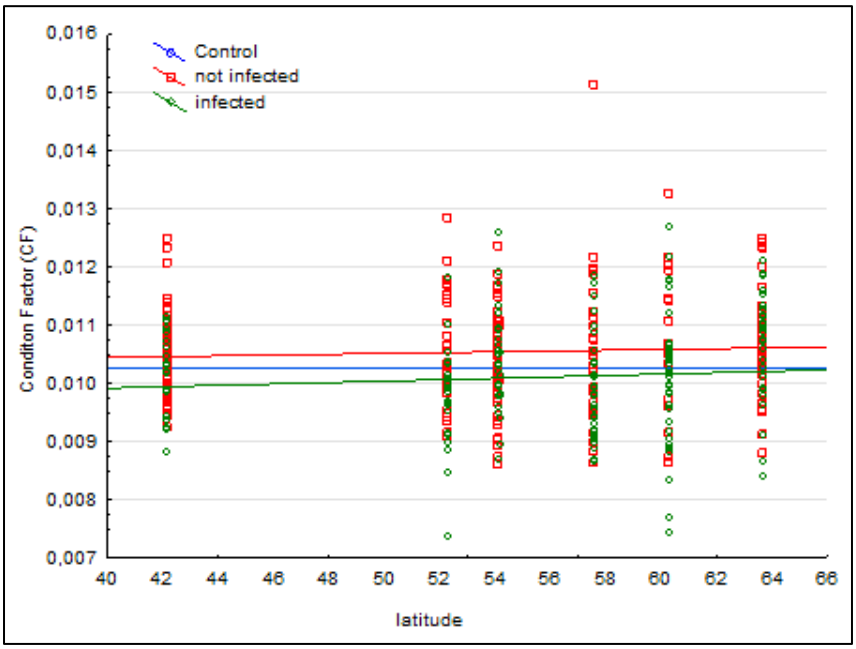
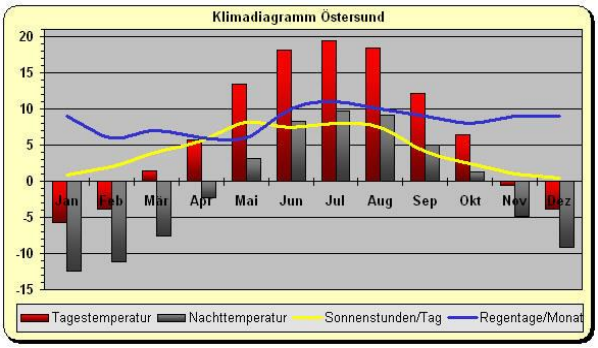
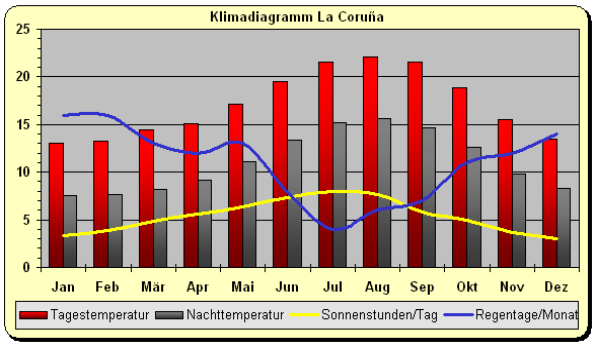


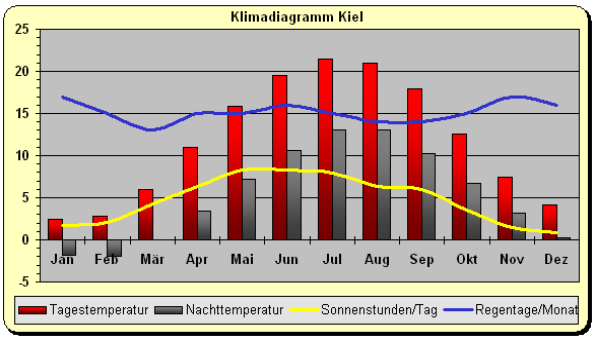
Figure 29: Conditon Factor

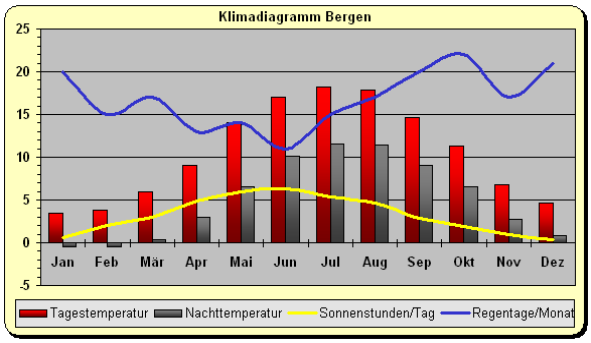
The Condition factor regression lines of infected and not infected fish show slight increase among latitude but no significance (not infected: $p = 0.4322$; $r = 0.0535$; $r^2 = 0.0029$; infected: $p = 0.3163$; $r = 0.0755$; $r^2 = 0.0057$). Furthermore the line of the infected has a higher CF value but the mean reveals the contrary. The infected fish have its mean at 0.010118 ±0.000072, not infected at 0.010544 ±0.000065 and Control at 0,010216 ±0.000110.

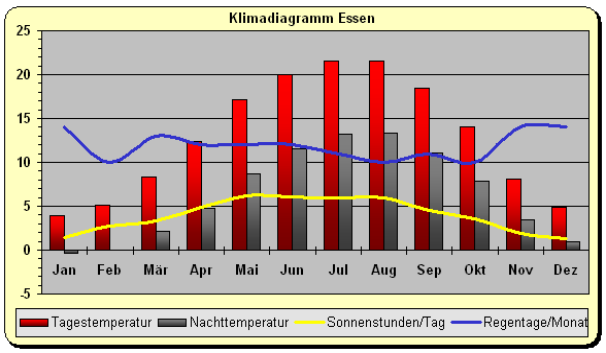
7.12 Climate diagrams

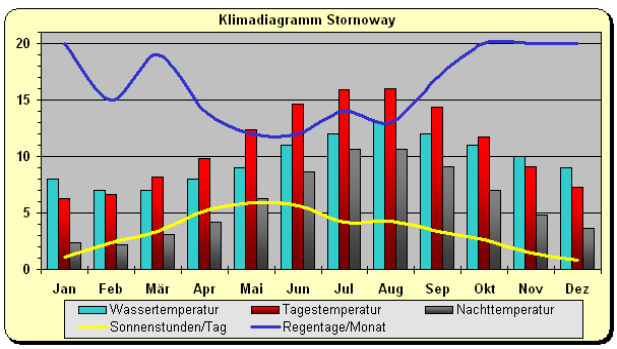












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9 Statement of authorship

I declare that this document and the accompanying code have been composed by myself, and describe my own work, unless otherwise acknowledged in the text. It has not been accepted in any previous application for a degree. All verbatim extracts have been distinguished by quotation marks, and all sources of information have been specifically acknowledged.

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